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Designing Potent and Druggable BRAF V600E Inhibitors to Overcome Acquired Resistance in Non-Small Cell Lung Cancer: An In Silico Study.

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ABSTRACT

The BRAF V600E mutation is an important oncogenic driver in non-small cell lung cancer (NSCLC) and other malignancies. The development of novel therapeutic agents is necessary to overcome acquired resistance to current inhibitors such as dabrafenib. In this study, a comprehensive in silico drug design strategy was used to identify new potent BRAF V600E inhibitors. The design of a new series of compounds (BBN1-BBN6) was guided by a statistically robust Quantitative Structure-Activity Relationship (QSAR) model ($R^2 = 0.8333$, $R^2_{\text{ext}} = 0.7962$). The designed candidates were tested by molecular docking, ADMET profiling and molecular dynamics (MD) simulations. The molecular docking results showed that the top candidates (BBN1, BBN4, BBN6) presented considerably better binding scores (e.g., -10.392 kcal/mol for BBN1) compared to dabrafenib (-5.429 kcal/mol). The designed compounds showed excellent drug-likeness, following Lipinski's rules and promising ADMET profiles, with high predicted intestinal absorption and no AMES or hERG toxicity. MD simulations confirmed the stable binding of the top candidates in the BRAF V600E active site over 100 ns. Compound BBN1 with the strongest binder (-51.77 kcal/mol) was identified as a promising candidate for BRAF V600E inhibition assays to be validated by experimental in vitro and in vivo studies.

Keyword; QSAR, BRAF V600E, NSCLC, Molecular Docking, MD simulation, ADMET

INTRODUCTION

Cancer is the second leading cause of mortality globally (Sung et al., 2021). In the United States, it is estimated to have 2,001,140 new cases of cancer and 611,720 deaths from cancer in 2024 (Saha et al., 2025). Non-small cell lung cancer NSCLC is the most prevalent among lung malignancies which accounts for about 85% of all lung cancers (Sbrana et al., 2024). Currently classical immunotherapy, chemotherapy and targeted therapy are in use for the treatment of NSCLC patients (Liu et al., 2023; Yang et al., 2022). At the molecular scale, NSCLC is highly heterogeneous, harboring mutations in a variety of tumor suppressor genes and oncogenes (Skoulidis & Heymach, 2019). Most common among them were changes in *TP53*, *KRAS*, *STK11* and *KEAP1*.

The RAS/RAF/MEK/ERK pathway is required for tumorigenesis and cancer progression (Umar et al., 2020). The mitogen-activated protein kinase (MAPK) pathway is a major transducer of extracellular signals to intracellular responses.

To pass messages from the cell surface, *RAF* proteins serve as important signal carriers, which is activated by to activate MEK and in turn ERK (Williams et al., 2015). This pathway is deranged in many NSCLC patients, where the *BRAF* gene is a strong driver of tumor growth (Dotsu et al., 2021). *BRAF* is located on chromosome 7q and encodes a 95 kDa serine-threonine kinase that tightly regulates cell division, development and programmed death (Riudavets et al., 2022). Three versions of this protein are expressed in mammalian cells, ARAF, BRAF and CRAF. Each isoform is composed of the same basic machinery with three highly conserved regions; a regulatory RAS-GTP binding domain (CR1), a serine-rich hinge region (CR2) and a catalytic, bilobed domain (CR3) which carries out the actual phosphorylation (Amini et al., 2026).

From a physiological point of view, *BRAF* proteins implement their functions downstream receptor tyrosine kinases (RTKs) and their activation is dependent on BRAF phosphorylation at THR-598 and SER-601 residues (B.-H. Zhang & Guan, 2001). This signaling chain makes ERK enter the cell nucleus where it activates particular genes (Leonetti et al., 2018). *BRAF* mutations are present in 2%–3% of patients with non-small cell lung cancer and half of these cases harbor the BRAF V600E alteration (Schmid & Buess, 2018). Position 600 in the *BRAF* protein is a hotspot for mutations, such as V600E, V600K, V600D, and V600R, where the normal valine building block is replaced with glutamate, lysine, aspartic acid, or arginine, respectively (Saha et al., 2025; Villaruz et al., 2015). These *BRAF* mutations, especially the V600E change in the protein's active

region, are potent cancer-driving events in melanoma and in a variety of spreading cancers including breast, colorectal, gastrointestinal, and non-small cell lung cancer(V. Yadav et al., 2025). In the care of patients, the combination of the MEK inhibitor trametinib and the BRAF inhibitor dabrafenib (Planchard et al., 2016) , dabrafenib and vemurafenib (Schreck et al., 2023) has become a mainstay in the treatment of people with *BRAF* V600E mutations. Reliable treatment options are now available in medical centers (single or combination) for patients with cancers driven by overactive RAS/RAF/MAPK signaling pathways. Nonetheless, despite good early clinical responses to BRAF inhibition, most *BRAF* and *KRAS*-mutated tumors eventually acquire resistance upon prolonged drug exposure (Dain Md Opo et al., 2022).

The development of novel, highly potent and structurally distinct therapeutic agents is required to overcome the problem of acquired resistance and improve therapeutic results in *BRAF*-mutated cancers. Therefore, the present study aims at the design of novel BRAF inhibitors by an exhaustive in silico drug design strategy. We developed a predictive Quantitative Structure-Activity Relationship (QSAR) model to relate the specific molecular descriptors of known inhibitors to their biological activities (pIC₅₀). Based on the information obtained from the QSAR model and structure based molecular docking against BRAF kinase domain (PDB: 4MNF), a series of new compounds were designed. Finally, these newly designed molecules were extensively evaluated for their binding affinities, pharmacokinetic (ADMET) profiles, and drug-likeness properties to evaluate their potential as viable preclinical candidates in comparison with standard reference drugs such as dabrafenib and trametinib.

Materials and Methods

QSARINS v_224 software was used to develop the 2D QSAR predictive model. The 2D structure of the compounds was sketched by chemdraw professional 16.0. The energy minimization of the 2D structure was done using the MM2 force field in Chem3D pro 16.0. (Moulishankar & Sundarrajan, 2023; Sathishkumar et al., 2022) PaDEL Descriptor software (version 2.20) was used to construct the molecular descriptors. Schrödinger Maestro 2024-3 was used for Docking, Molecular dynamic simulation and MM/GBSA.

Data collection,

A collection of 35 compounds with NSCLC inhibitory characteristics were retrieved from pubchem (AID_1126887). The IC₅₀ values generated for the above 35 compounds was converted to molar pIC₅₀ [$-\log_{10}$ (IC₅₀ in M)] (table S1 in supplementary file).

Molecular sketching and 3D optimization

The compounds structure was drawn by ChemSketch and saved as a mol file format. The energy minimization of the 2D structure was done by using the MM2 force field in Chem3D pro. 16.0. Additionally, the geometry of the molecules concerned was optimized using the density functional theory (DFT) method, with a 6-31G basis set and the B3LYP functional and saved as the sdf file format. The molecular descriptor for 35 compounds was calculated by the PaDEL Descriptor version 2.20 software (Ibrahim et al., 2020)

QSAR model Development

QSARINS software was used to develop the MLR-QSAR predictive models by ordinary least squares. QSARINS uses a hybrid approach that combines genetic algorithms (GAs) and multiple linear regressions (MLRs) (Bathula et al., 2023; Subramani et al., 2022). Multiple linear regression was used to correlate the relationship between the biological activity (pIC₅₀) and molecular descriptors. The obtained data set was split into two sets, namely the training set (25) and the test set (10). The training set is used for model development and the test set is used for validation of the developed model. This work uses the MLR using the ordinary least-square approach to construct a good QSAR model. The best QSAR model was selected on the basis of lower number of descriptors, the lowest Lack-of-Fit (LOF) and the highest predicted accuracy on the external test set (R^2_{ext}).

Applicability domain

The applicability domain (AD) of a QSAR model is the theoretical physicochemical space defined by the structural properties of the training set that ensures that subsequent predictions are reliable (Roy et al., 2015). In the present study, the AD was visualized and defined by the use of a Williams plot in which the standardized residual of each compound was plotted against its leverage value (h). A compound is classified as reliable if it is within two bounds: a leverage value smaller than

the critical warning leverage (h^*) and a standardized residual between ± 3 standard deviations. Molecules that are outside these limits are considered as outliers. Specifically, compounds with $h > h^*$ fall outside the structural domain of the training set, whereas residuals beyond ± 3 are considered prediction errors (Moussaoui et al., 2024; Zhang et al., 2025). The equation below was used to calculate the warning leverage (h^*)

$$h^* = \frac{3(p + 1)}{n}$$

Y-randomization

To assess the robustness of the developed QSAR model, Y-randomization was performed in an effort to eliminate the possibility of chance correlation between the molecular descriptors and biological activity values (Er-raji et al., 2022). In this procedure, experimental pIC50 values were randomly permuted, keeping the original descriptor matrix. Afterward, new QSAR models were developed using the same set of descriptors that were selected for the original non-randomized model (Du et al., 2019). If the correlation coefficients of the randomized models are much lower than that of the original model, the reliability of the QSAR model is ensured. Therefore, the mean coefficient of determination of the randomized models (R^2_r) must be lower than the R^2 of the original model (Younis et al., 2022). The model robustness was also evaluated by the cRp^2 parameter as proposed by (Todeschini & Valsecchi, 2022):

$$cRp^2 = R \times \sqrt{R^2 - R_r^2}$$

where R is the correlation coefficient of the original model, R^2 is its coefficient of determination, and R_r^2 is the mean of the coefficient of determination obtained from the randomized models. A cRp^2 . The Y-randomization was carried out in MLRplusValidation1.3_10Oct20

molecular docking studies

The 3D crystal structure of BRAF-V600E kinase domain (PDB ID: 4MNF); resolution: 2.80Å) was retrieved from the Protein Data Bank and prepared using the Protein Preparation Wizard in Schrödinger Maestro 24-3 (kumar et al., 2022). Bond orders were assigned, missing hydrogen atoms added, disulfide bonds created. Water molecules further than 5.0 Å from the active site were

removed and the protein structure was energy-minimized with a root mean square deviation (RMSD) cutoff of 0.30 Å . The ligands were prepared by using the Ligprep module with suitable parameters like optimization, tautomers, ring conformation, 2D to 3D conversion, determination of promoters, ionization states at pH 7.0 with partial atomic charges using OPLS4 force field (R. Yadav et al., 2021). The receptor grid defining the active-site binding pocket was then generated using the Glide Grid Generation tool in Maestro. The grid was used as a guide for molecular docking and analysis of ligand–protein interactions in the active site of BRAF V600E kinase domain. molecular docking tool was used for performing docking studies into the active site region of BRAF-V600E (PDB ID: 4MNF). Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The best pose was selected on the basis of Glide score which considers various favorable interactions of ligands with active site amino acid residues (Rathi Suganya et al., 2016).

