

Identification of Novel Raf-kinase Inhibitors Using in Silico Virtual Screening for Targeted Cancer Therapy

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Abstract

Cancer has long been a major health problem; the overexpression of a protein kinase in cell signaling pathways is a common cause of it. Targeted therapy has become a more popular focus of research due to its specificity towards particular protein kinases, compared to conventional chemotherapy, which does not differentiate between normal and cancerous cells. B-Raf is one such protein kinase that is commonly mutated in various types of cancer, including melanoma, colorectal cancer, and prostate cancer. The objective of this study is to identify new inhibitors or candidates for anti-protein kinase drugs targeting the Raf-B protein using the dual strategy of screening novel compounds and repurposing drugs. This research focused on using in silico drug design tools, such as Autodock vina, for virtual screening. We docked and studied 13 candidates that had not yet passed the preclinical phase, and repurposed 29 medications that already had a temporary authorization for use for a different target. This allowed us to predict their physicochemical and pharmacokinetic properties, as well as their binding energies with the B-RAF protein kinase (ID: 6M3M). Our results were promising, yielding three candidates with an excellent binding energy above -10.2 kcal/mol, with candidate 8b (-10.7 kcal/mol, 6-(4-((phénylamino)(sulfanyl) méthyl]amino)sphényl)-2-phényl-2,3,4,5-tétrahydropyridazin-3-ol) being the most promising. We also found five molecules from the repurposing with a binding energy above -9.4 kcal/mol, with Uprosertib (-10.3 kcal/mol, N-[(2S)-1-Amino-3-(3,4-difluorophenyl)-2-propanyl]-5-chloro-4-(4-chloro-1-methyl-1H-pyrazol-5-yl)-2-furamide) having the highest binding energy, it used in trials studying the treatment of Cancer, Melanoma, Solid Tumours, Cervical Cancer, and HER2/Neu Negative, among others. Importantly, none of these candidates were predicted to pass the blood-brain barrier, making them harmless to the central nervous system. A QSAR study is recommended to provide valuable insights into the ligand-protein interactions and potentially optimize the design of these promising molecules with enhanced potency and selectivity. Also, it is important to conduct further in vitro and in vivo studies to verify their efficacy and safety.

Keywords: Cancer; Targeted therapy; Protein kinase; Molecular docking; Autodock vina; Drug repurposing.

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1. Introduction

Cancer has been prevalent in recent centuries. It arises from the uncontrolled and abnormal multiplication of cells that evade the body's normal cellular regulation [1]. These cells form a tumor that can invade nearby tissues or spread to distant parts of the body via the bloodstream or lymphatic system. This characteristic makes cancer one of the deadliest diseases [2]. For a long time, chemotherapy has been a primary clinical solution for cancer treatment [2]. Recent research has shed light on the significant side effects associated with conventional chemotherapy, primarily because it cannot differentiate between normal and cancerous cells during their rapid division [3]. However, a promising alternative known as targeted therapy has emerged, offering a more specific and precise approach to treatment. Compared with conventional methods, targeted therapy has a greater therapeutic index and lower toxicity. This novel form of anticancer treatment specifically targets molecular abnormalities present in tumor cells, such as Protein kinases [4].

Protein kinases are a large family of enzymes that catalyze the transfer of a phosphate group from ATP to a protein substrate [5]. This process, called phosphorylation, regulates many cellular processes, including cell growth, differentiation, and death. There are two main types: tyrosine and serine/threonine, which is the majority type [6].

Tumor survival, growth, and metastasis depend on tumor cell proliferation, differentiation, apoptosis, and angiogenesis, which are regulated by numerous protein kinases and signal transduction pathways [7].

The Ras-mitogen-activated protein kinase (MAPK) signaling pathway is the first signaling pathway elucidated from the cell membrane to the nucleus, consisting of the Ras/Raf/MEK/ERK signal transduction cascade. Raf is a serine/threonine kinase that includes three isoforms (Raf-1 or C-Raf, A-Raf, and B-Raf). Blockade of the RAF/MEK/ERK pathway at the level of RAF kinases can effectively inhibit tumors driven by this pathway [8].

B-Raf protein (SER/THR kinase) plays an important role in activating the RAS/RAF/MEK/ERK signaling pathway and promoting normal cell development [9]; hence, this protein is considered a promising therapeutic target for the discovery of new antitumor agents.

B-Raf kinase is mutated in approximately 7% of all human cancer cells, and the most common oncogenic mutation is V600E, which is found in 90% of B-Raf-mutated cancer cells and involves a substitution of valine by glutamic acid at codon 600. The deregulation of the normal B-Raf signaling pathway leads to its overexpression in different types of cancer, including colorectal (15%), hepatocellular (14%), mammary gland (10%), melanoma (60%), and prostate cancer (10%) [10].

The objective of this study is to identify new inhibitors or candidates for anti-protein kinase drugs targeting the Raf-B protein using the dual strategy of screening novel compounds and repurposing drugs. Firstly, to screen and predict the potential use of certain candidates retrieved from papers addressing the same target and to provide a synthetic overview of the results, thereafter to reposition several drugs currently in clinical trials to extend their spectrum of activity to tackle and overcome the threat of resistance to anti-Raf-B drugs, through the use of the virtual screening method.

2. Materials and Methods

2.1. Materials

— **Virtual screening software:** AutoDock is a program used for protein-ligand docking. It is a set of procedures, the aim of which is to predict the interaction of small molecules, such as « ligand » drug candidates or substrates, with a receptor whose 3D structure is known, in our docking study, we used AutoDock Vina [10, 11];

— **Results visualization software:** to visualize our results, we used Biovia Discovery Studio Visualizer version 4.0.100.13345 to depict various intermolecular interactions, including hydrogen, hydrophobic, Van der Waals, and electrostatic bonds. Discovery Studio is based on machine learning algorithms [12];

— **ADMET prediction servers:** The SwissADME tool is used to assess whether the molecule is likely to reach its target protein in the body, in terms of absorption, distribution, metabolism, and excretion (ADME). The pharmacokinetic parameters and physicochemical properties of the proposed inhibitors were predicted using this rapid server [13].

Physicochemical properties: The notion of « *Druggability* » or « *Drug-likeness* » establishes a rule of thumb based on the physicochemical properties of orally administered

drugs. This rule is called « Lipinski's Rule of Five » or « RO5 » [14] and is a standardized parameter for detecting oral drugs and estimating drug design potential from bioactive molecules. This rule is based on four physicochemical parameters for the molecule to have adequate pharmacokinetic properties [14]:

- Molecular weight (MW) ≤ 500 Daltons (Da);
- Lipophilicity; water/octanol partition coefficient $\log P \leq 5$;
- Number of hydrogen bond donors HBD ≤ 5 ;
- Number of hydrogen bond acceptors HBA ≤ 10 .

