Supplementary information

Quantification of buparlisib in the human liver microsomes employing ultra-fast sensitive UPLC-MS/MS method: In vitro and in silico metabolic stability evaluation

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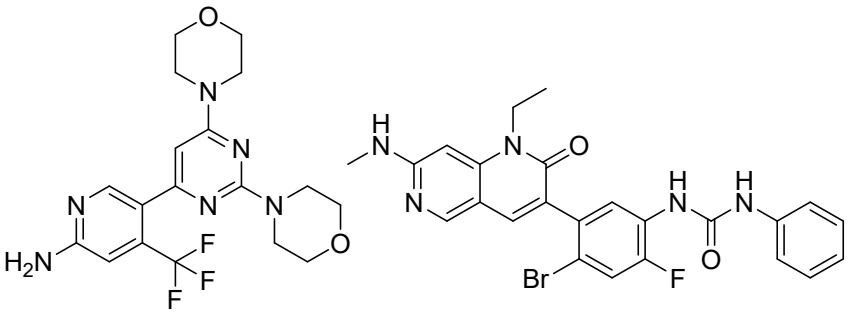
In silico ADME screening of BLB

The ADME properties of the BLB were characterized employing its SMILES name: [C1COCCN1C2=NC(=NC(=C2)C3=CN=C(C=C3C(F)(F)F)N)N4CCOCC4] on the SwissADME online website. An assessment was performed to ascertain the likelihood of BLB exhibiting drug-like features via the screening of its absorption, distribution, metabolism, and excretion (ADME) characteristics. The log p data indicates that BLB exhibits a moderate degree of solubility in water (Log S = -4.61). The pharmacokinetic data demonstrates substantial gastrointestinal absorption, with established impenetrability to the blood-brain barrier. The Log Kp score, indicative of penetrability through skin, is assessed at -7.74 cm/s. The documented bioavailability result is 0.55. The proposed action mechanism of BLB indicates no inhibition of P-glycoprotein, that acts as a substrate; rather, it displays inhibitory effects on CYP2D6, CYP1A2, and CYP3A4, while exhibiting no inhibition of CYP2C9 and CYP2C19. The drug similarity assessment conforms to the standards settled by Muegge, Veber, Egan, Ghose, and Lipinski [1-3]. Figure S2A presents the BLB ADME radar, supplemented by related details delineated in Table S1.

Figures:



Buparlisib

Molecular Weight: 410.39

Ripretinib (IS)

Molecular Weight: 510.36

Fig. S1. The chemical structures of the buparlisib (BLB) and the IS (ripretinib).

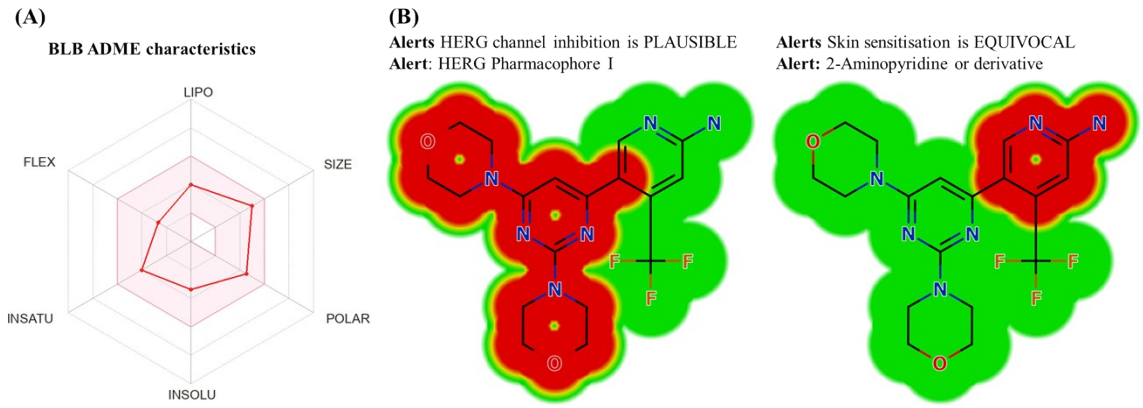


Fig. S2. The BLB ADME chart was generated by the accessible SwissADME online program. Molecular weight (SIZE): 410.39 g/mol; Polarity (POLAR): TPSA 89.63 Å²; Saturation (INSATU) section of carbons in sp^3 hybridization 0.50; Lipophilicity (LIPO) is counted as XLOGP3 = 1.50; Flexibility (FLEX): 4 rotatable bonds; Suggested solubility (INSOLU): log S ≤ -4.61 **(A)**. The BLB structural alerts were characterized employing the DEREK module and painted in red color **(B)**.