In silico ADMET profiling

Predicting the pharmacokinetic and safety profiles of potential drug candidates specifically their absorption, distribution, metabolism, excretion, and toxicity (ADMET); is critical for reducing late-stage attrition in drug development. In this study, Insilco ADMET profiling was conducted using two complementary web-based platforms. Metabolic interactions and potential toxicity risks was calculated in pkCSM (Momin et al., 2024), while was utilized to evaluate key drug-likeness, physicochemical properties and bioavailability of the compounds were calculated in SwissADME (Nyijime et al., 2025). A comprehensive assessment of the therapeutic potential and safety of the designed compounds was achieved by integrating the predictive abilities of both tools and this facilitate the reliable selection of optimal lead molecules.

Molecular dynamic simulation and MM-GBSA

Three compounds, designated BBN1, BBN4 and BBN6, were docked into the BRAF V600E kinase domain and are studied here. All three are fluorinated, a common feature of kinase inhibitors. The aim of the simulations was threefold: to confirm that each docked pose is retained under full solvation and thermal motion over 100 ns; to characterise the stability of both the kinase and each bound ligand; and to rank the three compounds by binding free energy using the MM/GBSA method. All simulations used GROMACS 2024.5 with the AMBER99SB-ILDN

protein force field and GAFF2 ligand parameters. Production runs were GPU-accelerated on NVIDIA A40 hardware. All three complexes reached the full 100 ns, each yielding 10,001 frames.

System Preparation

The three docked complexes (BBN1, BBN4 and BBN6) shared an identical BRAF V600E kinase domain (chain A, residues 449 to 720), verified by checksum of the protein coordinates, so the receptor was prepared once. It was repaired with PDBFixer to add missing heavy atoms, protonated at pH 7.0, and parameterised with GROMACS pdb2gmx using the AMBER99SB-ILDN force field and TIP3P water (Lindorff-Larsen et al., 2010). Each ligand was extracted from its docked complex with hydrogens and element assignments intact, converted to MOL2 with Open Babel to obtain a clean bond table, and parameterised with GAFF2. Atomic partial charges were derived with AM1-BCC via Antechamber (Shi et al., 2025), with bonded parameters assigned by parmchk2 and ACPYPE.

Simulation Protocol

Each complex was solvated in a rhombic dodecahedron box with a minimum solute-to-wall distance of 1.2 nm, filled with TIP3P water and neutralised with 0.15 M NaCl. The protocol was: steepest-descent energy minimisation; 100 ps NVT equilibration at 300 K with the V-rescale thermostat (Bussi et al., 2007) under position restraints; 100 ps NPT equilibration at 1 bar with the C-rescale barostat; and 100 ns unrestrained production (2 fs timestep, LINCS constraints, PME electrostatics, 1.0 nm cut-offs). Coordinates were saved every 10 ps, giving 10,001 frames per complex.

Trajectory Analysis

Analyses used GROMACS tools with index groups selected by name and a periodic boundary correction applied before all RMSD calculations. Protein backbone RMSD, ligand RMSD, per-residue C-alpha RMSF, radius of gyration, protein-ligand minimum distance, contact counts and hydrogen bonds were computed. Ligand RMSD was computed against the docked pose after backbone fitting, with image correction, to give a faithful measure of ligand movement within the pocket. The binding pocket for the dissociation assessment was defined from frame 0 as the protein residues within 0.5 nm of the docked ligand, and each ligand tracked against that fixed pocket. Unless stated, means are over 10 to 100 ns.

Binding Free Energy

MM/GBSA binding free energies were computed with gmx_MMPBSA 1.6.4 over the equilibrated 50 to 100 ns window (501 evenly spaced frames) on the PBC-corrected trajectory. The configurational entropy term was not included, so the values are effective binding energies suitable for ranking rather than absolute affinities (B et al., 2021).

Results and discussion

Model Development

A multiple linear regression (MLR) QSAR model has been developed based on a set of 35 compounds to predict the biological activity of novel BRAF inhibitors. The best predictive model was obtained by three molecular descriptors including GATS5i, SaasC, and IC3. The equation that is generated is:

$$\text{pIC}_{50} = -4.86246 + 4.12143(\text{GATS5i}) - 0.29931(\text{SaasC}) + 1.5506(\text{IC3})$$

GATS5i (Geary autocorrelation of lag 5 weighted by ionization potential) is a 2D autocorrelation descriptor that evaluates the spatial distribution of ionization potential across the molecular topology at a distance of five bonds (Mauri, 2020). It has the highest positive coefficient (+4.12143) in the model, i.e. it is the most contributor to biological activity. Recent analyses of QSAR interpretations suggest that optimization of such spatial-electronic properties, by introduction of substituents that favorably modify the ionization potential at this particular topological distance, will lead to a significant increase in the binding affinity of the inhibitors (Polishchuk, 2017).

IC3 (Information content index of neighborhood symmetry of order 3): is a topological information index that measures the structural complexity, branching and molecular symmetry (Mauri, 2020). This descriptor has positive coefficient (+1.5506) which implies that increase in this descriptor will increase the pIC₅₀ value. The positive coefficient indicates that increasing structural complexity in the 3rd-order topological neighborhood is beneficial for target engagement. Probably this is because it allows the molecule to adopt a more favorable conformation to the three-dimensional geometric constraints of the receptor binding pocket (Muratov et al., 2020). SaasC (Sum of atom-type E-state indices for secondary sp³ carbons) Unlike the other two descriptors, SaasC has a negative coefficient (-0.29931). This descriptor stands for the electrotopological state of secondary sp³-sp³ hybridized carbon atoms, mostly found in

aliphatic chains or saturated rings (Mauri, 2020). The negative sign indicates that an increase in number or E-state value for these specific aliphatic carbon atoms is not favorable for biological activity (Polishchuk, 2017). The reduction of aliphatic sp³ carbon character or the replacement of these groups by other rigid polar or unsaturated functional groups should therefore be a priority in the design of more potent derivatives.

Validation of Developed QSAR Model

The model was statistically significant and had predictive power. The internal validation of the training set was performed with a high coefficient of determination (R^2) of 0.8333 and adjusted R^2 (R^2_{adj}) of 0.8095. The internal predictability of the model was confirmed by a Leave-One-Out cross-validated Q-squared (Q^2_{LOO}) value of 0.7799 well above the accepted threshold of >0.5. The low root mean square error (RMSE_{tr} = 0.2370) and mean absolute error (MAE_{tr} = 0.1838) values also support the model's precision.

The model was externally validated with a test set of 10 compounds to evaluate its capability to correctly predict the activity of unseen molecules. The model predicted a R^2_{ext} of 0.7962. The model has passed all the strict external validation criteria of Golbraikh and Tropsha, including the important slope limits ($k = 0.9816$; $k' = 1.0171$) and residual limits, which confirms the strong external predictive ability of the model as shown in Table 1. The predicted biological activity of the dataset is in good correlation with the observed activity with very low residuals for both training and test sets (Table 2), Figure 1(supplementary).

Table 1. Criteria of Golbraikh and Tropsha for external validation

Name	Symbol	Threshold value	This study	
Internal validation (Training set)				
Coefficient of Determination	R^2	> 0.6	0.8333	passed
Adjusted R-squared	R^2_{adj}	> 0.5	0.8095	passed
Leave-One-Out Cross-Validation	(Q^2_{LOO})	> 0.5	0.7799	passed
Cross validated Q-squared (Q^2_{LOO})				
External validation (Test set)				
Predicted R^2 for test set	R^2_{Ext}	> 0.6	0.7962	passed
Number of tests set compounds	$N_{test\ set}$	≥ 5	10.000	passed
	$ r^2_0 - r'^2_0 $	< 0.3	0.06911	passed
	$\left r^2 - \frac{r'^2_0}{r^2} \right $	< 0.1	0.00101	passed

Slope of the plot of observed activity against predicted activity values at zero intercept (k')	K'	$0.85 < k < 1.15$	1.01713	passed
Slope of the plot of predicted against observed activity at zero intercept(k)	K	$0.85 < k < 1.15$	0.98163	passed

Table 2. Observed and predicted activities values of the dataset predicted by the best model equation.