Protein: Protein Data Bank (PDB), is a worldwide collection of data on the three-dimensional structures (3D structures) of biological macromolecules, mainly proteins and nucleic acids [15].

Ligands: PubChem is an American database of chemical molecules; it currently contains several million compounds, with their structures and physico-chemical properties freely accessible. This chemical library, comprising over 102 million compounds, was used to highlight structural similarities with the ligand used in this study [16].

2.2. Methods

2.2.1. Target cavity determination

The active site of our protein has been identified and analyzed by the PrankWeb [17]. The number of active pockets was also determined using CASTp [18]. The presence of hydrogen bonds, lipophilic or hydrophilic interactions, and ionizable charges all affect the protein and ligand's binding affinity. The AutoDock Tool software was used to generate the grid by selecting the active sites of the proteins [19]. The dimensions of the grid box obtained are as follows: center_x = 11.690; center_y = -8.020; center_z = -17.412.

2.2.2. Validation of virtual screening protocol

1. RMSD test: The correct positioning of the ligand relative to its receptor is usually judged by the root-mean-square deviation (RMSD) between the position of the ligand in the crystallographic complex and that of the ligand docked by the docking program. A maximum threshold of 2 Å is often used to discriminate a good position from a bad one [20].

2. Visual analysis: confirms the results of the RMSD test and verifies the reliability of the simulations. This

visualization allows us to determine whether a ligand simulated by AutoDock overlaps with its reference position.

3. Screening protocol: our study will focus on the interaction between the active site of the enzyme (B-RAF) and the inhibitors to form a stable complex. Interactions are carried out using "AutoDock Vina" software [19].

2.2.3. Protein and ligand preparation

1. Ligand preparation

Ligands previously constructed in ChemDraw 3D and saved in PDB format were downloaded from the PubChem database. They were then processed by AutoDock Tools, which controlled their movements while defining their aromatic atoms, flexible bonds, and torsion angles. All these data were saved in pdbqt format.

2. Protein preparation

The therapeutic target studied in this work is the B-Raf proto-oncogene serine/threonine-protein kinase (PDB: 3IDP), whose structure was obtained from the PDB. The protein is prepared using the AutoDock Tools subroutine, adding the missing polar hydrogens. Kollman charges were then assigned before the protein was exported in pdbqt format.

3. Virtual screening with molecular docking

Docking studies were performed by AutoDockTools 1.5.7 graphical interface to search for the best conformation of the enzyme-ligand complex. We analyzed the conformational energies of the candidates and the B-Raf protein kinase (PDB: 3IDP) to determine the binding energy. The binding results were analyzed in Biovia Discovery Studio Visualizer software to assess potential interactions between the ligand and target proteins.

4. Pharmacokinetics

Predictions of passive Human gastroIntestinal Absorption (HIA) and Blood-Brain Barrier (BBB) permeability both involve reading the Boiled-Egg model. Knowledge of which compounds are substrates or non-substrates of the permeability glycoprotein (P-GP) is essential for assessing active efflux across biological membranes, for example, from the gastrointestinal wall to the lumen or brain. One of P-GP's main roles is to protect the central nervous system (CNS) from xenobiotics. It is also important to note that P-GP is

overexpressed in certain tumor cells, leading to multi-resistant cancers [21].

3. Results and Discussion

3.1. Presentation of the studied protein

B-Raf is a 766-amino acid, regulated signal transduction serine/threonine-specific protein kinase. Broadly speaking, it is composed of three conserved domains characteristic of the Raf kinase family: conserved region 1 (CR1), a Ras-GTP-binding self-regulatory domain, conserved region 2 (CR2), a serine-rich hinge region, and conserved region 3 (CR3) (**Figure 1**) [22, 23].

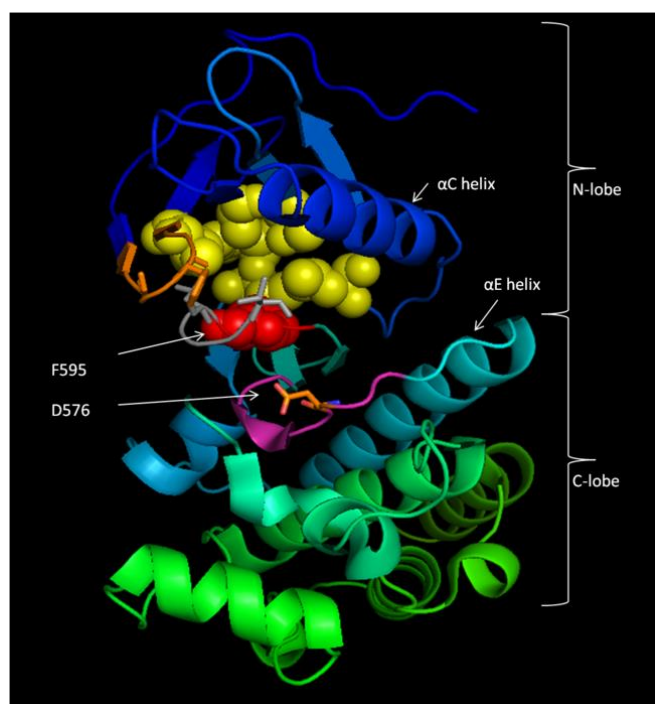


Figure 1. Inactive conformation of the B-Raf kinase (CR3) domain. P-Loop (orange) hydrophobic interactions with activation loop (gray) residues that stabilize the inactive kinase conformation are shown with sticks. F595 (red) blocks the hydrophobic pocket where the ATP adenine binds (yellow). D576 (orange) is shown as part of the catalytic loop (magenta). Figure modified from PDB id 1UWH [23].

3.2. Validation of the virtual screening protocol

3.2.1. RMSD test

Once the protein has been prepared, the co-crystallized ligand is redocked into the target's active site using AutoDock Vina software. The RMSD is calculated by comparing the geometry of the best position with the original ligand obtained directly from the PDB using

Discovery Studio. The results of this test are shown in **Table 1**.

Table 1. Redocking results.

Protein	ID PDB	Ligand	RMSD	Score
B-Raf V600E kinase domain	3IDP	Aminoisoquinoline inhibitor	0.5230	-10.0

3.2.2. Visual analysis

As shown in **Figure 2**, the Aminoisoquinoline inhibitor ligand models simulated by AutoDock (colored purple) exhibit spatial conformations that are perfectly superposable at a distance of 0.2615 Å with those determined experimentally by crystallography (colored green). This is further evidence of the reliability of the AutoDock program for in silico studies of RAF-B inhibitors.

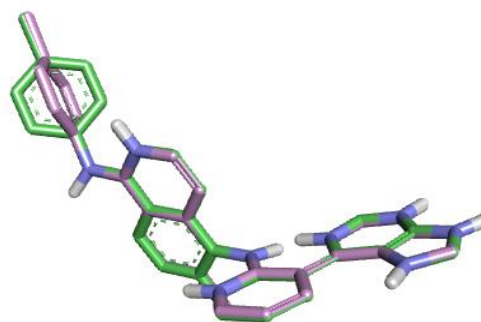


Figure 2. The Aminoisoquinoline inhibitor ligand models simulated by AutoDock.