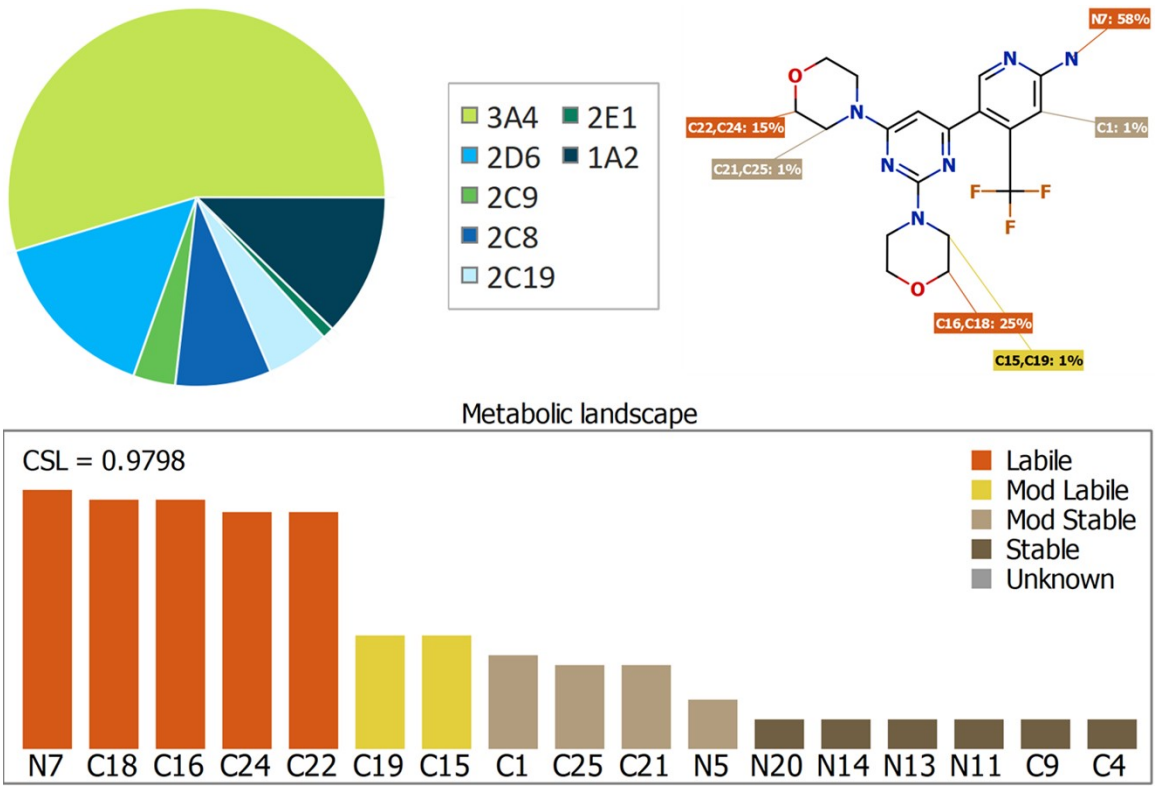


Fig. S3. The predicted CSL value (0.9798) reveals that buparlisib (BLB) has a significant lability to metabolic reactions. The statistics were gathered, acquired, and analyzed applying the WhichP450 module.