Comp.ID	Status	GATS5i	SaasC	IC3	Exper. Activity	Pred. Activity	Residuals
1	Training	0.959014	2.072187	4.230641	5.0773	5.0299	-0.0474
2	Test	1.019664	0.94	4.777021	6.3565	6.4659	0.1094
3	Test	1.053272	1.097135	4.671402	6.3872	6.3936	0.0064
4	Test	0.983958	1.101829	4.923856	6.1487	6.498	0.3493
5	Training	0.878271	1.372978	4.378783	5.0391	5.1361	0.097
6	Training	0.975657	1.055185	4.869747	6.9208	6.3938	-0.527
7	Training	0.92777	1.077011	4.868523	6.1549	6.1881	0.0332
8	Training	0.986593	1.091573	4.958844	6.2147	6.5662	0.3515
9	Training	0.967048	1.109031	4.8125	6.4089	6.2535	-0.1555
10	Test	0.876539	1.240602	4.869631	6.0706	5.9297	-0.1409
11	Training	0.830898	1.251343	4.902569	6.1308	5.7894	-0.3414
12	Training	1.014431	0.99963	4.871928	6.4202	6.5737	0.1534
13	Training	0.943256	1.066343	4.868523	6.1249	6.2551	0.1301
14	Training	0.921403	1.193935	4.430723	5.0255	5.448	0.4225
15	Training	1.012022	2.312086	4.59716	6.1135	5.7449	-0.3687
16	Test	0.98086	3.242649	4.746115	5.1379	5.5689	0.431
17	Training	0.848552	0.694171	5.064869	6.2291	6.2806	0.0515
18	Training	0.960511	2.586454	4.997677	6.2218	6.0715	-0.1504
19	Training	1.003792	2.569502	5.064869	6.1871	6.3591	0.172
20	Test	1.045414	2.31662	5.033184	6.8539	6.5572	-0.2967
21	Test	0.980932	0.964769	5.083128	6.8239	6.7735	-0.0504
22	Training	0.989405	2.493554	5.064869	6.2518	6.3226	0.0707
23	Training	0.95479	2.489362	5.033184	6.2076	6.132	-0.0756
24	Training	1.110114	1.073102	4.896821	6.9586	6.9846	0.026
25	Test	1.166412	1.115052	4.838563	6.8539	7.1138	0.2599
26	Training	1.008274	1.130407	5.002268	7.1549	6.7112	-0.4437
27	Training	0.948308	1.269259	4.976155	6.0177	6.3821	0.3643
28	Training	1.179389	2.576061	4.998563	7.0969	6.978	-0.1189
29	Training	1.017304	2.594923	5.1313	6.2441	6.5102	0.2661
30	Test	1.134306	2.59816	5.078639	6.3665	6.9098	0.5433
31	Training	1.060757	2.583538	4.918563	6.301	6.3628	0.0618

32	Training	0.94718	0.722829	5.078639	6.7696	6.6999	-0.0697
33	Training	1.164559	2.345278	5.054381	7.0458	7.0726	0.0268
34	Training	1.10065	1.89742	5.136842	7	7.0711	0.0711
35	Test	1.105917	0.993426	5.099436	7.301	7.3053	0.0043

Analysis of Correlation Matrix of Descriptors

The statistical validity of the MLR model requires the molecular descriptors used in the equation to be independent of each other. Multicollinearity refers to the high correlation between the independent variables which destabilizes the regression model and inflates variance making the coefficients unreliable (Wilcox, 2022).

The intercorrelation between all the three descriptors is very low as seen from the descriptor correlation matrix (Table 3). The highest correlation was identified between GATS5i and SaasC ($r = 0.3107$), which is far below the accepted statistical limit for multicollinearity strictly followed in recent QSAR guidelines (usually $r < 0.70$) (Goel et al., 2018; Suvannang et al., 2018). Likewise, the correlations between GATS5i and IC3 ($r = 0.2877$) and between SaasC and IC3 ($r = 0.1019$) are negligible. This confirms the fact that the descriptors are strictly orthogonal and are not redundant in information. Thus, each descriptor provides a distinct independent dimension of structural and physicochemical data to the QSAR model, thus establishing its robustness, statistical significance and predictive reliability.

Table 3. Descriptor correlation matrix for the best model

	GATS5i	SaasC	IC3
GATS5i	1		
SaasC	0.3107	1	
IC3	0.2877	0.1019	1

Applicability domain (AD)

The applicability domain (AD) analysis was performed using the Williams plot (Figure 1), which revealed the applicability domain of the model and indicated that the compounds are well within the warning leverage boundaries ($h^* = 0.480$). and standardized residuals (± 3). It implies that the developed QSAR model has a sufficiently wide and well-defined applicability domain and can accurately predict the biological activities of new, structurally similar compounds.

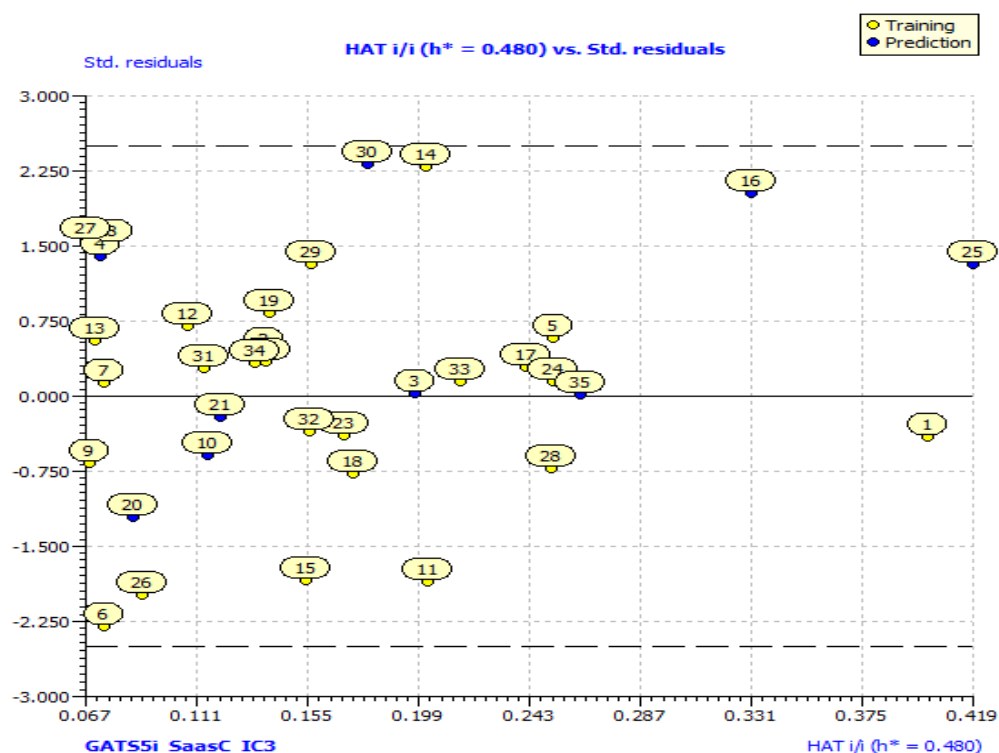


Fig. 1. William's plot of the best model. The dashed lines are the cut-off 3σ and the warning value of $HAT(h^*, 0.600)$.

Y-randomization

The Y-randomization was performed by creating 10 random models (Table S2 in supplementary file) to avoid chance correlation. The randomized models had very low mean R^2 (0.1350) and negative mean Q^2 LOO (-0.2314) values. The calculated value of $cR^2_p = 0.7783$ which exceeded the standard acceptability threshold of 0.5 indicates that the developed original QSAR model is highly robust and not due to random chance.

Design of new compounds

Based on the structural requirements from the QSAR descriptors (GATS5i, SaasC and IC3), the compound with highest inhibitory activity and lowest residue (compound 35) was used as a template (Figure 2) to design a novel series of six compounds (BBN1 – BBN6) (Figure 3). The biological activities of these new compounds were predicted using the validated QSAR equation and were found to have very promising pIC_{50} values (Table 4) ranging from 7.7055 to 8.0085. Note that compound BBN4 has the highest predicted pIC_{50} (8.0085) followed by BBN5 (7.8214).