3.3. Review of tested chemical compounds

In this part, we describe the physico-chemical parameters and the interaction results of 13 chemical compounds taken from the bibliography with B-Raf kinase (PDB: 3IDP).

3.3.1. Classification of chemical compounds according to liposolubility

Figure 3 represents the classification of chemical compounds according to their liposolubility.

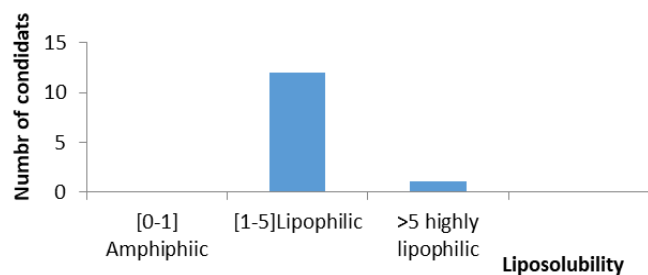


Figure 3. Classification of chemical compounds according to their liposolubility.

3.3.2. Classification of the molecules according to the topological polar surface

Figure 4 represents the classification of compounds according to their topological polar surface area.

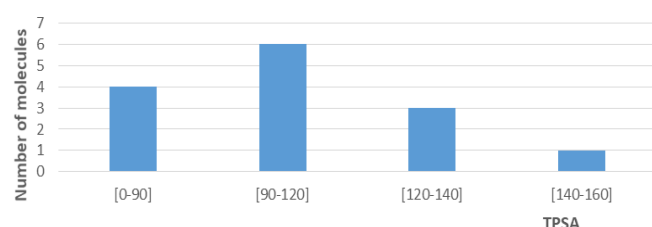


Figure 4. Classification of chemical compounds according to their TPSA.

3.3.3. Hydrogen bonds donor/acceptor

Figure 5 shows the number of hydrogen-bond donor/acceptor groups for the candidates.

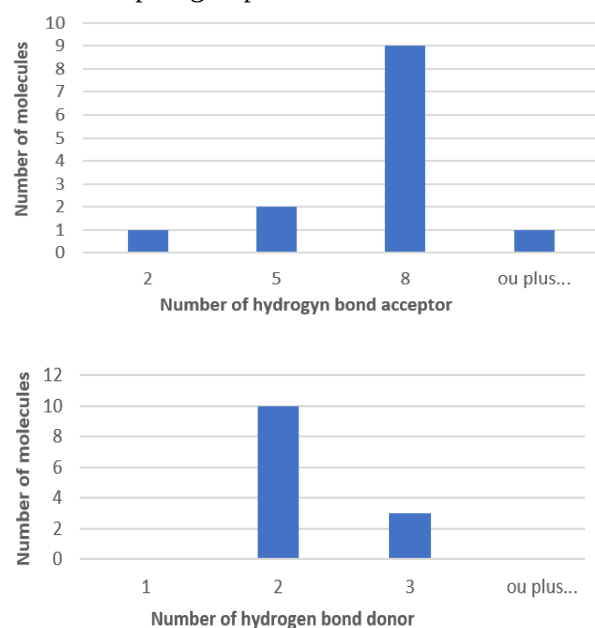


Figure 5. Number of hydrogen bond donor/acceptor groups for candidates.

3.3.4. Classification of compounds according to molecular weight

Table 2 represents the classification of compounds according to molecular weight.

Table 2. The molecular weight of compounds.

Compounds	MW (g/mol)	Compounds	MW (g/mol)
1i	584.38	25d	487.37
15j	558.65	38a	471.56
17b	549.59	12e	462.45
18a	535.56	12b	458.51
15b	526.58	12	426.47
14a	494.56	8b	400.50
12f	494.49	/	/

3.3.5. Study of Druglikeness

The results obtained using the SwissADME server are shown in **Table 3**.

Table 3. Comparative table of the druglikeness rules results.

Compounds	Lipinski	Bioavailability score
1i	No	0.17
8b	Yes	0.55
17b	Yes	0.55
18a	Yes	0.55
12b	Yes	0.55
12f	Yes	0.55
14a	Yes	0.55
15b	Yes	0.55
15j	Yes	0.55
25d	Yes	0.55
35	Yes	0.55
38a	Yes	0.55
12e	Yes	0.55

3.3.6. Study of the IC50

Table 4 represents the IC50 values of the compounds.

Table 4. Representative table of the IC50 value.

Compounds	IC50 (nM)	Compounds	IC50 (nM)
38a	2	15b	86.49
1i	7	25d	94
8b	24.79	18a	114
12f	33.1	17b	128
12b	37	12e	490
15j	68.17	/	/

3.3.7. Post-screening analysis

1. Analysis of ligand receptor affinity

The interactions between the active site of the B-Raf enzyme and the ligands are calculated using molecular docking, and the results are presented in [Table 5](#).

Table 5. The docking result of candidates.

Compounds	Binding energy (kcal/mol)	Compounds	Binding energy (kcal/mol)
8b	-10.7	12e	-9.3
12b	-10.6	38a	-9.2
14a	-10.2	18a	-9.2
15j	-10.0	25d	-8.4
12f	-9.8	17b	-8.4
15b	-9.4	1i	-7.3
12	-9.4	/	/

2. Analysis of the active site

The [Figure 6](#) represent the active site's amino acids that bond with candidates.

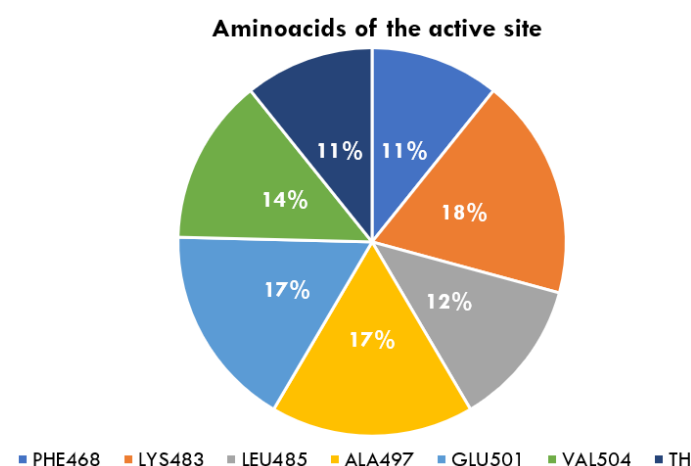


Figure 6. Representation of the active site's amino acids that bind to candidates.

3. Review of compounds with high affinity

[Table 6](#) represents the classification of the top three compounds based on their binding energy.

Table 6. Classification of the top three compounds based on their binding energy.

