Supplementary file

Developing and validation of a sensitive and fast UPLC-MS/MS method for estimating the in-vitro metabolic stability of crenolanib in HLMs: Identification of structural alarms related to the in-silico toxicity and metabolic lability

Mohamed W. Attwa *, Awadh M. Ali*, Haitham AlRabiah, Adnan A. Kadi

Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

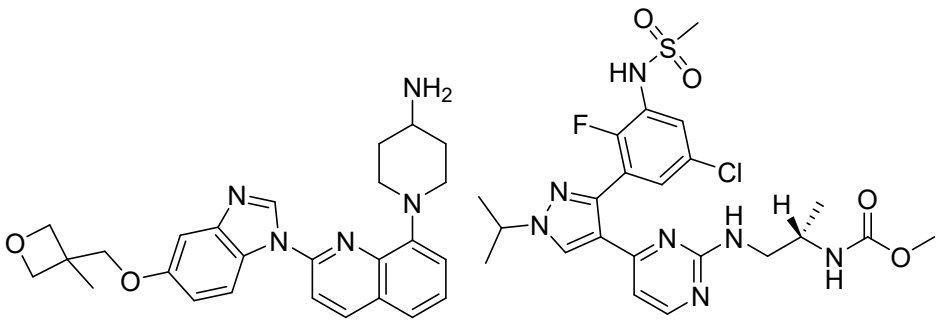
Mohamed W. Attwa: https://orcid.org/0000-0002-1147-4960
Awadh M. Ali: https://orcid.org/0009-0004-6410-5909
Haitham AlRabiah: https://orcid.org/0000-0002-7953-5969
Adnan A. Kadi: https://orcid.org/0000-0001-8115-4228

*Correspondence to:
Dr. Mohamed W. Attwa
E-mail: mzeidan@ksu.edu.sa, aali1@ksu.edu.sa
Fax: +966 1146 76 220
Tel.: +966 1146 70237

In-silico ADME profiling of CLB

The ADME-specific properties of CLB were evaluated using its Smiles depiction CC1(COC1)COC2=CC3=C(C=C2)N(C=N3)C4=NC5=C(C=CC=C5N6CCC(CC6)N)C=C4 via the SwissADME platform. An assessment was done to determine the potential of CLB to exhibit drug-like effects by screening its ADME features. The log p mark generated by the SwissADME approach suggests that CLB demonstrates a moderate degree of water solubility (Log S = -5.03). The predicted pharmacokinetic profile indicates significant GI absorption and implies possible penetration of the blood-brain barrier. The documented bioavailability score is 0.55. The suggested mechanism of CLB action includes the inhibition of particular cytochrome P450 enzymes, namely CYP2C9, CYP3A4, CYP2C19, and CYP2D6, in addition to P-glycoprotein, that functions as a substrate. The declaration states that CLB does not display an inhibitory effect on other cytochrome P450 enzymes, involving CYP1A2. The Log Kp value, which signifies skin permeability, is quantified at -6.39 cm/s. The assessment of drug similarity conforms to the standards set by Veber, Muegge, Egan, and Lipinski (Motiwale, Verma, Silakari, & Sapra, 2024; Muegge, 2003); however, it does not meet the Ghose criteria (No; 1 violation: MR>130) (Attwa, Abdelhameed, & Kadi, 2024). Fig. S2 displays the ADME radar map for CLB, with relevant information specified in Table S1.

Figures:



Crenolanib
Molecular Weight: 443.55

Encorafenib (IS)
Molecular Weight: 540.01

Fig. S1. The chemical structure of crenolanib and encorafenib (IS).