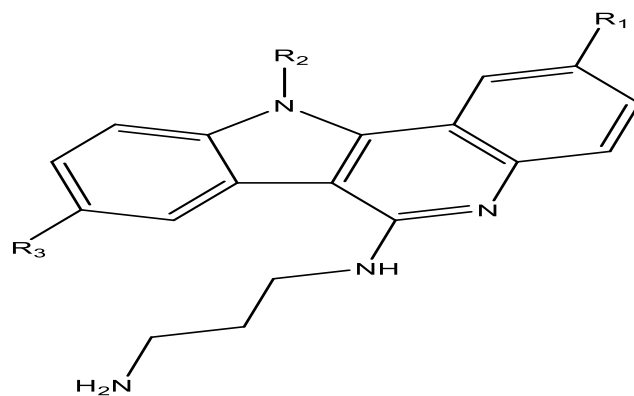


Fig.2 Structure of Template

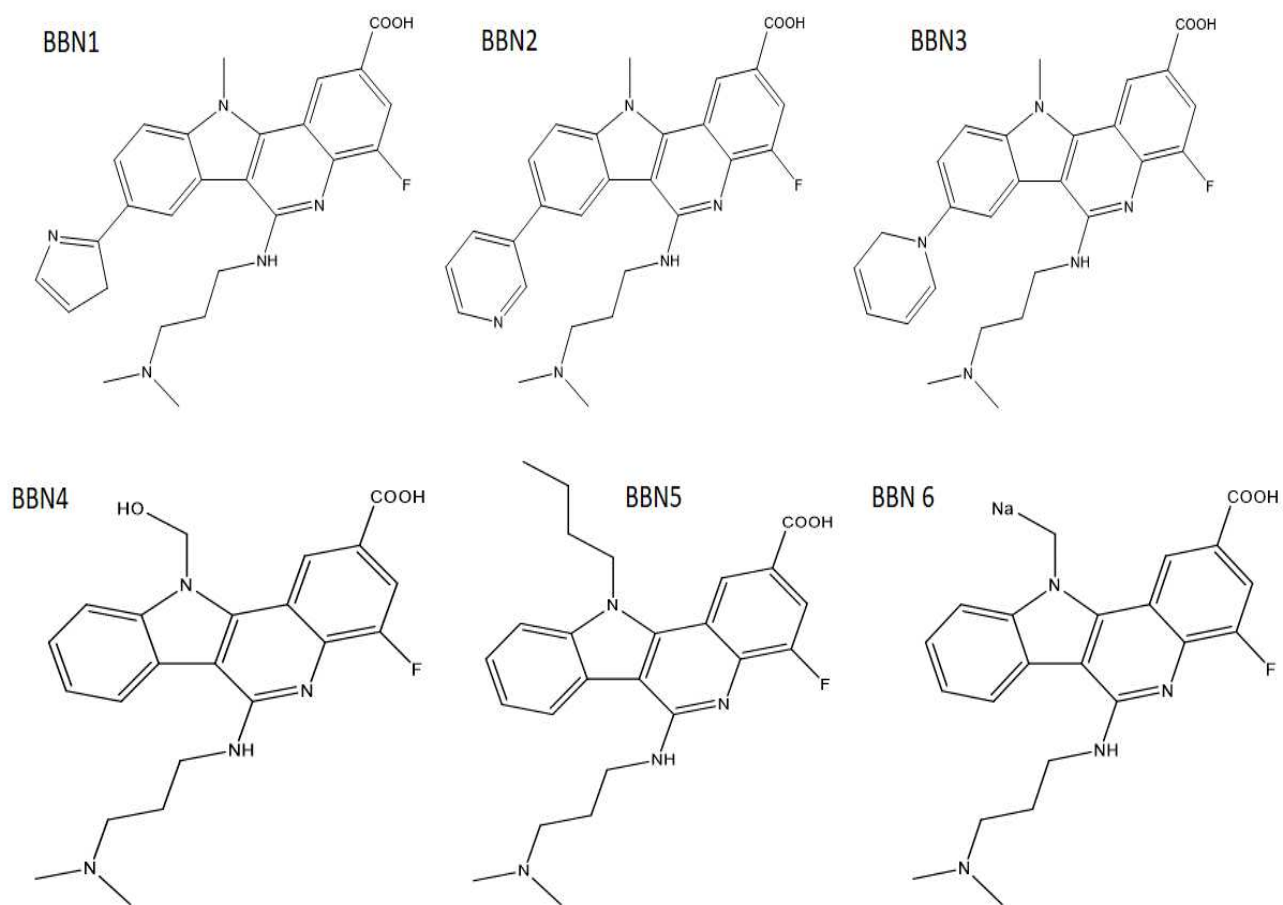


Fig.3. structures of the designed compounds

Molecular docking and binding mode studies

Structure-based molecular docking simulations were performed to validate the structural validity of the ligand-based QSAR predictions and to decode the molecular interactions of the designed inhibitors at the atomic level. The compounds were docked in the active site of mutated BRAF kinase domain (PDB ID: 4MNF) co-crystallized with selective inhibitor. Table 4 presents the calculated docking scores, the predicted activities (pIC₅₀) and some key molecular descriptors of the best-performing designed compounds (BBN1, BBN4 and BBN6). The specific residues involved in hydrogen, carbon–hydrogen, π – π stacking, along with other hydrophobic or electrostatic interactions are summarized in Table 5.

For a comparative baseline, the clinical BRAF inhibitor Dabrafenib was docked using identical parameters. Interestingly, the designed compounds BBN1 (docking score: – 10.392 kcal/mol), BBN4 (– 9.897 kcal/mol), and BBN6 (– 9.428 kcal/mol) showed significantly lower (more favorable) docking scores than Dabrafenib (– 5.429 kcal/mol). This indicates the remarkable thermodynamic stability and binding compatibility of these newly designed scaffolds in the ATP-binding pocket of the BRAF V600E mutant kinase.

In this study, compound BBN1 was found to have the highest binding affinity with a docking score of – 10.392 kcal/mol and a predicted pIC₅₀ of 7.7104 (Table 4). The 2D interaction map (Figure 4, left) indicates that the high affinity can be attributed to an equilibrium of hydrophilic and hydrophobic interactions. BBN1 forms two classical high affinity hydrogen bonds with SER-536 and ASN-580 in kinase domain. The compound is held in the binding cleft by these bonds. An extensive network of carbon-hydrogen bonds with the residues SER-465, CYS-532 (a key hinge residue), GLN-530 further supports the conformational stability of BBN1. These weak electrostatic forces keep the molecule in a very favorable bioactive conformation. On other side, the aromatic rings of the core and the terminal substituents of BBN1 interact with important hydrophobic residues such as PHE-583, TRP-531, LYS-483 and VAL-471 through strong hydrophobic (alkyl and π – π -alkyl) interactions. This hydrophobic contact maximizes the desolvation entropy of binding and contributes considerably to its low docking score.

The compound BBN4 is the most structurally optimized candidate according to our QSAR model with the highest predicted biological activity (pIC₅₀=8.0085). This excellent predicted potency is assisted with a strong docking score of -9.897 kcal/mol (Table 5). As illustrated in the 2D diagram (Fig. 4, right), An important classic hydrogen bond is formed between the backbone amide of

CYS-532 and BBN4. CYS-532 is a critical hinge region residue and targeting this residue mimics the hydrogen bonding pattern of ATP and is a hallmark of potent Type-I kinase inhibitors (Xing et al., 2015). The aromatic core of BBN4 also forms a strong face-to-face π - π stacked interaction with the residue TRP-531. The interaction serves as an energetic anchor stabilizing the planar core of the inhibitor. BBN4 is also stabilized by a carbon-hydrogen interaction with GLU-501 and strong hydrophobic/electrostatic interactions with ALA-481, VAL-471 and LYS-483. The low SaaSC value of BBN4 (-0.1568) minimizes structural bulkiness and steric clashes of secondary sp³ carbons. This optimized profile enables the molecule to fit into the shape of the pocket with little strain (Wu et al., 2023).

As shown in Figure 5 (left), BBN6 assumes a tightly coordinated binding orientation in the catalytic cavity. BBN6 forms two classical hydrogen bonds with SER-465 and the hinge region residue CYS-532. Good directional stability is provided by this two-hinge contact. Like BBN4, BBN6 has a strong π - π stacked interaction with TRP-531 via the central aromatic ring. This interaction positions the core of the molecule in the hydrophobic pocket. The aliphatic and aromatic portions of BBN6 are stabilized by strong hydrophobic interactions with LYS-483, VAL-471, ALA-481, ILE-463 and carbon-hydrogen bonds with GLU-501 and ASP-594 (Table 5).

Dabrafenib binds the BRAF kinase domain thru similar structural motifs, but with less overall stability (Figure 5, right). Conventional hydrogen bonds are formed by dabrafenib with CYS-532 and SER-536. π - π stacking with TRP-531, and forms a halogen bond between its fluorine atoms and SER-536 (shown as the blue-green dashed line). The thiazole sulfur atom of Dabrafenib engages in a π -sulfur interaction with PHE-583 (shown by the orange dashed line). Furthermore, it is also engaged in hydrophobic contacts with ALA-481, LYS-483, VAL-471 and ILE-463.

The molecular docking simulations indicate that the designed inhibitors (BBN1, BBN4, and BBN6) exhibit significantly higher binding affinities than the clinical reference drug, Dabrafenib. This thermodynamic advantage results from several important structural aspects:

1. Optimized Hinge-Region Engagement: Dabrafenib engages CYS-532 via conventional hydrogen bonds, but our designed inhibitors establish highly coordinated, multi-directional hydrogen bond networks, for example the dual SER-465/CYS-532 coordinate in BBN6 and the SER-536/ASN-580 coordinate in BBN1. These interactions stabilize the kinase in the inactive state more strongly (Xing et al., 2015).

2. Minimization of Steric Strain: The molecular weight of Dabrafenib is high (519.56 g/mol) and lipophilicity (LogP) of 5.3649 is also high. The high in molecular weight may result in a sub-optimal geometrical fit and slight steric clash in the tight ATP-binding cleft (Wu et al., 2023; Zheng & Du, 2013) . Conversely, the designed compounds (e.g., BBN4: MW=410.44 g/mol, LogP=3.4934; BBN6: MW=416.42 g/mol, LogP=3.6696) are more compact and chemically optimized.

3. Favorable Electrotopological State (SaasC): Our designed compounds show increased structural rigidity by decreasing secondary sp^3 hybridized aliphatic carbon groups (represented by lower SaaSC descriptor values (table 4), especially negative value of -0.1568 for BBN4). This decrease in rotatable aliphatic chains results in a decrease of the loss of conformational entropy upon binding (DeLorbe et al., 2009) . This allows the ligands to bind strongly to the residues of the active site (VAL-471, LYS-483, ALA-481 and ILE-463) without steric strain.

In brief, these molecular docking analyzes are consistent with the design principles of the QSAR model. They show that the newly designed BBN series, especially BBN1, BBN4 and BBN6, has higher structural compatibility, hinge-binding capacity and pocket-fitting efficiency than the standard therapeutics such as Dabrafenib.

