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Molecular docking studies of 3a,4-dihydro-3*H*-[1,3,2]oxazaphospholo[3,4-a]Indole-1-oxide derivatives for anticancer activity

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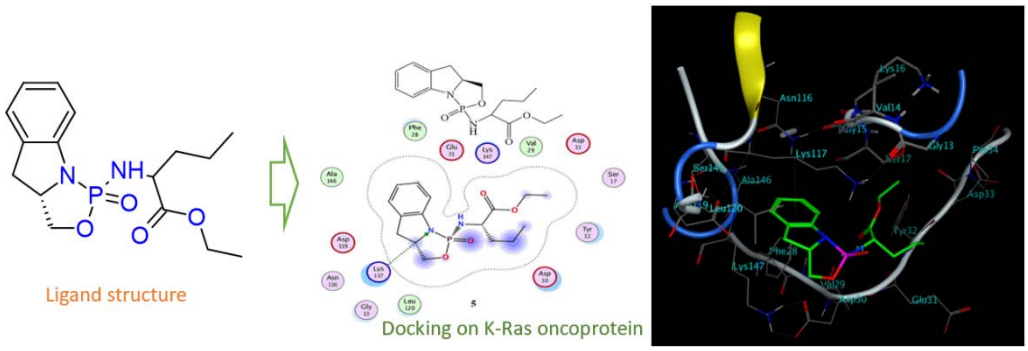
ABSTRACT



This study aimed to evaluate anticancer activity efficacy of eight novel 3a,4-dihydro-3*H*-[1,3,2]oxazaphospholo[3,4-a]indole-1-oxide derivatives (**1-8**) for their potential as drug candidates by using online Swiss ADME and molecular docking studies. Lipinski's Rule of Five and Topological Polar Surface Area (TPSA) analysis suggest favourable properties for drug-likeness, pharmacokinetics, and potentially high intestinal absorption with estimated absorption rates above 80% for most compounds. Furthermore, we investigated the anticancer potential of these compounds using computer simulations by molecular operating environment (MOE). The binding energy and the simulations showed interactions between the compounds and a protein called K-Ras, a target in various cancers. The simulations revealed promising interactions to the protein, particularly for compound **5** which exhibited the strongest binding affinity (-6.62 Kcal/mol) among all tested, which nearby value to the positive control, sotoracib (-7.28 Kcal/mol). This study highlights the promise of these compounds for their potential as both drugs and anticancer agents.

Keywords: 3a,4-dihydro-3H-[1,3,2]oxazaphospholo[3,4-a]indole-1-oxide derivatives, Lipinski's Rule of Five,, In silico docking , K-Ras oncoprotein , Anticancer activity.

INTRODUCTION

Researchers are developing new cancer therapies by creating phosphonamides linked to amino acid esters. This approach aims to improve drug delivery to cancer cells by leveraging the amino acid group. Organophosphorus compounds, while widely used in various applications, can have unintended environmental consequences. In the fight against aggressive cancers like triple-negative breast cancer,[1] limited treatment options necessitate the development of more effective and targeted drugs. This research into phosphonamides with amino acid esters holds promise for a new generation of selective and potent antineoplastic agents. Heterocycles, especially nitrogen-containing ones, are crucial for developing new cancer drugs.[2,3] In fact, nearly 75% of U.S. Food and Durg Administration (FDA) approved anticancer drugs with heterocyclic structures contain nitrogen.[4] Among these, indole stands out as a prominent scaffold. Indole derivatives exhibit potent cell death effects in various cancer cell lines and can modulate key biological pathways in cancer progression.[5] These pathways include cell signaling, cell cycle control, blood vessel formation in tumors, deoxyribonucleic acid (DNA) repair, and triggering cell death. Due to its versatility, indole is a highly valuable platform for designing and developing new and effective anticancer agents.[6]