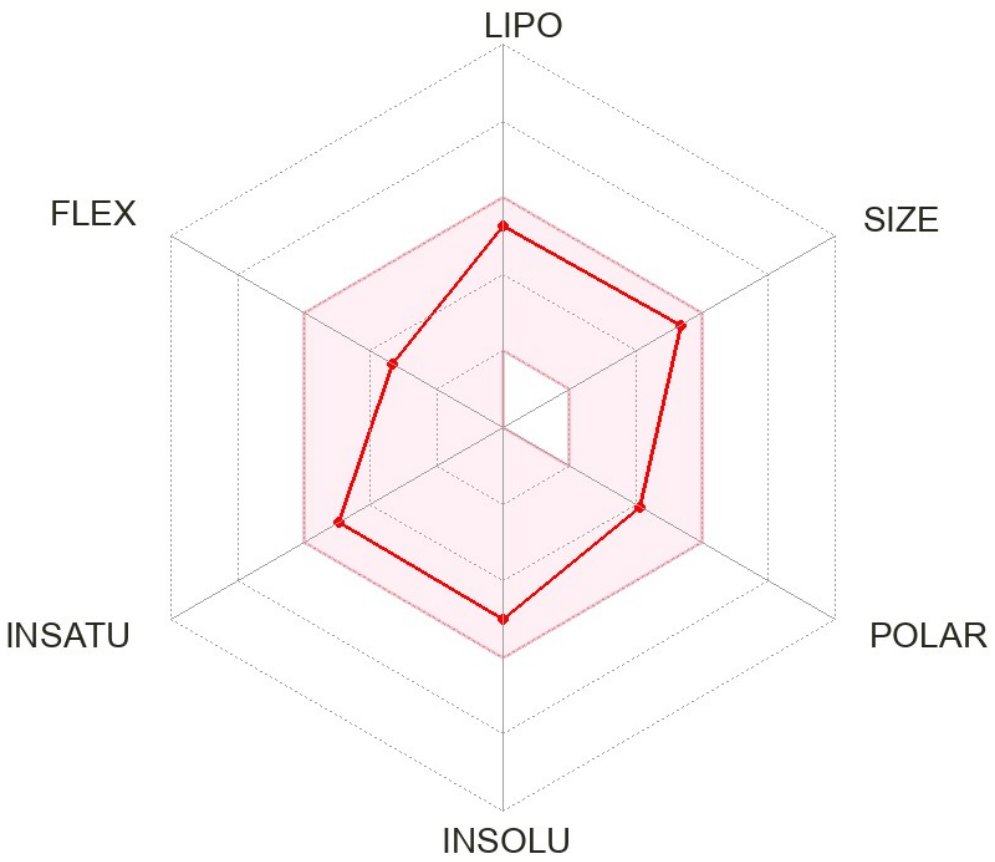


Fig. S2. The CLB ADME radar chart was generated with the SwissADME web application as an in-silico data. Lipophilicity (LIPO) is measured as XLOGP3 = +3.69. Polarity: TPSA 78.43 Å^2; Molecular weight: 443.54 g/mol; Solubility: log S ≤ -7.01; Saturation: proportion of carbons in sp^3 hybridization 0.38; Flexibility: 5 rotatable bonds.