Table 4. Descriptor, pIC50 and docking score of the designed compounds

Name	GATS5i	SaasC	IC3	pred. PIC50	docking score
BBN 1	1.097026	1.084059	5.401808	7.7104	-10.392
BBN 2	1.075356	1.090414	5.465142	7.7174	-8.901
BBN 3	1.069417	1.112677	5.495865	7.7339	-5.056
BBN 4	1.138063	-0.1568	5.245447	8.0085	-9.897
BBN 5	1.068131	0.147045	5.369282	7.8214	-8.063
BBN 6	1.115986	0.092664	5.156889	7.7055	-9.428

Table 5. Binding interactions of designed compounds with 4MNF Amino acid residues

Compound ID	Hydrogen bond	Carbon-hydrogen bond	Pi-pi stacked	Others	Docking score
BBN1	SER-536, ASN-580	CYS-532, GLN-530 SER-465	PHE-583	TRP-531, ALA,481, LYS-483, VAL-471	-10.392
BBN4	CYS-532	GLU-501	TRP-531	ALA-481, VAL-471, LYS-483	-9.897
BBN6	SER-465, CYS-532	GLU-501, ASP-594,	TRP-531	LYS-483, VAL-471, ALA-481, ILE,463	-9.428
Dabrafenib	CYS-532, SER-536	TRP-531, SER-535	TRP-531	PHE-583, ALA-481, LYS-483, VAL-471, ILE-463	-5.429

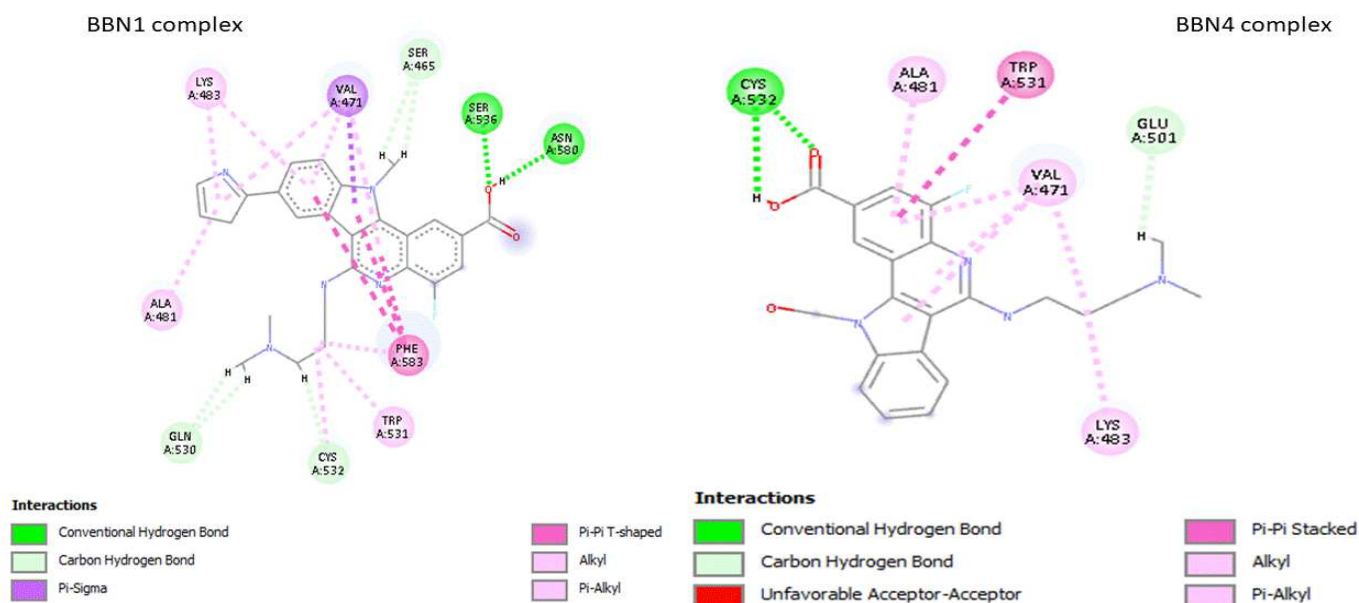


Fig.4. 2D structure of BBN1 and BBN4 in complex with 4MNF

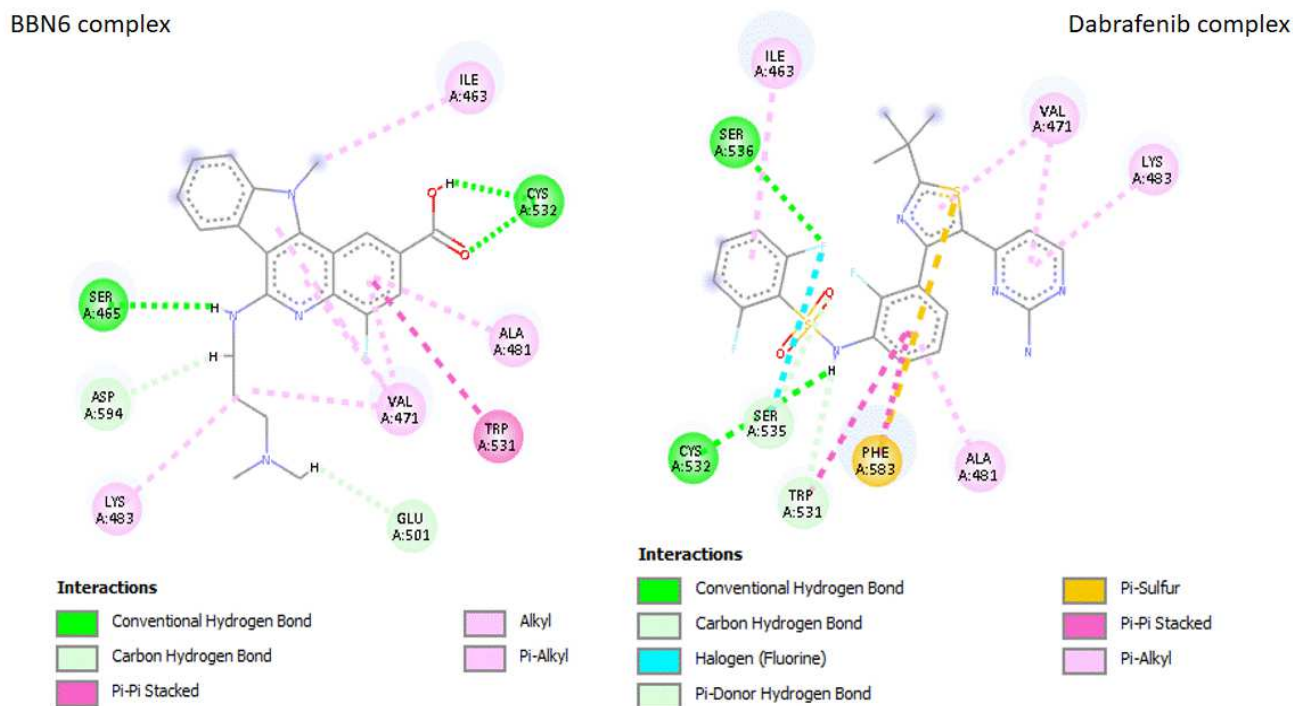


Fig.5. 2D structure of BBN6 and Dabrafenib in complex with 4MNF

In Silico ADMET and Drug Likeness Profiling

For a drug candidate to be successful it must have high potency and good pharmacokinetic profile. Consequently, the most promising novel inhibitors (BBN1, BBN4 and BBN6) were subjected to in silico ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profiling and compared with Dabrafenib (Table 7).

All of the three BBN compounds exhibited excellent Human Intestinal Absorption (HIA) values, ranging from 89.13% to 94.93% similar to the reference drugs. They exhibited good water solubility and moderate permeability in Caco-2. Importantly, BBN4 and BBN6 are predicted to be non-inhibitors of CYP2D6 and CYP3A4, thus potentially presenting a lower risk of drug-drug interactions compared to Dabrafenib (which is inhibitors of CYP3A4). Moreover, the designed compounds showed no AMES toxicity, no hERG inhibition, and no skin sensitization indicating a preliminary safe toxicity profile. Furthermore, the synthetic accessibility scores of these compounds (3.11-3.89) are lower than the one of dabrafenib (4.05), which means that they are relatively easier to be synthesized.

Finally, the compounds were evaluated for drug-likeness using multiple established criteria (Tables 8 and 9). The designed inhibitors (BBN1, BBN4, BBN6) adhere strictly to Lipinski's Rule of Five with optimal molecular weights (<500 Da), LogP values (<5) and suitable numbers of hydrogen bond donors and acceptor. Conversely, the reference drug dabrafenib failed several drug-likeness rules (Lipinski, Veber, Egan and Ghose filters) mainly due to its higher molecular weight and lipophilicity. The BBN series showed better drug-likeness and bioavailability scores, suggesting great potential for further optimization and in vitro validation as next-generation BRAF inhibitors.

Table 7. Insilico ADMET prediction of the newly designed inhibitors

ADMET properties	Compounds			
	BBN1	BBN4	BBN6	Dabrafenib
Water solubility	- 4.639	-3.464	-3.57	-3.995
CaCO ₂ permeability	0.921	1.011	1.032	0.702
Human Intestinal Absorption (HIA)	89.133	94.93	94.794	93.334
Skin permeability (Logkp)	-2.869	-2.735	-2.733	-2.735

p-glycoprotien substrate	YES	-YES	YES	NO
p-glycoprotien inhibitor	YES	NO	NO	YES
BBB permeability (LogBB)	-0.998	-1.213	-1.136	-0.711
CNS permeability	-2.176	-2.764	-2.631	-3.166
CYP2D6 substrate	YES	NO	NO	NO
CYP3A4 substrate	NO	NO	NO	YES
CYP2D6 inhibitor	NO	NO	NO	NO
CYP3A4 inhibitor	YES	NO	NO	YES
Total clearance (mL/min/kg)	0.418	0.657	1.682	0.101
Renal OCT2 inhibitor	NO	NO	NO	NO
AMES toxicity	NO	NO	NO	NO
hERG inhibitor	NO	NO	NO	NO
Skin sensitization	NO	NO	NO	NO
synthetic Accessibility	3.89	3.11	3.23	4.05
Bioavailability score	0.55	0.55	0.55	0.55

Table 8. Lipinski's properties, TPSA and NROTB of the newly designed compound

Inhibitors	property					
	MW	LOGP	HBD	HBA	nrotb	TPSA
BBN1	459.52	4.7871	2	6	7	82.75
BBN4	410.44	3.4934	3	6	7	90.62
BNN6	416.42	3.6696	2	5	7	70.39
Dabrafenib	519.56	5.3649	2	8	6	147.48

MD simulations

All three inhibitors remained bound in the BRAF V600E pocket for the full 100 ns, each with a bound fraction of 1.000 and no dissociation events. The results below concern the degree of stability and the ranking of binding strength.