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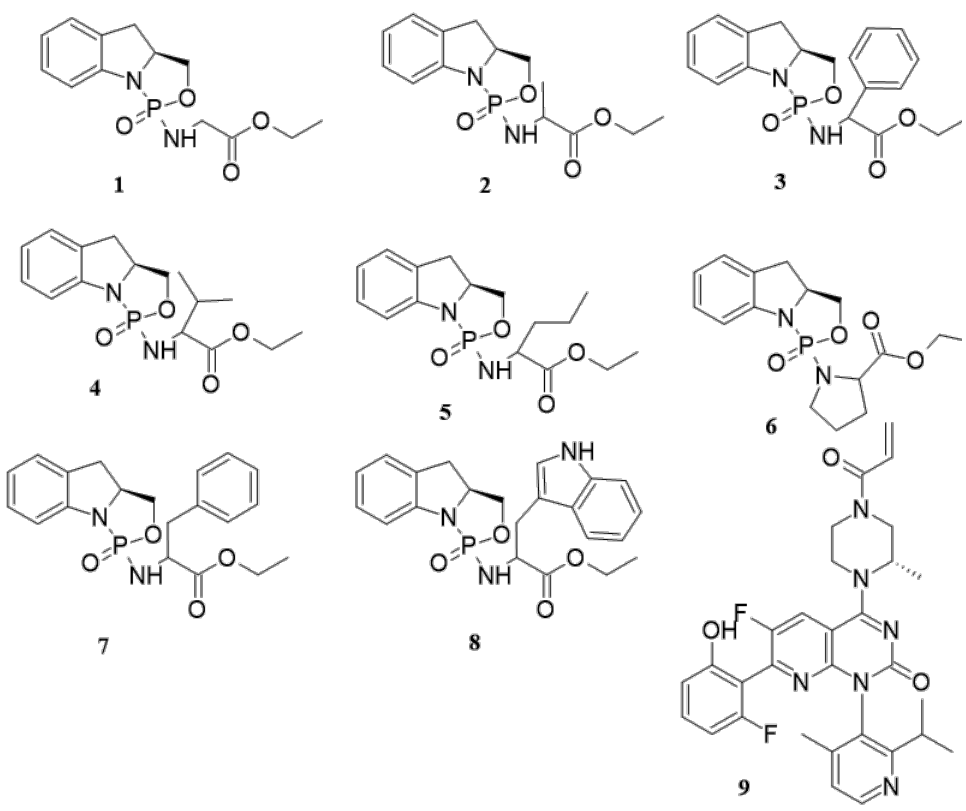

Figure 1. Chemical structure of the molecules used in the study.

Indole alkaloids, like the well-established vincristine and vinblastine used for various cancers, demonstrate the remarkable potential of this natural scaffold in anticancer drug development. These successes are not isolated, as other indole derivatives like indole-3-carbinol and 3,3′-diindolylmethane (DIM) show promise in cancer treatment[7]. This growing list of effective indole-based drugs, both natural and synthetic, reaching clinical use or evaluation, underscores its prominent role in the field8-10. The prevalence of indole among nitrogen-containing heterocycles in FDA-approved drugs further emphasizes its value[11]. While morpholine ranks slightly higher, indole sits firmly within the top 10 most frequent nitrogen heterocycles in these medications, paving the way for continued discovery of potent and selective anticancer agents. Indole, a ring structure containing nitrogen and oxygen, has been a valuable building block for drugs due to its diverse biological activities[12]. These include pain relief, inflammation reduction, antioxidant effects, weight management, cholesterol control, and even combating infections, neurodegeneration, and cancer. The key to indole's effectiveness is its oxygen atom. This atom interacts with target molecules, forming strong bonds, and simultaneously reduces the basicity of the nitrogen atom, improving overall drug performance. Additionally, incorporating morpholine into drugs often enhances their bioavailability and solubility in water, making them more readily absorbed by the body.[13,14]