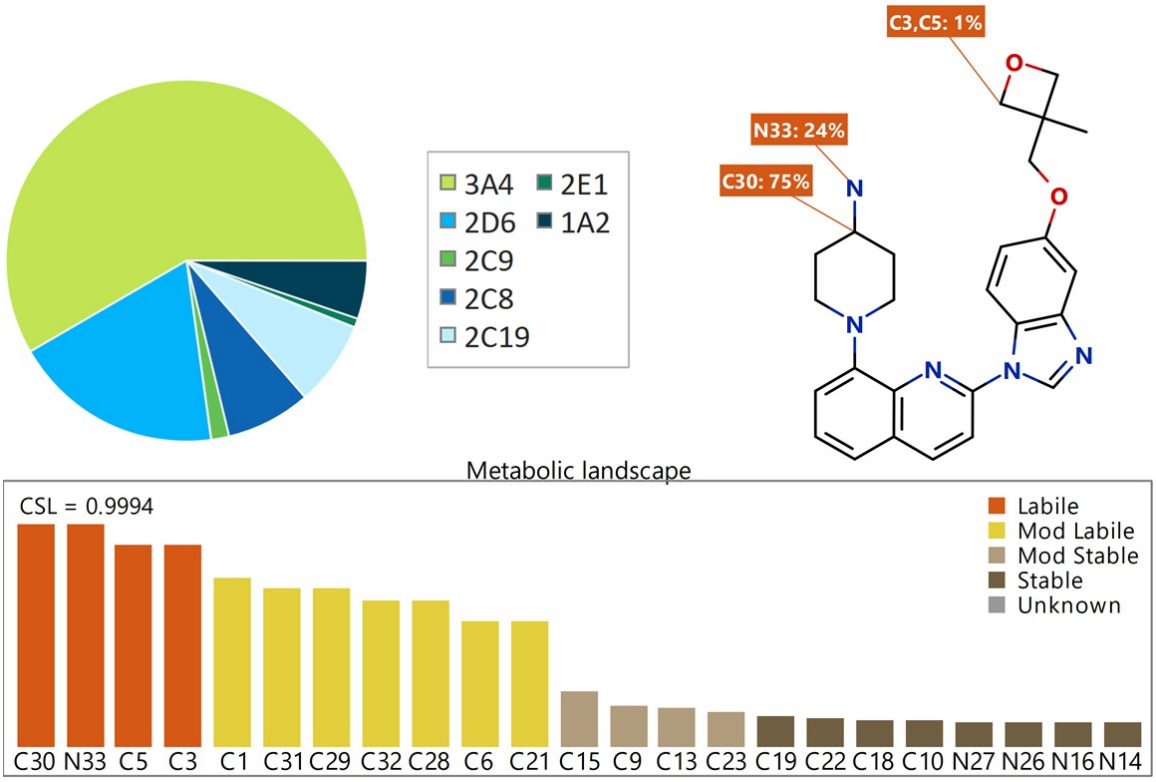


Fig. S3. A CSL rating of 0.9994 indicates that CLB demonstrates significant susceptibility to various metabolic pathways. The results were evaluated using the P450 metabolic software.