Rang	Complex	Energy (kcal/mol)	Number of hydrogen bonds
1	8b	-10.7	2
2	12b	-10.6	1
3	14a	-10.2	2

4. Interaction study of the three top ligands with B-Raf

[Figure 7](#) represents the 2D Diagrams of the amino acid active sites that bind to the top three candidates.

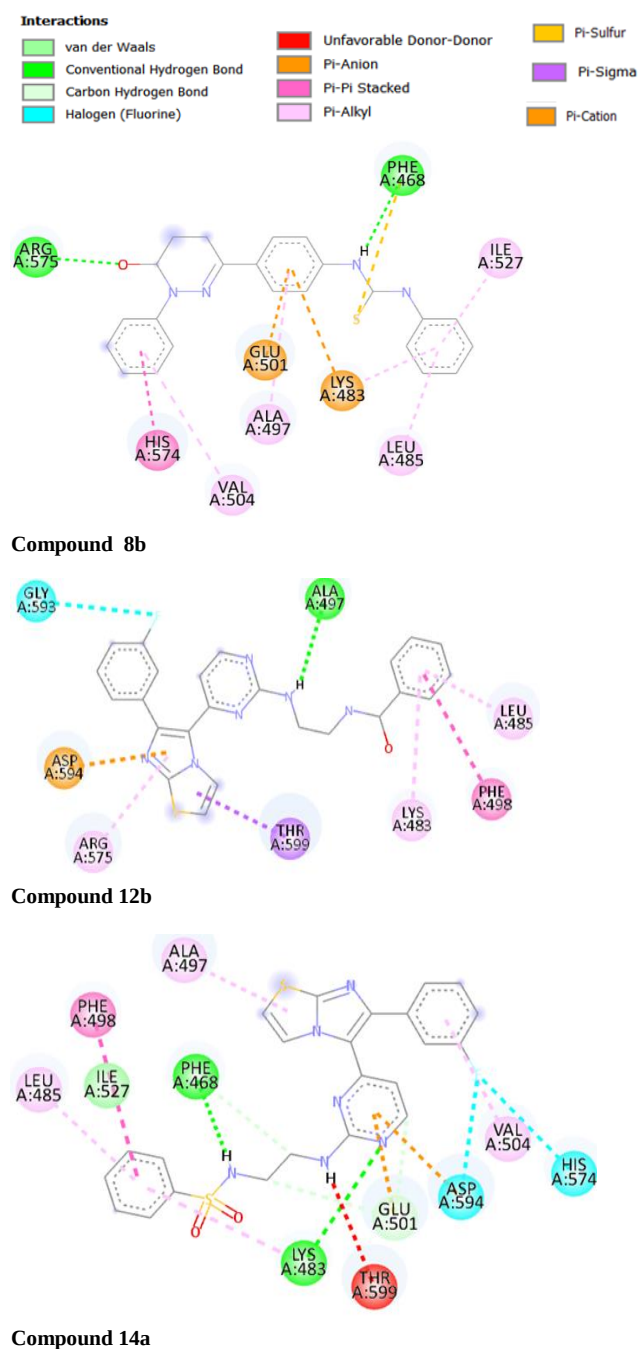


Figure 7. 2D Diagrams of the amino acids' active site that bind with the three top candidates.

5. Pharmacokinetics

Predictions of passive human gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeability both rely on interpretation of the Boiled-Egg model ([Figure](#)

8). The results from SwissADME show that none of these three candidates pass the BBB; compound 14a is outside the egg, and only compound 12b is a PGP substrate (Figure 8).

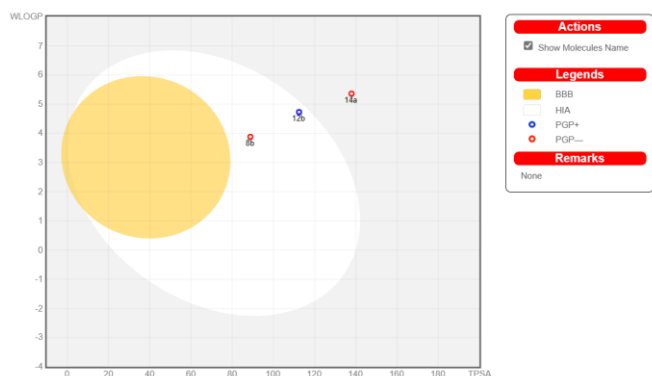


Figure 8. Boiled-Egg model for the three top candidates.

3.4. Drug repurposing

Drug repurposing is the process of identifying new therapeutic uses for existing drugs that regulatory agencies have already approved for other indications. This strategy can reduce the time and cost of drug development and the risk of failure in clinical trials. Drug repurposing has gained increasing attention in recent years as a promising approach to address unmet medical needs and accelerate the development of new treatments for a wide range of diseases.

3.4.1. Classification of molecules according to their mode of action

Figure 9 represents the classification of molecules according to their mode of action.

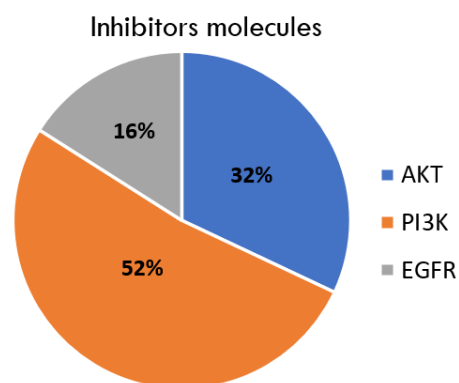


Figure 9. Repartition of molecules based on the mechanism of action.

3.4.2. Classification of molecules according to liposolubility

Figure 10 represents the classification of molecules according to their liposolubility.

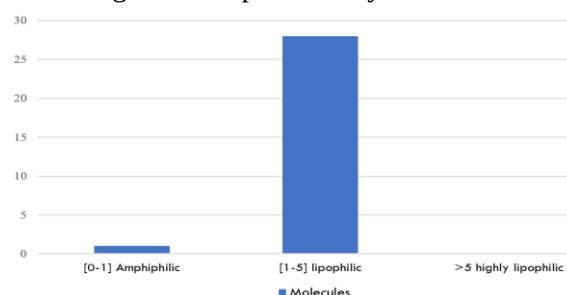


Figure 10. Classification of molecules according to their liposolubility.

3.4.3. Classification of the molecules according to the topological polar surface

Figure 11 represents the classification of the molecules according to the TPSA.

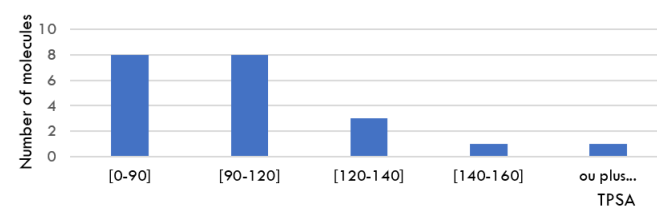


Figure 11. Classification of molecules based on their TPSA.