Similar to morpholine, indole-1-oxide derivatives, another class of nitrogen-containing compounds, have gained significant interest in drug discovery. Their value lies in their ability to readily form new carbon-carbon (C-C) and carbon-nitrogen (C-N) bonds during organic synthesis, making them versatile building blocks for creating novel drugs[15,16]. Compounds containing oxazaphospholo[3,4-a]indol-1-oxides structural units have been recognized as potential anticancer,[17] antifungal,[18] antibacterial,[19] antituberculosis,[20] anti-anxiety,[21] and anti-arrhythmic agents.[22]

We have selected a set of novel experimental compounds viz., 3a,4-dihydro-3H-[1,3,2]oxazaphospholo[3,4-a]indole-1-oxide derivatives were identified in our previous studies for their antimicrobial activity[23]. This efficient synthetic approach allows for the rapid generation of diverse potential drug candidates, accelerating the drug discovery process. Building on this foundation, and the oxazaphospholo[3,4-a]indol-1-oxideclass of the compounds we have predicted on these compounds to be potential for anticancer activity. Since in silico methods offer a valuable and cost-effective way to assess the initial potential of drugs before further testing, we employed them to confirm the anticancer activity of these compounds. Given their promise against cancer cells, these compounds warrant further exploration, and this in silico study serves as the first step in confirming their potential as anticancer agents.

EXPERIMENTAL

Lipinski rule of five for drug likeliness studies to assess the ADME parameters, drug likeliness and pharmacokinetic properties an in silico online search has been conducted for the compounds (1-8) by using SwissADME. Each and every produced compound complies with Lipinski's five-factor criterion. The molecules were predicted by SwissADME to have a molecular weight of below 500 Da, hydrogen bonds showed < 10, and a lipophilicity of five, all of which follow the Lipinski rule of five.[24]

In silico molecular docking studies

The anticancer activity of 3a,4-dihydro-3H-[1,3,2]oxazaphospholo[3,4-a]indole-1-oxide (ligand) using molecular operating environment (MOE) docking simulations against K-Ras oncoprotein (PDB ID: 4epx) (protein) was performed. The S-score was used to assess binding affinity, where more negative values indicate stronger binding. The active

site of the protein was validated using a reference compound, and co-crystallized ligands were re-docked to confirm the identified active site.[25,26]

Results and Discussion

Drug likeness analysis

Scientists use a set of guidelines called Lipinski's Rule of Five (or simply Rule of Five) to assess a molecule's potential as a drug. This rule considers how the drug might behave in the body, including how well it's absorbed (absorption), distributed throughout the body, metabolized (broken down), and eliminated (excretion) – all aspects combined under the term ADME. The Rule of Five also helps determine if the molecule might have a biological effect.The Rule of Five has four key components viz., the molecule's weight should be less than 500 Daltons (Da), a molecule should have no more than 5 hydrogen bond donors (like O-H and N-H groups), a molecule should have no more than 10 hydrogen bond acceptors (atoms like oxygen and nitrogen) and, Octanol-Water Partition Coefficient (log P) value representing the molecule's preference for oil or water, should not be greater than 5.

The compounds **1-8** all comply with these criteria (Table 1). They have less than 500 Da of molecular weight, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, a log P value less than 5 and since they violate no more than one aspect of the Rule of Five, we can expect them to have good characteristics for dissolving and passing through cell membranes (permeability). Following Lipinski's Rule of Five, all eight compounds appear to be drug-like substances. This suggests they might possess favourable properties for how they behave in the body (pharmacokinetics) and could potentially have biological activity.

Table 1. *In silico* drug likeness and K-Ras oncoprotein (PDB ID: 4epx) Binding affinityof compounds (1-8) by using SwissADME

Compound	Structure Analysis using Lipinski's rule of five				Lipinski violations	TPSA (Å²)	% ABS	Binding affinity (Kcal/mol)
	MW	HBD	HBA	Log P				
1	296.26	1	5	2.25	0	77.68	82.2	-6.47
2	310.29	1	5	1.89	0	77.68	82.2	-6.40
3	372.35	1	5	3.16	0	77.68	82.2	-4.06
4	338.34	1	5	2.67	0	77.68	82.2	-6.14
5	338.34	1	5	2.97	0	77.68	82.2	-6.62
6	336.32	0	5	2.72	0	68.89	82.2	-6.12
7	386.38	1	5	2.86	0	77.68	82.2	-6.15
8	425.42	2	5	2.81	0	93.47	76.8	-6.30