Protein Structural Stability

Backbone RMSD (Figure 6) confirms the kinase remained stable in every complex. Mean values over 10 to 100 ns were 0.183 nm for BBN4, 0.256 nm for BBN6 and 0.349 nm for BBN1, all low and characteristic of a well-folded kinase domain that stays close to its starting structure.

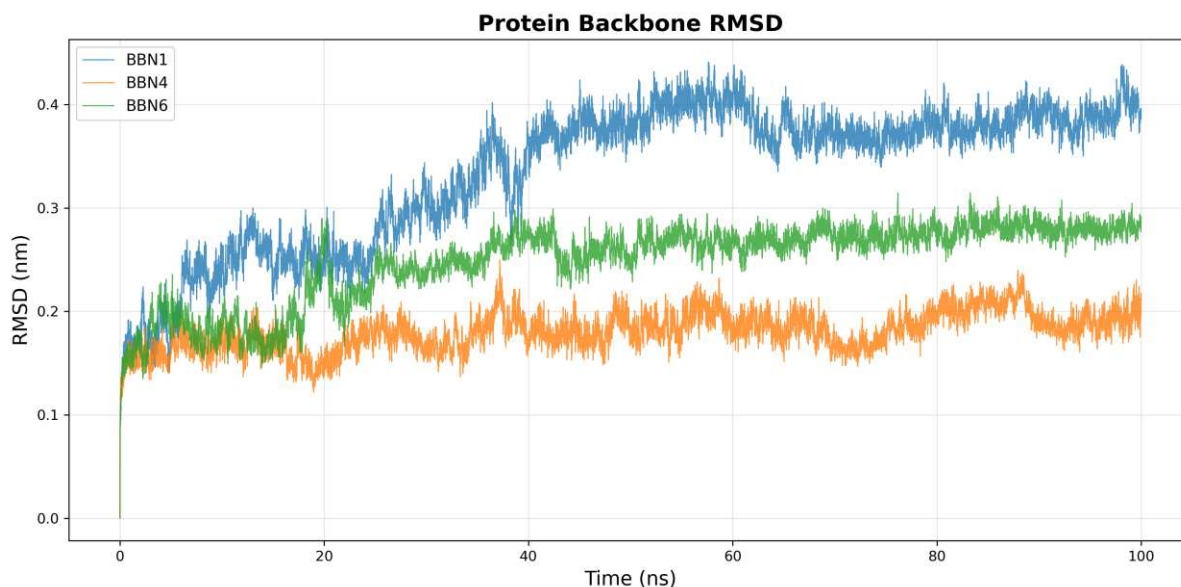


Fig.6. Protein backbone RMSD over 100 ns for the three BRAF V600E complexes. All remained low and stable, indicating the kinase fold was preserved throughout.

Radius of Gyration

The radius of gyration (Figure 7) was stable across the full 100 ns for every complex, with means between 1.890 and 1.913 nm and fluctuations of around 0.01 nm. The absence of any sustained drift confirms the compact fold of the kinase domain was maintained in all three simulations.

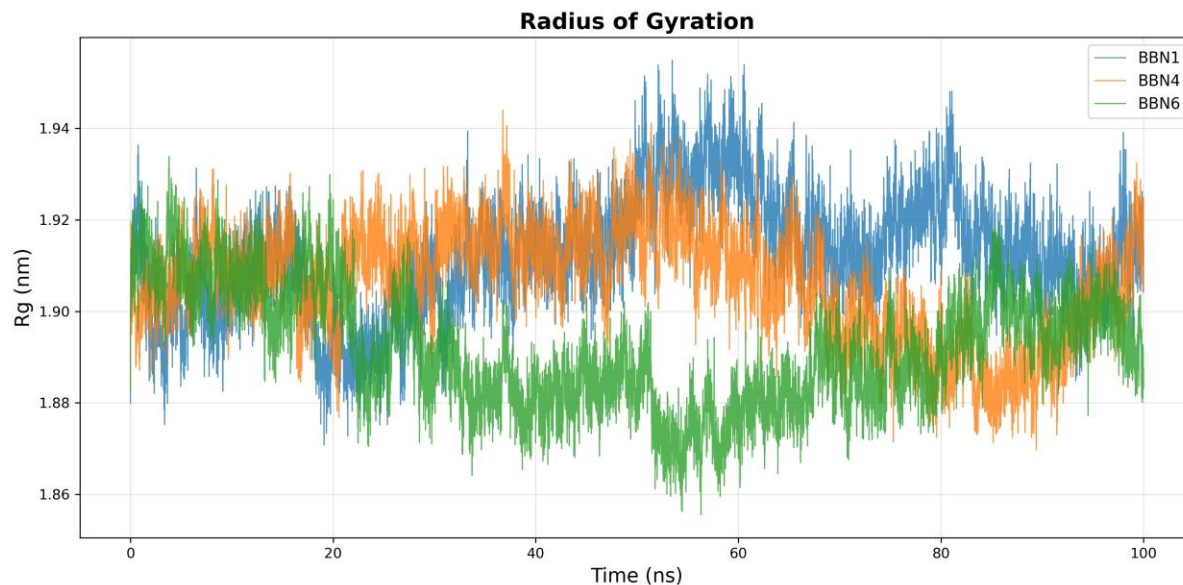


Fig.7. Radius of gyration over 100 ns. Stable, tightly clustered values confirm the kinase fold was preserved in every complex.

Per-Residue Flexibility

The per-residue C-alpha RMSF profiles (Figure 8) were closely comparable across the three complexes, with flexibility confined to the chain termini and surface loops while the structured core and the residues lining the ATP-binding site remained rigid. This indicates that ligand binding did not introduce compound-specific changes to the kinase dynamics.

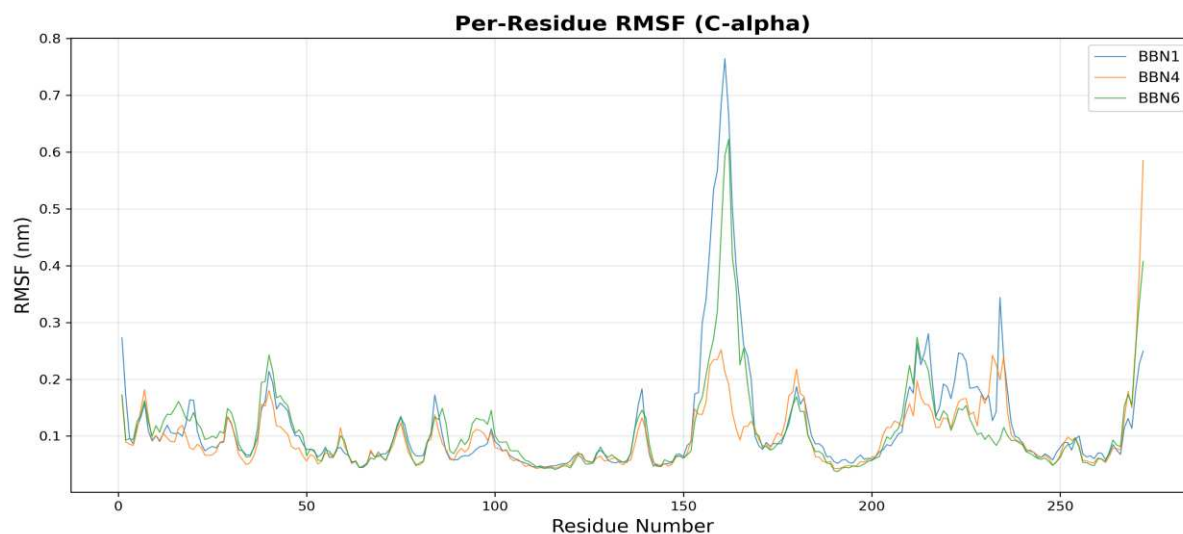


Fig.8. Per-residue C-alpha RMSF over 100 ns. Consistent profiles across all three complexes, with flexibility limited to termini and surface loops.

Ligand Binding Stability

Table 9 summarizes the per-ligand stability metrics. Every compound recorded a bound fraction of 1.000. Ligand RMSD values, computed against the docked pose after backbone fitting, were low for all three: BBN1 held its pose most tightly (0.274 nm), followed closely by BBN6 (0.285 nm) and BBN4 (0.382 nm). All three values are small and stable, the signature of ligands that settle into a well-defined binding geometry and remain there.

Table 9. Ligand binding stability. Protein RMSD, ligand RMSD and Rg are means with standard deviations over 10 to 100 ns.

Compound	Protein (nm)	RMSD Ligand (nm)	RMSD Rg (nm)	Bound fraction	Verdict
BBN1	0.349 ± 0.053	0.274 ± 0.042	1.913 ± 0.013	1.000	Stably bound
BBN4	0.183 ± 0.018	0.382 ± 0.070	1.906 ± 0.013	1.000	Stably bound
BBN6	0.256 ± 0.031	0.285 ± 0.054	1.890 ± 0.012	1.000	Stably bound

The minimum ligand-pocket distance (Figure 9) stayed well below the 0.45 nm contact criterion for all three compounds across the whole trajectory, confirming that no ligand left the pocket. At the end of the run the pocket distances were around 0.20 nm for all three, and contact counts remained high throughout, indicating deep and sustained engagement with the binding site.