3.4.4. Number of hydrogen bond donor/acceptor groups

Figure 12 represents the number of hydrogen bond donor/acceptor groups for candidates.

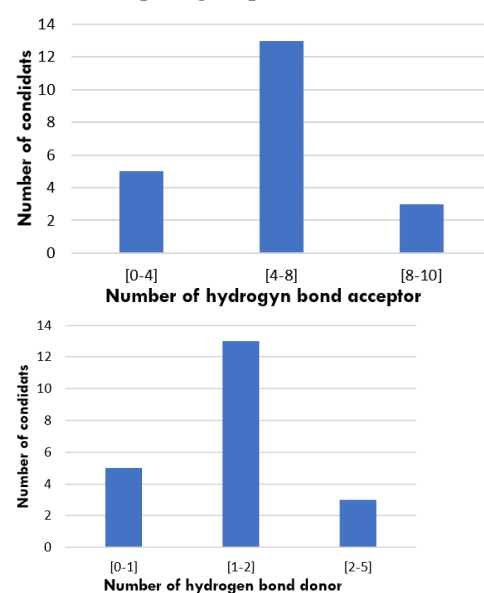


Figure 12. Number of hydrogen-bond donor/acceptor groups for candidates.

3.4.5. Post-screening analysis

1. Analysis of ligand receptor affinity

The interactions between the active site of the B-Raf enzyme and the ligands are calculated by molecular docking, and the results are presented in [Table 7](#).

Table 7. The docking results of drug repurposing.

Molecules	Energy of Bending Kcal/mol	Molecules	Energy of Bending Kcal/mol
Uprosertib	-10.3	Duvelisib	-8.7
Alpelisib	-9.9	Tamnorzatinib	-8.7
Afuresertib	-9.6	Nazertinib	-8.6
Eganelisib	-9.5	Copanlisib	-8.5
Poziotinib	-9.4	Lazertinib	-8.4
Ipatasertib	-9.2	Serabelisib	-8.4
Pilaralisib	-9.2	Buparlisib	-8.3
Capivasertib	-9.1	BAY1125976	-8.0
MSC2363318A	-9.0	Mirdamentib	-7.7
Miransertib	-8.9	Ivosidenib	-7.6
Pictilisib	-8.9	Parsaclisib	-7.5
AZD3759	-8.8	Lorlatinib	-7.4
Idelalisib	-8.8	Sonolisib	-6.7
Taselisib	-8.8	Omdpi	-5.9
MK_2206	-8.7	/	/

2. Analysis of the active site

The Glu 501 amino acid bonds with most molecules; secondly, there is Ala 497, and in third place comes Lys 483 ([Figure 13](#)).

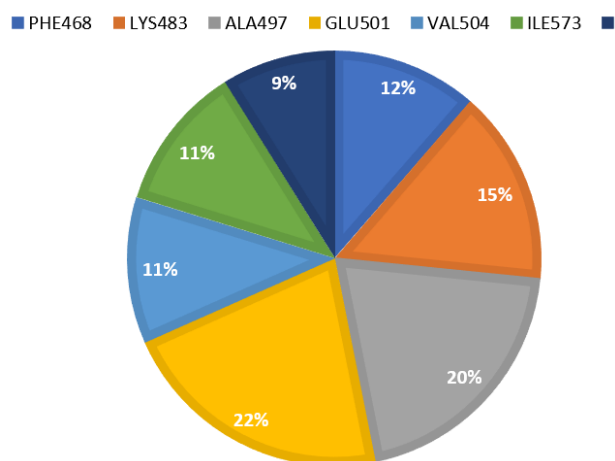


Figure 13. Representation of the active site's amino acids that bind to candidates.

3. Review of molecules with high affinity

[Table 8](#) represents the top five molecules based on their binding energy.

Table 8. Classification of the top five molecules based on their binding energy.

Rank	Ligand name	Energy (kcal/mol)	Number of hydrogen bond
1	Uprosertib	-10.3	2
2	Alpelisib	-9.9	0
3	Afuresertib	-9.6	4
4	Eganelisib	-9.5	1
5	Poziotinib	-9.4	1

4. Interaction study of the five top molecules with B-Raf

Uprosertib had the highest binding energy score of -10.3 Kcal/mol. It bonded to the active site's amino acids through two hydrogen bonds with Glu 501 and Lys 483, and through three Pi-alkyl interactions with Leu 485, Ala 497, and Val 504, and one halogen bond with Met 484. Alpelisib had the second-highest binding energy score and formed only hydrophobic bonds with the active site's amino acids. It forms four halogen bonds: fluorine with Met 484, Phe 468, Lys 483, and Leu 525; two alkyl bonds with Leu 485, Val 504, and Glu 501; and no hydrogen bonds. Afuresertib had a score of -9.6 Kcal/mol. It forms four hydrogen bonds with Lys 483 and Ala 497, and two with Glu 501; hydrophobic interactions include two Pi-alkyl bonds with Val 504 and Leu 485, one Pi-sigma bond with His 574, and one halogen bond with Met 484. Eganelisib had a binding energy score of -9.5 Kcal/mol and formed several bonds with the active site's amino acids, including one hydrogen bond with Ile 573 and multiple hydrophobic interactions, including three Pi-anion/cation bonds with Lys 483, Glu 501, and Arg 575, and one Pi-alkyl bond with Val 504. Poziotinib scored -9.4 Kcal/mol. When docked with the B-Raf protein, it formed multiple bonds with the active site's amino acids, including one hydrogen bond with Ala 497 and multiple hydrophobic bonds; one fluorine-type halogen bond with Asn 500, two pi-anion/cation bonds with Lys 483 and Glu 501 ([Figure 14](#)).