TPSA is often used in drug discovery to predict how well a drug candidate will be absorbed by the body. Drugs that have a high TPSA are less likely to be absorbed well because they are less able to pass through cell membranes. Cell membranes are made up of a phospholipid bilayer, which is a fatty layer with polar heads and nonpolar tails. Polar molecules can form hydrogen bonds with the polar heads of phospholipids, which helps them to pass through the membrane. Nonpolar molecules, on the other hand, are repelled by the polar heads and attracted to the nonpolar tails, which makes it more difficult for them to pass through the membrane. Generally, below 60 Å² suggests near-complete absorption, meaning the molecule can easily pass through cell membranes, below 140 Å² indicates good permeability, suggesting the molecule can likely pass through cell membranes reasonably well and, above 140 Å² orhigher values indicate lower permeability. Molecules with very high TPSA may struggle to cross cell membranes. All the experimental compounds **1-8** have below 140 Å². This indicates that the molecule has a relatively small polar surface area, allowing it to form minimal hydrogen bonds with the membrane's polar heads and hindering its penetration. Consequently, the molecule can more readily pass through the intestinal lining.

Intestinal absorption percentage (%ABS) refers[27] to the percentage of an orally administered substance (drug, nutrient, etc.) that actually enters the bloodstream from the intestine. It's a crucial factor in determining the effectiveness of orally taken medications and the bioavailability of nutrients from food. While TPSA offers a good initial assessment, a more precise prediction of %ABS can be made using formulas that consider TPSA alongside other factors. One such formula, developed by Zhao et al[27], uses TPSA to estimate %ABS:

%ABS = 109 – (0.345 x TPSA)

This formula suggests that higher TPSA values correspond to lower %ABS, as the molecule faces greater difficulty crossing the intestinal membrane. In practice, other factors like, molecule size, shape, charge, and interactions with transporters in the intestine can all influence %ABS. This formula provides an estimate, and the actual %ABS might deviate slightly depending on the specific molecule and its properties. Intestinal Absorption which reflects when the drug has taken (medicine, nutrient, etc.) orally, it travels to intestine. Here, the intestine absorbs some of the substance into the bloodstream, allowing it to reach different parts of your body. In this regard, our compounds **1-8** showed the TPSA below 140 Å² suggests good permeability and, potentially high %ABS of likely 82.2 for compounds **1-7** and 76.8 for compound **8**, this means that out of every 100 units of the substance taken orally, 82.2 for **1-7** and 76.8 for **8** units are estimated to enter the bloodstream from the intestine.