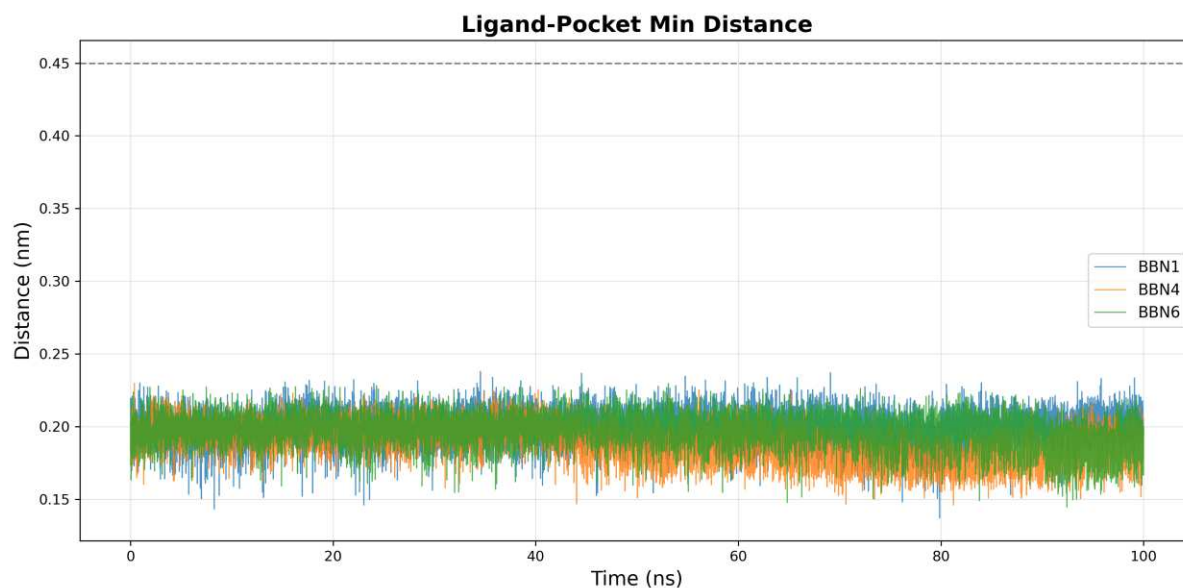


Fig.9. Minimum ligand-pocket distance over 100 ns. All three compounds remained far below the 0.45 nm contact criterion (dashed line), confirming sustained binding.

Ligand RMSD relative to the docked pose (Figure 10) stayed low and stable for all three compounds throughout the run, with no upward drift. BBN1 held its pose most tightly, consistent with it being the strongest binder.

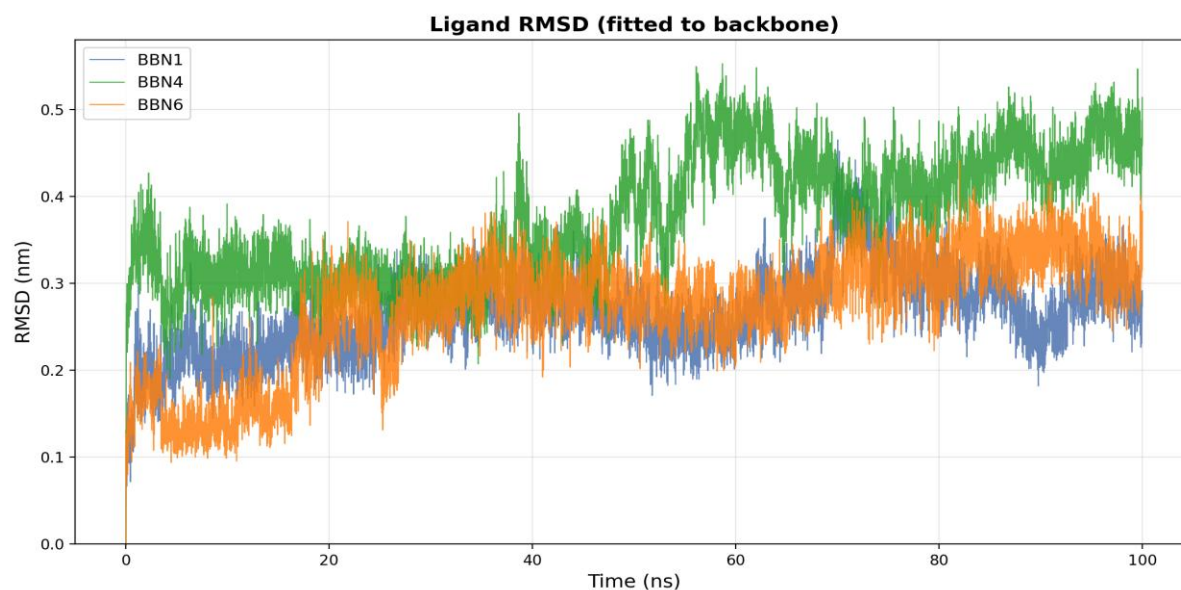


Fig.10. Ligand RMSD over 100 ns after backbone fitting, computed against the docked pose. Low, stable values for all three compounds confirm they held their binding geometry.

Protein-Ligand Contacts and Hydrogen Bonds

Contact counts within 0.4 nm (Figure 11) were high and sustained for every compound throughout the trajectory, with no systematic decline. BBN1 maintained the highest contact count, consistent with it being the strongest binder. Hydrogen bonding (Figure 12) was likewise maintained across the run for all three, supporting stable, specific binding in each case.

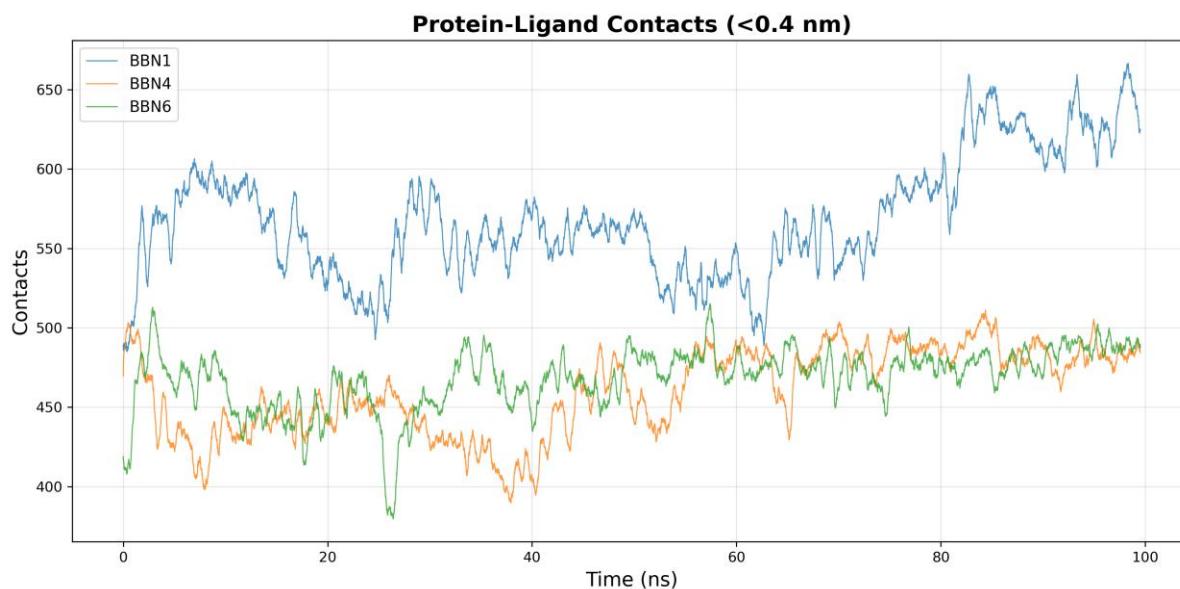


Fig. 11. Protein-ligand contacts within 0.4 nm over 100 ns, smoothed. Sustained contact for all three compounds throughout, highest for BBN1.

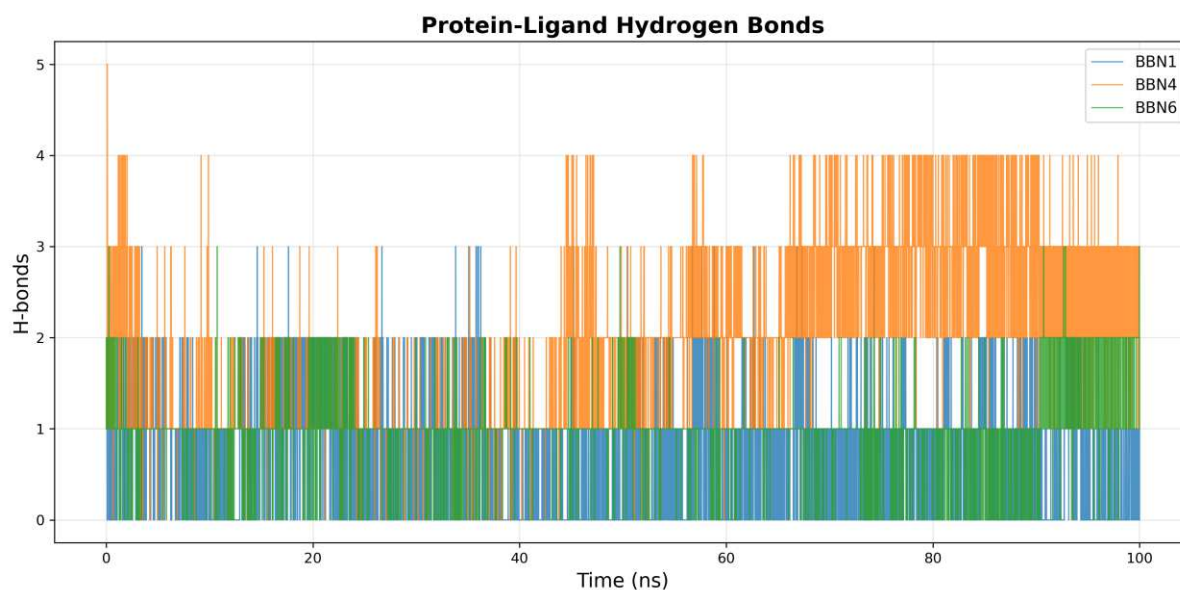


Fig. 12. Protein-ligand hydrogen bonds over 100 ns for the three complexes.