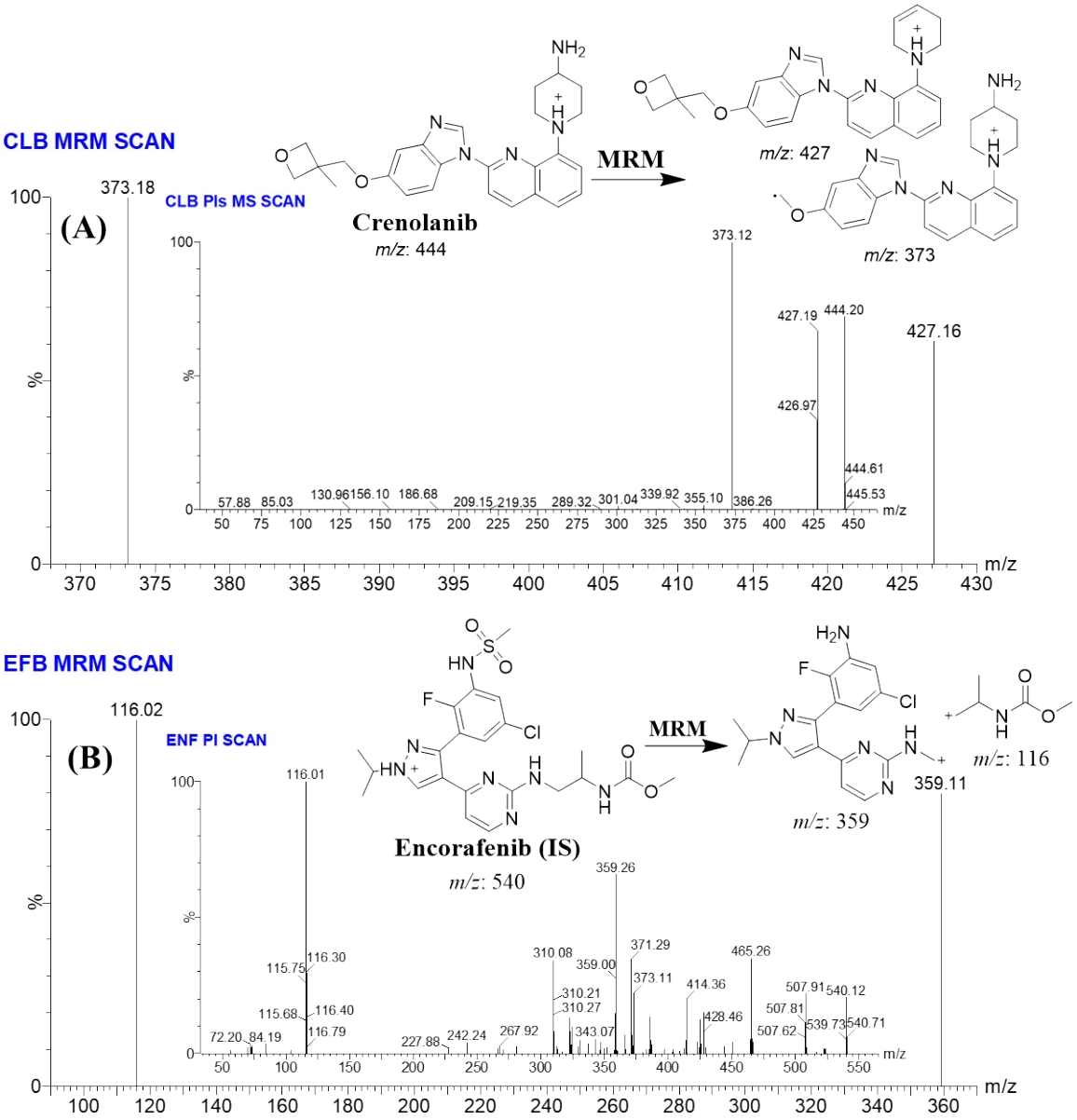


Fig. S4. MS spectra of CLB (**A**) and EFB as IS (**B**) attained via MRM analysis showing product ion MS spectra for the target analyte (CLB) and the IS (EFB). The proposed fragmentation behaviors are displayed.

Minor structural alterations in the the piperidin-4-amine moiety or substitution of the moiety may improve the metabolic stability of crenolanib

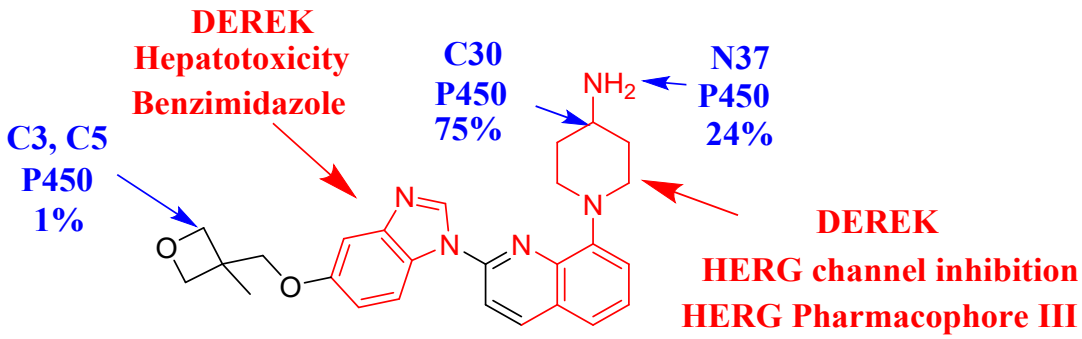


Fig. S5. The CLB metabolic labile sites (blue color) was produced employing StarDrop's WhichP450 module, whereas CLB DEREK alerts (red color) indicate that the piperidin-4-amine moiety is accountable for CLB's metabolic lability.