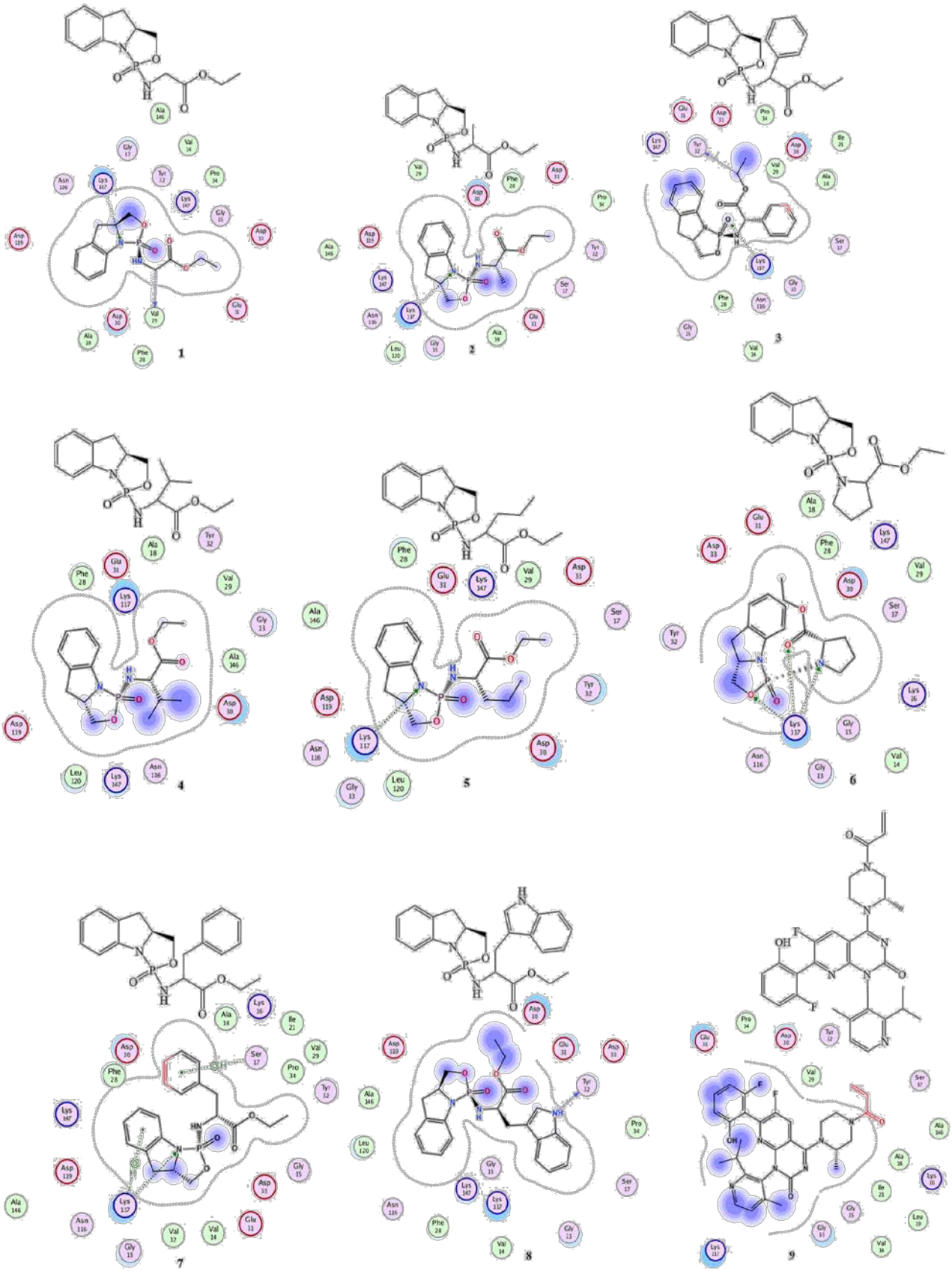

Figure 2: Interactions of K-ras Protein with compounds 1-8 and 9.

However, for a more precise prediction of %ABS, need to consider using the Zhao et al[27]., formula or other methods that account for additional factors like size, shape, and charge can also play a role in intestinal absorption.

In silico anticancer activity

Based on promising research suggesting β-amino carbonyl compounds have potential for antibacterial and antioxidant activity[28,29], and given our prior findings of *in vitro* antimicrobial activity for our synthesized compounds[23], we investigated their anticancer potential using in silico methods. This study aimed to assess the anticancer activity of compounds **1-8** through computer simulations. The docking method, molecular operating environment (MOE) was selected as it is user friendly and gives receptor–ligand binding affinities with all possible binding geometrieson the basis of a numerical value called S-score. Also, it identifies hydrogen bonds, hydrophobic interactions, salt bridges and solvent exposure, and gives the S-score. The proteins PDB ID: 4epx of K-Ras oncoproteins was downloaded from protein databank and the co-crystallised ligands were extracted from the receptors and subjected to dock again and the active site has been validated with the reference compound. The structures of ligands were energy minimised using MMFF94X forcefield[28,29]. Most of the amino acids Val12, Gly13, Val14, Gly^{15}, Ala^{18}, Val^{29}, Asp^{30}, Glu^{31}, Tyr^{32}, Pro^{34}, Asn1^{16}, Lys1^{17}, Asp1^{19}, Leu1^{20}, Ala1^{46} and Lys1^{47} have also interacted with all the experimental ligands including positive control, Sotoracib (**1-9**).