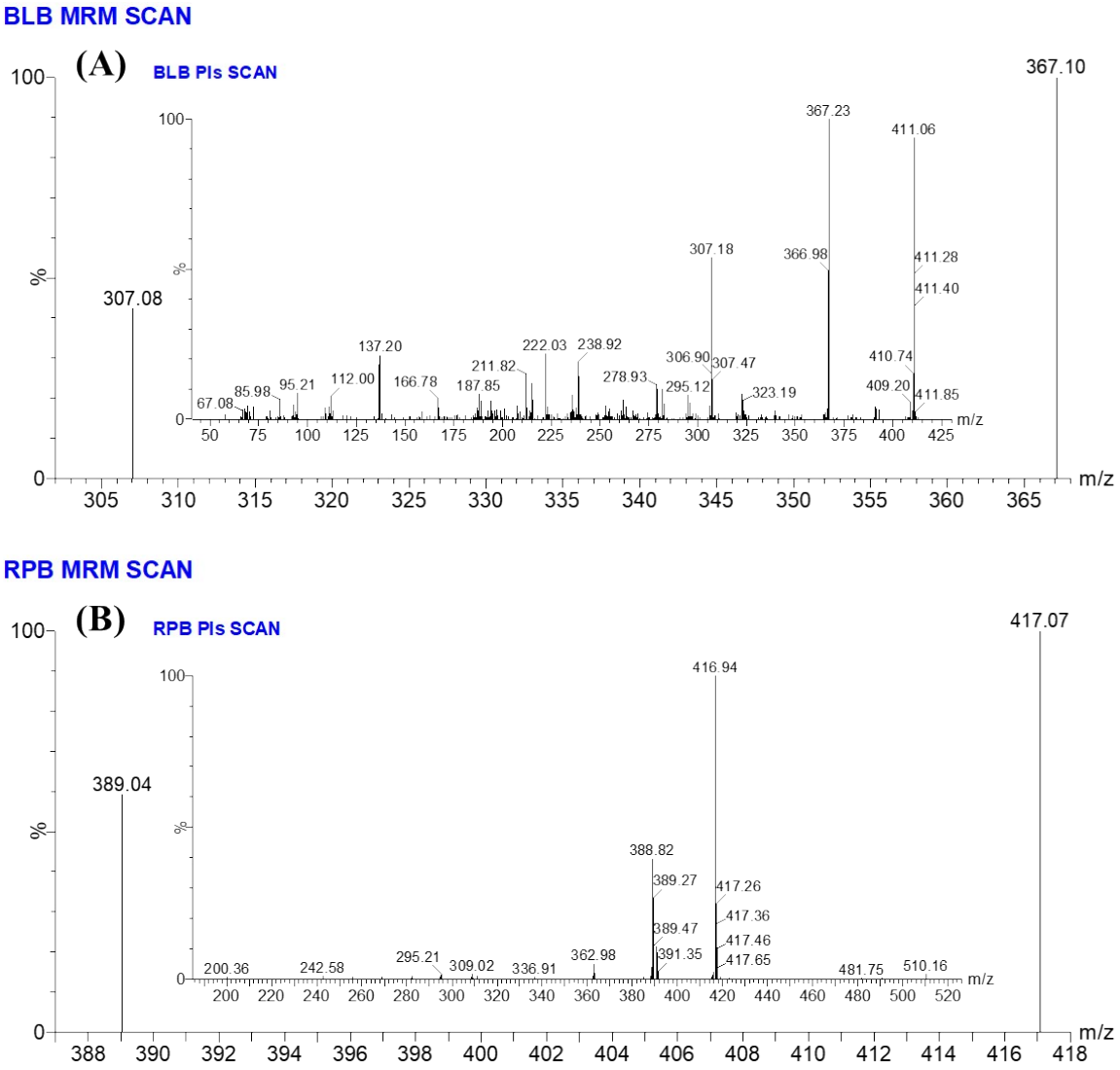


Fig. S4. MS spectrum of RPB (**A**) and MS spectrum of BLB (**B**) attained via the MRM analyzer modes. The fragments MS spectrum for BLB and RPB overlapping the MRM MS spectrum showed the related product ions.

Minor structural alterations in 2-aminopyridine moiety (lability: 58%) and morpholine groups (lability: 42%) may increase the buparlisib metabolic stability