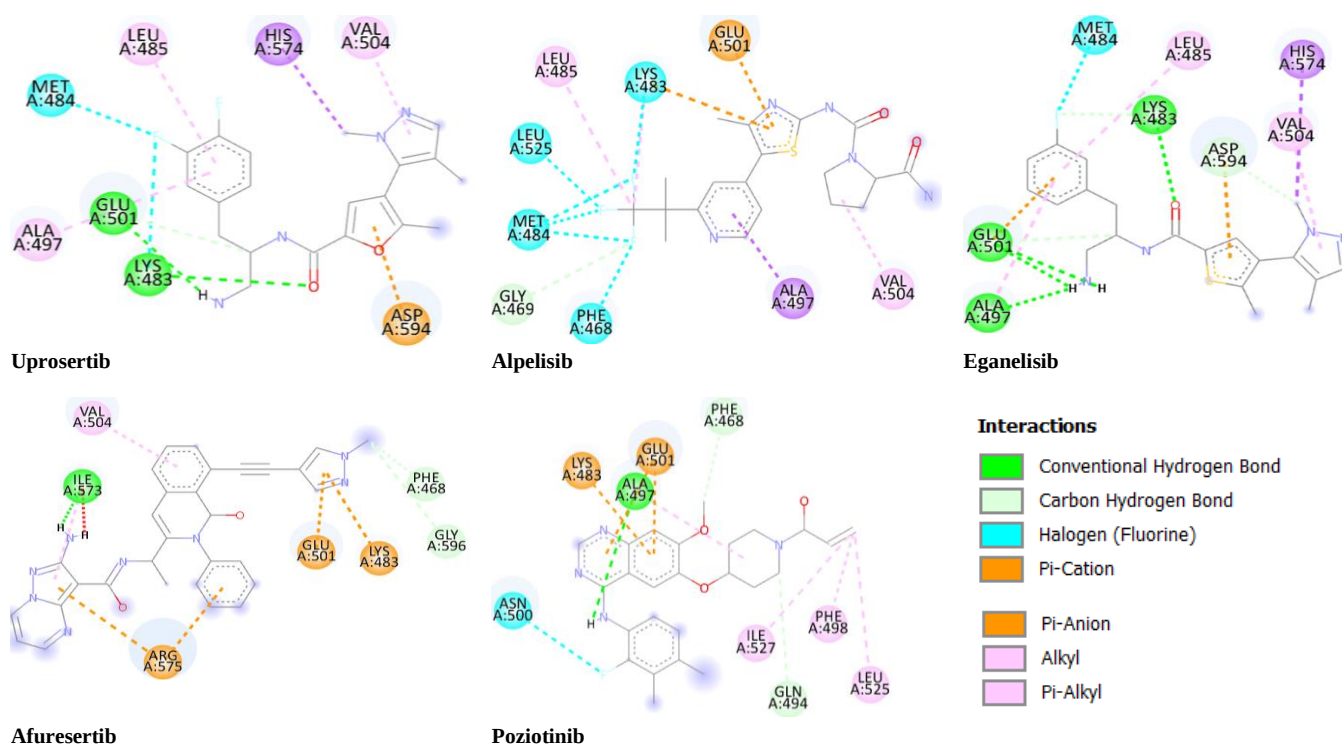


Figure 14. 2D Diagrams of the amino acids' active site that bind with the five top molecules.

5. Pharmacokinetics

The results from SwissADME show that none of these three candidates pass the BBB; only Alpelisib is outside the egg. Alpelisib and Eganelisib are PGP substrates (Figure 15).

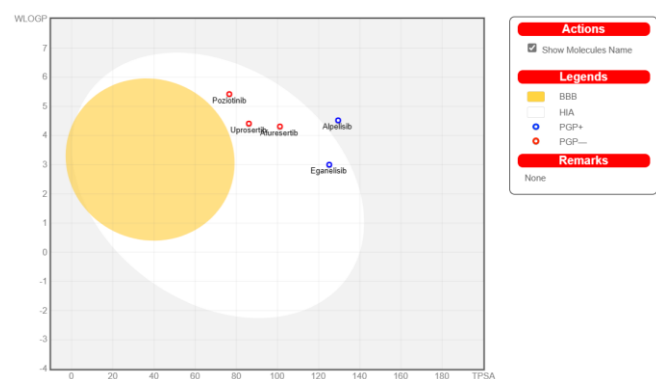


Figure 15. Boiled-Egg model of the top five molecules.

In the Boiled-Egg study, the five candidates are all highly absorbed in the gastrointestinal tract except for Alpelisib, and three of them (Poziotinib, Uprosertib, and Afuresertib) are PGP- so they have an augmented oral bioavailability and Cmax (risk of toxicity). These compounds (Uprosertib, Alpelisib, Afuresertib,

Eganelisib, and Poziotinib) can't pass the BBB; therefore, they are unable to reach the CNS, so this system is protected from any adverse effects these compounds may cause (Figure 15).

3.5. Discussion

Our aim was to identify lead compounds through computer-based drug design that would be effective against the overexpression of the B-RAF protein kinase. Our strategies included testing of molecules extracted from previous articles and drug repurposing.

The RMSD value calculated for the B-Raf V600E kinase domain is 0.5230 Å. The prediction is acceptable if its value does not exceed 2 Å. This result indicates that AutoDock Vina is a suitable modeling tool for the in silico study of the inhibition of this protein. The low RMSD of the Aminoisoquinoline inhibitor kinase domain complex, with a dock score of -10.0 kcal/mol, demonstrated the validity of the used docking protocol. Following the RMSD test, visual analysis is an essential step to verify the reliability of AutoDock docking simulations further and to confirm the RMSD results. This visualization enables us to determine whether a

simulated ligand overlaps with its reference pose. The result shows a spatial conformation perfectly superposable at a distance of 0.2615 Å. We can conclude that AutoDock is a high-performance program that can be used with minimal risk of error.

Firstly, we screened a total of 13 chemical compounds extracted from previous articles targeting the V600E B-RAF protein kinase. The partition coefficient (P) describes the propensity of a neutral compound to dissolve in an immiscible biphasic system of lipids and water [24]. A negative value for logP means the compound has a higher affinity for the aqueous phase (it is more hydrophilic); when logP = 0, the compound is equally partitioned between the lipid and aqueous phases; a positive value for logP denotes a higher concentration in the lipid phase (it is more lipophilic). LogP = 1 indicates a 10:1 partitioning between the Organic and Aqueous phases [25]. The majority of them have a lipophilic character ranging from 1 to 5 (**Figure 3**).

Topological Polar Surface Area (TPSA) is a computational method used to calculate the polar surface area of a molecule. This is important for drug design, as a molecule's polarity can affect its ability to bind to biological targets [26]. All molecules with TPSA greater than 140 Å tend to penetrate cell membranes, whereas those with TPSA less than 90 Å can pass through the blood-brain barrier [27]. More than half of these candidates have a TPSA included in the interval. Fewer than five of them can pass through the blood-brain barrier (**Figure 4**).

Hydrogen bonds are a type of non-covalent interaction between a hydrogen atom and a highly electronegative atom. The number of hydrogen bond donors and acceptors reflects the number of hydrogen bonds that form between the molecules, so the more hydrogen bonds formed, the more stable the complex formed is [28]. Most of them have (5-8), and they are strong acceptors and are weak donors with (0-3) hydrogen bonds (**Figure 5**). As for the molecular weight, the compound 1i has the heaviest molecular weight, 584.38 g/mol, while the compound 8b is the lightest with 400.5 g/mol (**Table 2**).

The results obtained using the SwissADME server in **Table 3** reveal that only the compound 1i violated the Druglikeness rules, with only one parameter

incompatible with the Lipinski rule, but it also had the lowest bioavailability score of 0.17.

The IC₅₀ is the concentration of a drug or other compound that inhibits 50% of a biological process or reaction. It is commonly used in pharmacology to assess drug potency and compare the effectiveness of different compounds [28]. The compound 38a has the lowest IC₅₀, and the compound 12e has the highest IC₅₀ value (**Table 4**).