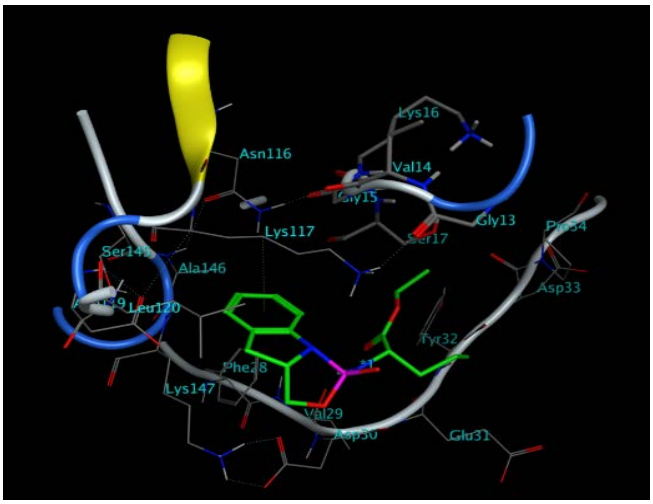

Figure 3. 3D diagram of the compound **5** interactions at the binding site of 4epx.

Analysis of the docking results revealed that the synthesized compounds exhibited various interactions. The nitrogen atom of phosphoramides ((3a,*S*)-1-(amino acid ester) -3a,4-dihydro-3*H*-1λ^5-[1,3,2]oxazaphospholo[3,4-*a*]indol-1-oxides) of ligands **1-9** has strongly interacted with the binding energies of -6.47, -6.40, -4.06, -6.14, -6.62, -6.12, -6.15, -6.30 and -7.28 Kcal/mol, respectively. All these compounds have strong interactions including the positive control (**9**) with the basic amino acid Lys117 of the protein at the active site (Figure 3).

Hydrophobic interactions of compounds **3** and **7** with Ile21 and, **2**,**4**,**5** and **8** with Leu120 due to aromatic substitution and simple alkyl substitutions, respectively, were observed. It has been observed the Val29 has hydrophobic interactions with the flanked methylene between the nitrogen and ester group of the side chains. The compound **5** has shown highest binding energy with -6.62 Kcal/mole and the 3D diagram has shown below (Figure 3).

CONCLUSION

This study investigated the potential of eight novel 3a,4-dihydro-3*H*-[1,3,2]oxazaphospholo[3,4-a]indole 1-oxide derivatives (**1-8**) as drug candidates. Two approaches were employed viz., in Lipinski's Rule of Five and TPSA, all compounds complied with Lipinski's Rule of Five, suggesting favourable characteristics for drug-likeness, pharmacokinetics, and potential biological activity. TPSA analysis revealed good permeability across cell membranes, particularly the intestine, translating to potentially high intestinal absorption percentages (estimated above 80% for most compounds). In Molecular docking simulations showed interactions between the compounds and the K-Ras oncoprotein, a protein implicated in various cancers. All compounds exhibited strong binding affinities, with compound **5** (-6.62 Kcal/mol) with the positive control, Sotoracib (**9**) (-7.28 Kcal/mol) demonstrating the most favourable interaction. These findings are encouraging and suggest that these compounds warrant further investigation for their potential as both drugs and anticancer agents. Future studies involving *in vitro* and *in vivo* assays are recommended to validate the *in-silico* predictions and assess their efficacy in biological systems.

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CONFLICT OF INTEREST STATEMENT

Authors do not have any financial or academic or otherwise conflict of interest for this work.

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