Binding Free Energy (MM/GBSA)

Because all three compounds remained bound, the MM/GBSA binding free energy is the primary basis for ranking them. The results are shown in Figure 13 and Table 10.

Binding Free Energy Ranking

All three compounds are strong binders, and BBN1 is the clear leader. BBN1 returned the most favourable value (-51.77 kcal/mol), ahead of BBN4 (-47.44 kcal/mol) and BBN6 (-46.72 kcal/mol). BBN1 is separated from the other two by roughly 4 to 5 kcal/mol, a margin comparable to or larger than the uncertainties, so its lead is meaningful. BBN4 and BBN6 are close to each other and should be regarded as comparable, forming a strong second tier behind BBN1.

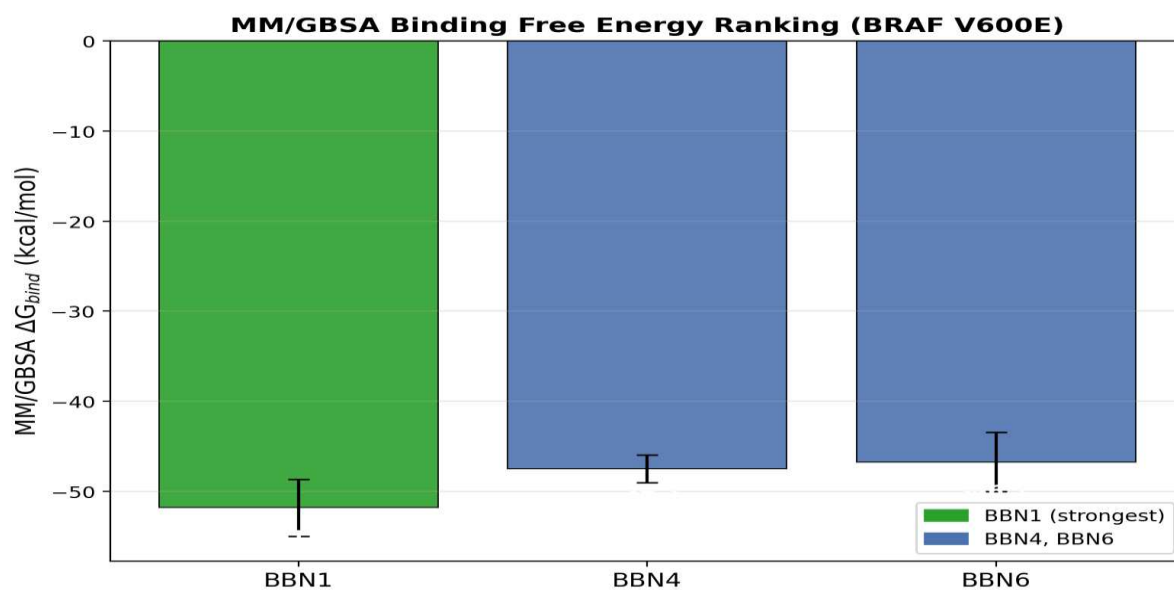


Fig. 13. MM/GBSA binding free energy for the three complexes over 501 frames of the 50 to 100 ns window. More negative indicates stronger predicted binding. Error bars are one standard deviation.

Energy Decomposition

Table 10 breaks the binding free energy into its components. Van der Waals interactions are the dominant favourable term for every compound, as expected for ligands buried in the hydrophobic ATP-binding cleft. BBN1 owes its leading position to a substantially larger van der Waals contribution (-70.53 kcal/mol) than either BBN4 or BBN6, reflecting more extensive shape-complementary contact with the pocket, which is also consistent with its higher contact count and

its being the largest of the three ligands. Its polar desolvation penalty is correspondingly larger, but not enough to offset the gain, leaving it the strongest binder overall.

Table 10. MM/GBSA binding free energy and its components (kcal/mol), over the 50 to 100 ns window, ranked strongest to weakest.

Compound	ΔG_{bind}	$\Delta v\text{dW}$	ΔE_{elec}	$\Delta P_{\text{polar solv}}$	$\Delta P_{\text{nonpolar solv}}$
BBN1	-51.77 ± 3.17	-70.53	-19.44	+46.19	-7.98
BBN4	-47.44 ± 1.53	-52.68	-20.39	+32.52	-6.89
BBN6	-46.72 ± 3.28	-55.91	-10.26	+26.33	-6.88

The central outcome of this study is that all three candidate inhibitors are stable binders of BRAF V600E over 100 ns, with strong binding free energies, and that BBN1 is the standout candidate. The kinase was structurally stable in every complex, the docked poses were retained with low ligand RMSD, and contacts and hydrogen bonds were sustained throughout. There were no dissociation events.

BBN1 is the strongest candidate on the combined evidence. It returned the most favourable MM/GBSA energy by a clear margin, held its docked pose most tightly of the three, and maintained the highest number of protein contacts. Its advantage traces to an unusually large van der Waals contribution, indicating that its larger scaffold makes more extensive complementary contact with the ATP-binding cleft. BBN4 and BBN6 form a strong and closely-matched second tier; both are stable, favourable binders, but neither reaches the binding strength of BBN1.

All three are fluorinated, which is a well-established medicinal-chemistry strategy for improving the potency and metabolic stability of kinase inhibitors. The results here are consistent with all three achieving a favourable balance of interactions in the pocket, with BBN1 the most complementary.

Limitations. These are single unbiased 100 ns simulations, one per compound. Confirming the ranking, in particular the closeness of BBN4 and BBN6, would benefit from replicate simulations

with independent starting velocities. MM/GBSA provides relative binding energies for ranking, not absolute affinities, and omits the entropy term. No approved BRAF inhibitor was included as a reference in this set, so the compounds are ranked relative to one another rather than against a clinical benchmark. Within these limits, the finding that all three are stable, strong binders and that BBN1 is the strongest is robust.

Dissociation Assessment

A dedicated dissociation analysis tracked each ligand against its docked pocket across all 10,001 frames. A frame counted as bound at or below 0.45 nm from the pocket; a dissociation event required the distance to exceed 0.60 nm continuously for at least 1 ns.

No dissociation event was recorded for any of the three compounds. Every ligand returned a bound fraction of 1.000, meaning each remained in contact with its pocket in every frame of its 100 ns trajectory. This confirms that all three docked poses are stable on the simulated timescale.

Table 11. Dissociation assessment.

Compound	Bound fraction	First dissociation	Verdict
BBN1	1.000	None	Stably bound
BBN4	1.000	None	Stably bound
BBN6	1.000	None	Stably bound

Conclusions

In the present study, a comprehensive computational workflow was successfully implemented in the design and evaluation of novel inhibitors targeting the BRAF V600E kinase. A statistically robust and highly predictive MLR-QSAR model was developed and key molecular descriptors (GATS5i, SaasC and IC3) correlating structural features with biological activity were identified. The model provided a clear rationale for lead optimization, showing that the ionization potential should be modulated, the structural complexity increased and the aliphatic character reduced. Based on these QSAR knowledge new series of compounds (BBN) were designed. Molecular docking studies showed that these new inhibitors, especially BBN1, BBN4 and BBN6, have much

higher predicted binding affinities to the BRAF V600E active site than the clinical drug Dabrafenib. The binding mode analysis confirmed the success of the design strategy, showing optimized binding to key residues in the hinge region and good binding interactions in the binding pocket.

Further, *in silico* ADMET and drug-likeness predictions suggested better pharmacokinetic and safety profiles of the designed candidates. They showed good predicted intestinal absorption, a better safety toxicity profile with no predicted AMES or hERG liabilities, and better overall drug-likeness scores than the reference drug.

MD simulations of 100 ns were completed for the three candidate inhibitors (BBN1, BBN4, BBN6) in complex with the BRAF V600E kinase domain, using AMBER99SB-ILDN, GAFF2, TIP3P water and PME electrostatics at 300 K and 1 bar, with GPU-accelerated production. Each trajectory produced 10,001 frames. The kinase fold was preserved in every complex, with backbone RMSD between 0.18 and 0.35 nm and stable radius of gyration throughout. All three ligands remained bound for the full 100 ns with a bound fraction of 1.000 and no dissociation events, holding their docked poses with low ligand RMSD (0.27 to 0.38 nm).

By MM/GBSA binding free energy, BBN1 was the strongest binder (-51.77 kcal/mol), ahead of BBN4 (-47.44 kcal/mol) and BBN6 (-46.72 kcal/mol). BBN1 leads by a clear margin, driven by its larger van der Waals contribution, while BBN4 and BBN6 form a comparable second tier.

On this evidence, all three merit experimental validation against BRAF V600E. BBN1 is the priority candidate for progression and experimental BRAF V600E inhibition assays as the lead compound, with BBN4 and BBN6 as secondary candidates.

Authorship contribution statement

A H: Writing - Original Draft, S.E.A: Supervision, M.W: validation, A.U; Formal Analysis, G.A.S: Data curation, Z.Y.I: Validation, S.H.A: Software. All authors review and approved the manuscript

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

Not applicable.

Funding

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Data availability statement

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

Clinical trial number:

Not applicable.

Consent for publication

Not applicable.

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