As for the interactions between the active site of the B-RAF enzyme and the ligands are calculated by molecular docking, the compound 8b got an affinity of -10.7 kcal/mol which is the highest, the compound 12b got -10.6 kcal/mol and the compound 14a in the third place with -10.2 kcal/mol. In contrast, the compound 1i had the lowest affinity (-7.3 kcal/mol).

The analysis site showed that the majority of candidates bonded with Lys 483 (18%), with Ala 497 and Glu 501 having the second-highest percentage at 17 % (**Figure 6**).

Among the 13 compounds, a shortlist of three was selected on the grounds of their binding energies. The candidate 8b leading them with an affinity of -10.7 kcal/mol (**Table 6**). The compound 8b formed many bonds with the active site's amino acids, multiple hydrophobic interactions were formed, including four pi-alkyl bonds with Leu 485, Ala 497, Val 504, and Ile 527, and two Pi-cation bonds with Lys 483 and Glu 501, and two hydrogen bonds with Phe 468 and Arg 575 (**Figure 7**). In the study entitled « *The Crystal Structure of BRAF in Complex with an Organoruthenium Inhibitor Reveals a Mechanism for Inhibition of an Active Form of BRAF Kinase* » realized by P Xie et al. [29], found that 8b compound, the tested ligand with highest binding energy displayed a hydrogen bond with the Phe 468 of the activation segment blocking the B-RAF in its inactive form, a hydrophobic bond with the β3 strand via Lys 483. Comprehensive analyses indicate that the β3-lysine participates in the formation of a salt bridge with the αC-glutamate for the formation of an active protein kinase conformation, which corresponds to an "αCin" arrangement [29]. In many inactive enzymes, these residues fail to form this salt bridge, thereby adopting an inactive "αC out" structure. 8b Compound also formed a hydrophobic bond with Leu 485 near the end of the β5-strand, Ala 497, Val 504 within the αC-helix [29], Ile 527

near the end of the $\beta 5$ -strand and His 574 of the catalytic loop, a hydrophobic bond with αC helix via Glu 501 and a hydrogen bond with the catalytic loop via Arg 575 [29].

The compound 12b formed several bonds with the active site's amino acids, mostly through hydrophobic interactions, it bonds with three Pi-alkyl to each of the amino acids Lys 483, Leu 485, Arg 575, one Pi-Sigma, Pi-anion, Pi-Pi stacking bond types were formed with Thr 599, Asp 549, Phe 498 consecutively and one halogen bond which is fluorine formed with Gly 593, and only one hydrogen bond formed with Ala 497 (Figure 7). In the study entitled « *The Crystal Structure of BRAF in Complex with an Organoruthenium Inhibitor Reveals a Mechanism for Inhibition of an Active Form of BRAF Kinase* » realized by P Xie et al. [29], found that 12b compound in the other hand displayed a hydrogen bond with Ala 497, a hydrophobic bond with the $\beta 3$ strand via Lys 483, a hydrophobic bond with Leu 485 and Phe 498, a hydrophobic bond with the catalytic loop via Arg 575, a fluorine bond with the activation loop via Gly 593, a hydrophobic bond with the DFG motif via Asp 594 at the end of the catalytic loop blocking the placement of the Mg^+ ions [29] and a hydrophobic bond with the activation loop via Thr 599 a residue that after being phosphorylated participate in the activation of the Raf kinases [29].

The compound 14a formed multiple hydrophobic bonds including three Pi-alkyl bonds with Leu 485, Ala 497, Val 504, two halogen bonds which are fluorine bonds with both His 579 and Asp 594, only one Pi-Pi to Phe 498 and lastly one Van Der Waals bond and two hydrogen bonds with Phe 468 and Lys 483, with Ile 527 with an overall binding energy score of -10.2 kcal/mol (Figure 7). In the study entitled « *Targeting oncogenic Raf protein-serine/threonine kinases in human cancers*, » realized by R Roskoski et al. [30], found that the 14a compound displayed a hydrogen bond with the Phe 468 of the activation segment blocking the B-RAF in its inactive form, a hydrogen bond with the $\beta 3$ strand via Lys 483, a hydrophobic bond with Leu 485, Ala 497, Phe 498, Val 504, Ile 527 and His 574, a hydrophobic bond with αC helix via Glu 501 and a hydrogen bond with the catalytic loop via Arg 575, a fluorine bond with the DFG motif via Asp 594 blocking the placement of the Mg^+ ions and a hydrophobic bond with the activation loop via Thr 599 [30].

The results from SwissADME show that these three candidates have molecular weights under 500g/mol, thus they can easily pass through the cell membrane, and they are all in agreement with the Lipinski rule, which suggests they should have a decent oral bioavailability score. All ligands have hydrogen acceptor numbers below 10 and hydrogen donor numbers below five, so they are stable. They have a Log p less than five and a TPSA between 90-140Å. Hence, they have good water solubility, better gastric tolerance, efficient elimination by the kidneys, and good permeability across the cell membrane. Then, when the value for the number of Lipinski rule violations is $n=1$ or $n<0$, ligands bind more easily to the receptor.

In the Boiled-Egg model, the high probability of passive absorption of the gastrointestinal tract is represented by the white region. In contrast, the yellow region represents the high probability of the BBB penetration. In addition, the blue color indicator indicates that the molecule is actively effluxed by P-gp (PGP+), whereas the red color indicator indicates that the molecule is a nonsubstrate of P-gp (PGP-) [31]. The candidates 8b and 12b are highly absorbed by the gastrointestinal tract; only 12b is a PGP substrate, which implies that 8b and 14a have an augmented oral bioavailability and C_{max} (risk of toxicity) (Figure 8). None of them can't pass the BBB; therefore, they are unable to reach the CNS, so this system is protected from any adverse effects these compounds may cause (Figure 8). The SwissADME results show that all compounds comply with the Lipinski rules, suggesting that they would not have problems with oral bioavailability.

Secondly, we used the drug repurposing strategy; we randomly selected 29 drugs currently in clinical phase that had in common interference with the B-RAF signaling pathway (anti-EGFR, AKT inhibitors, and PI3K inhibitors).

The majority of the molecules are PI3-kinase inhibitors (52%), 32% are AKT inhibitors, and only 16 % are EGFR inhibitors (Figure 9). All molecules are lipophilic, with A values between 1 and 5, except for Copanlisib, which is amphiphilic, and none of them are hydrophilic (Figure 10). Eight molecules have TPSA below 90 and pass through the blood-brain barrier, and two molecules have TPSA above 140 (Figure 11). Most of the candidate molecules are strong hydrogen bond

acceptors (4-8) and weak hydrogen bond donors (0-2) (**Figure 12**).