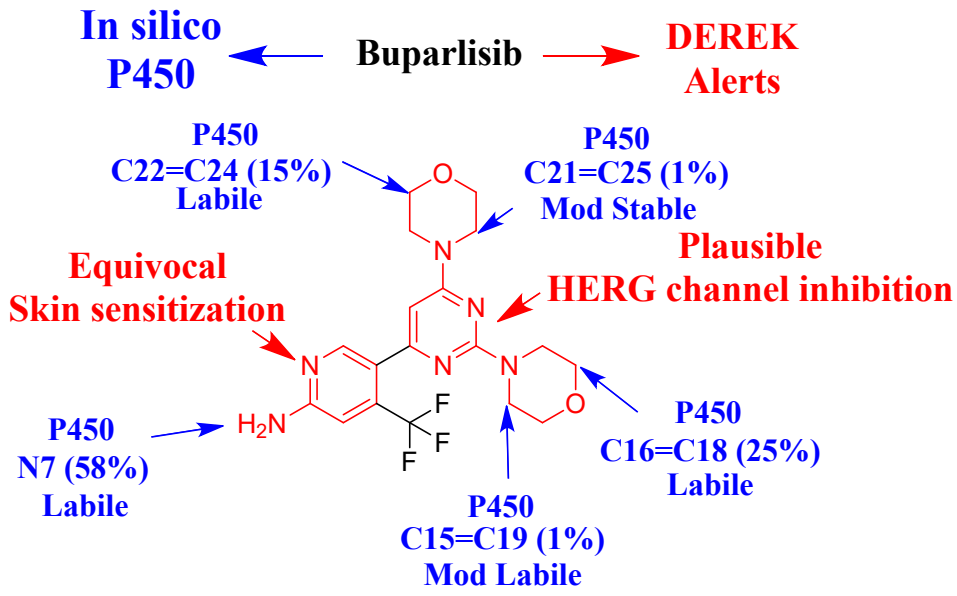


Fig. S5. The BLB chemical structure depicts the WhichP450 in silico metabolic sites highlighted in blue color, signifying that the 2-aminopyridine ring (lability: 58%) and morpholine groups (lability: 42%) influence BLB's metabolic lability. Furthermore, the BLB DEREK testing outcomes indicated proposed side effects (Plausible HERG channel inhibition and skin sensitization) due to the structural alerts in the BLB chemical structure painted in red color.

Tables:

Table S1. The ADME features of BLB were evaluated employing the freely online accessible SwissADME application.

Physicochemical Characteristics	
Molecular weight	410.39 g/mol
Formula	$C_{18}H_{21}F_3N_6O_2$
Rotatable bonds num.	4
Heavy atoms num.	29
Arom. heavy atoms num	12
Fraction Csp3	0.50
Num. H-bond acceptors	8
Num. H-bond donors	1
TPSA	89.63 Å²
Molar Refractivity	106.12
Lipophilicity	
Log Po/w (SILICOS-IT)	1.96
Log Po/w (XLOGP3)	1.50
Log Po/w (WLOGP)	2.21
Log Po/w (iLOGP)	2.64
Log Po/w (MLOGP)	1.09
Consensus Log Po/w	1.88
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Egan	Yes
Muegge	Yes
Veber	Yes
The score of bioavailability	0.55

Water Solubility	
Solubility	1.74e-01 mg/mL; 4.25e-04 mol/L
Log S (ESOL)	-3.37
Class	Soluble
Solubility	4.20e-01 mg/mL; 1.02e-03 mol/L
Log S (Ali)	-2.99
Class	Soluble
Solubility	1.01e-02 mg/mL; 2.46e-05 mol/L
Log S (SILICOS-IT)	-4.61
Class	Moderately soluble
Medicinal Chemistry	
Synthetic accessibility	3.46
PAINS alert	0 alert
Brenk alert	0 alert
Leadlikeness	No; 1 violation: MW>350
Pharmacokinetics	
BBB permeant	No
P-gp substrate	Yes
GI absorption	High
CYP3A4 inhibition	Yes
CYP2C9 inhibition	No
CYP2C19 inhibition	No
CYP1A2 inhibition	Yes
CYP2D6 inhibition	Yes
Permeation of Skin (Log Kp)	-7.74 cm/s

Table S2. The ten characteristics of the AGREEprep evaluation of the UPLC-MS/MS approach.

	Criterion	Score	Weight
1	**Placement of sample preparation**	0.66	1
	Sample preparation location: On-line/In situ		
2	**Hazardous substances**	0.33	5
	Mass (g) or volume (mL) of hazardous components: 0.9		
3	**The durability, recycling, and reuse of materials**	0.5	2
	Materials are neither sustainable nor renewable; however, they are utilized multiple times		
4	**Waste**	0.63	4
	Mass [g] or volume [mL] of waste: 0.9		
5	**Economies of scale within the sample**	1.0	2
	Automated and miniaturized approaches should be selected		
6	**Throughput of samples**	0.96	3
	Each hour's output of samples is 60		
7	**Collaboration and automating**	0.38	2
	Number of sample preparation steps: 2 or fewer; level of automation: semi-automatic devices		
8	**Energy utilization**	1.0	4
	Estimated power use per assessment: 10 W		
9	**Configuration for analysis following sample preparation**	0.25	2
	Gas chromatography, liquid chromatography with quadrupole detection, etc.		
10	**Operator's security**	1.0	3
	No. of distinct hazards: No hazards or no exposure		

References

1. L. Huang, H. Luo, S. Li, F. X. Wu and J. Wang, *Brief Bioinform*, 2021, **22**.
2. H. Komura, R. Watanabe and K. Mizuguchi, *Pharmaceutics*, 2023, **15**.
3. H. Sardar, *Phytonutrients*, 2023, 02-08.