Tables:

Table S1. The ADME properties of CLB were evaluated utilizing the publicly accessible SwissADME web platform.

Physicochemical characteristics		**Water Solubility**	
Formula	$C_{26}H_{29}N_5O_2$	Solubility	4.33e-03 mg/ml ; 9.76e-06 mol/l
Heavy atoms num.	33	ESOL (Log S)	-5.01
Molecular weight	443.54 g/mol	Class	Moderately soluble
Rotatable bonds num.	5	Solubility	4.16e-03 mg/ml ; 9.39e-06 mol/l
Arom. heavy atoms num	19	Ali (Log S)	-5.03
Fraction Csp3	0.38	Class	Moderately soluble
		Solubility	4.29e-05 mg/ml ; 9.68e-08 mol/l
Num. H-bond donors	1	SILICOS-IT (Log S)	-7.01
Num. H-bond acceptors	5	Class	Poorly soluble
TPSA	78.43 Å²	**Medicinal Chemistry**	
Molar Refractivity	132.96	PAINS	0 alert
Lipophilicity		Brenk	0 alert
Log Po/w (XLOGP3)	3.69	Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5
SILICOS-IT (Log Po/w)	3.21	Synthetic accessibility	3.47
iLOGP (Log Po/w)	3.49	**Pharmacokinetics**	
WLOGP (Log Po/w)	3.54	P-gp substrate	Yes
MLOGP (Log Po/w)	2.24	GI absorption	High
Consensus Log Po/w	3.24	Inhibition of CYP2D6	Yes
Druglikeness		Permeant to BBB	Yes
Lipinski	Yes; 0 violation	CYP1A2 Inhibition	No
Ghose	No; 1 violation: MR>130	CYP2C9 Inhibition	Yes
Egan	Yes	CYP3A4 Inhibition	Yes
Muegge	Yes	CYP2C19 Inhibition	Yes
Veber	Yes	Skin permeation (Log Kp)	-6.39 cm/s
The score of bioavailability	0.55		

Table S2. The sustainability results of the UPLC-MS/MS methodology.

Greenness Features	**Recording**	**Weight**
1. Utilize straight analytical techniques to obviate the necessity for sample making.	0.3	3
2. The goals are to attain a small size of samples and a limited amount of samples.	0.98	2
3. On-site observations are advised whenever feasible.	0.33	2
4. The amalgamation of analytical techniques and working protocols yields reduced reagent usage and energy upkeep.	1.0	2
5. One should choose automated and miniaturized methods.	0.75	2
6. It is advised to avoid derivatization.	1.0	2
7. It is important to prevent the emergence of noteworthy analytical excess and ensure that appropriate measures are implemented for its management.	0.79	2
8. Methods that simultaneously analyze various analytes or parameters are favored over those that evaluate a single analyte in succession.	1.0	2
9. Energy consumption must be reduced.	0.0	2
10. It is advisable to use the reagents produced from renewable sources.	0.5	2
11. It is authoritative to eradicate or replace hazardous reagents.	0.8	2
12. The safety standards for operators should be enhanced.	0.6	3

References

Attwa, M. W., Abdelhameed, A. S., & Kadi, A. A. (2024). An Ultra-Fast Validated Green UPLC-MS/MS Approach for Assessing Revumenib in Human Liver Microsomes: In Vitro Absorption, Distribution, Metabolism, and Excretion and Metabolic Stability Evaluation. *Medicina, 60*(12), 1914.

Motiwale, M., Verma, H., Silakari, O., & Sapra, B. (2024). 3 The utilization of descriptors in convoluted Lipinski's rule of five. *Computational Drug Delivery: Molecular Simulation for Pharmaceutical Formulation*, 39.

Muegge, I. (2003). Selection criteria for drug-like compounds. *Medicinal Research Reviews, 23*(3), 302-321. doi:https://doi.org/10.1002/med.10041