The interactions between the active sites of the B-RAF enzyme and the ligands showed that Uprosertib had the highest affinity (-10.3 kcal/mol), Alpelisib the second highest (-9.9 kcal/mol), Afuresertib third (-9.6 kcal/mol), and Omdpi the lowest (5.9 kcal/mol) (**Table 7**).

The analysis of the active site showed that the Glu 501 amino acid binds to the most molecules, followed by Ala 497, and Lys 483 comes in third place (**Figure 13**).

Among the 29 molecules, a selection of the top five molecules was made based on their binding energies. Uprosertib had the highest binding score (-10.3 kcal/mol) and formed two hydrogen bonds; Eganelisib and Poziotinib formed only one hydrogen bond each; Alpelisib did not form any; but Afuresertib formed four hydrogen bonds (**Table 8**).

Uprosertib had the highest binding energy score of -10.3 Kcal/mol. It bonded to the active site's amino acids through two hydrogen bonds with Glu 501 and Lys 483, and through three Pi-alkyl interactions with Leu 485, Ala 497, and Val 504, and one halogen bond with Met 484. Alpelisib had the second-highest binding energy score and formed only hydrophobic bonds with the active site's amino acids. It forms four halogen bonds: fluorine with Met 484, Phe 468, Lys 483, and Leu 525; two alkyl bonds with Leu 485, Val 504, and Glu 501; and no hydrogen bonds. Afuresertib had a score of -9.6 Kcal/mol. It forms four hydrogen bonds with Lys 483 and Ala 497, and two with Glu 501; hydrophobic interactions include two Pi-alkyl bonds with Val 504 and Leu 485, one Pi-sigma bond with His 574, and one halogen bond with Met 484. Eganelisib had a binding energy score of -9.5 Kcal/mol and formed several bonds with the active site's amino acids, including one hydrogen bond with Ile 573 and multiple hydrophobic interactions, including three Pi-anion/cation bonds with Lys 483, Glu 501, and Arg 575, and one Pi-alkyl bond with Val 504. Poziotinib scored -9.4 Kcal/mol. When docked with the B-RAF protein, it formed multiple bonds with the active site's amino acids, including one hydrogen bond with Ala 497 and multiple hydrophobic bonds; one fluorine-type halogen bond with Asn 500, two pi-anion/cation bonds with Lys 483 and Glu 501 (**Figure 14**).

In the study entitled « *Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF* » realized by PTC Wan et al. [32], it was found that the docking and active site analyses of Uprosertib displayed one hydrogen bond with $\beta 3$ stand via Lys 483, a hydrogen bond with αC helix via Glu 501, a hydrophobic bond with the DFG motif via Asp 594, blocking the placement of the Mg^{+} ions. In contrast, Afuresertib displayed three hydrogen bonds: one with the αC helix via Glu 501, one with the $\beta 3$ strand via Lys 483, and one with Asp 594 of the activation loop DFG motif, which appropriately positions the terminal ATP γ -phosphate through Mg^{2+} ions [32]. Alpelisib also induces hydrogen and fluorine interactions via Gly 465 and Phe 467, respectively, in the P-loop, thereby establishing an inactive conformation of the B-RAF kinase domain. And displayed a hydrophobic bond with the $\beta 3$ strand via Lys 483 and the carboxylate side chain of the catalytic αC helix via Glu 501 [32]. In addition, Eganelisib and Poziotinib exhibited similar hydrogen bonds with Phe 468 of the p-loop, a hydrophobic interaction with the αC helix via Glu 501, with the $\beta 3$ strand via Lys 483, and one more interaction for Eganelisib with the C lobe catalytic loop via Arg 575 [33].

The results from SwissADME show that these five candidates have hydrogen acceptor numbers below 10 and hydrogen donor numbers below 5. And the molecular weight of all ligands is below 500 DA, so they can easily pass through the cell membrane, except for Eganelisib, which is above 500 DA. All Ligands have Mi logP values below five. So, they have good water solubility, better gastric tolerance, efficient renal elimination, and good permeability across the cell membrane. Then, when the value of the number of violations is $n=1$ or $n<0$, ligands bind to the receptor more easily.

In the Boiled-Egg study, the five candidates are all highly absorbed in the gastrointestinal tract except for Alpelisib, and three of them (Poziotinib, Uprosertib, and Afuresertib) are PGP- so they have an augmented oral bioavailability and C_{max} (risk of toxicity). These compounds (Uprosertib, Alpelisib, Afuresertib, Eganelisib, and Poziotinib) can't pass the BBB, therefore, they are unable to reach the CNS, so this system is protected from any adverse effects these compounds may cause (**Figure 15**). The SwissADME

results show that all compounds comply with the Lipinski rules, suggesting that they would not have problems with oral bioavailability.

This study is limited; a Quantitative Structure-Activity Relationship (QSAR) study is necessary. By determining the pharmacophore, a QSAR model can provide valuable insights into the essential features and molecular properties that contribute to ligand binding affinity and specificity. Consequently, incorporating a QSAR investigation into our research would enable us to gain deeper insights into the ligand-protein interactions and potentially optimize the design of novel compounds with enhanced potency and selectivity.

Also, the size of our chemical library was reduced (13 new candidates, 29 repurposed drugs), as were the calculation assumptions.

4. Conclusion

Cancer is one of the deadliest diseases; it arises from the uncontrolled and abnormal multiplication of cells that evade the body's normal cellular regulation. B-Raf protein (SER/THR kinase) plays an important role in activating RAS/RAF/MEK/ERK signaling pathway and promoting normal cell development. The B-RAF was targeted in this study to discover new inhibitors that could constitute a good strategy for cancer treatment.

A total of 13 chemicals compounds extracted from previous articles targeting B-RAF protein kinase and 29 selected drugs that are currently in their clinical phase with the same signaling pathway that of B-RAF were chosen for virtual screening targeting the B-RAF protein kinase with code: 3IDP to choose the top five repurposes medicines and the best chemicals compounds with the greatest binding energy to this protein.

The top five drug repurposing candidates were Uprosertib, Alpelisib, Afuresertib, Eganelisib, and Poziotinib, with binding energies of -10.3, -9.9, -9.6, -9.5, and -9.4 Kcal/mol, respectively. The top three chemical compounds were 8b, 12b, and 14a, with binding energies of -10.7, -10.6, and -10.2 kcal/mol, respectively, no violations of Lipinski's rules, and a bioavailability score of 0.55. A QSAR study is recommended to provide valuable insights into the ligand-protein interactions and potentially optimize the

design of novel compounds with enhanced potency and selectivity.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

Authors Contributions

MATMOUR. D: Problematic, Methodology, results interpretation, writing original draft and corrections required by reviewers; BEKKADOUR. M: Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft; MIRAOU. D, SAHNINE. M, MAAFA. A, BENABDI. S, KANDOU. C, MERAD. Y: results interpretation, references, Formal analysis, Investigation.

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