Supplementary file

A Hydrophilic Interaction UPLC-MS/MS Quantitative Method for the Quantification of Saracatinib in the Human Liver Microsomes matrix and its Application to In-Vitro Metabolic Stability Assessment

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Saracatinib In-Silico ADME Characteristics

SwissADME is an online in silico tool created by the Swiss Institute of Bioinformatics, utilized to predict the ADME features of SRB. The program can be used online at the URL that follows: http://www.swissadme.ch/. The program's webpage was reached on May 14, 2025 [1,2]. The ADME-specific parameters of SRB were assessed utilizing its SMILES representation: CN1CCN(CC1)CCOC2=CC3=C(C(=C2)OC4CCOCC4)C(=NC=N3)NC5=C(C=CC6=C5OCO6)Cl via the SwissADME platform. An assessment was performed to screen for the potential of SRB to exhibit drug-like effects by examining its ADME features. The log p score generated by the SwissADME approach indicates that SRB has a moderate level of water solubility (Log S = -5.66). The anticipated pharmacokinetic profile suggests substantial GIT absorption; however, permeability through the BBB has not been recognized. The reported bioavailability score is 0.55. The suggested mechanism of SRB action involves the inhibition of particular cytochrome P450 enzymes, specifically CYP3A4, CYP2C9, CYP2D6, and CYP2C19 as well as P-glycoprotein, that doesn't function as a substrate. The assertion indicates that SRB does not prove inhibitory possessions on other cytochrome P450 enzymes, such as CYP1A2. The Log Kp value, representing skin permeability, is measured at -6.72 cm/s. The assessment of drug similarity adheres to the criteria established by Veber, Muegge, [1], Egan, and Lipinski (Yes; 1 violation: MW>500) [3, 4], while it doesnot adhere to the criteria established by Ghose (No; 2 violations: MW>480, MR>130). Fig. S1 illustrates the ADME radar map for SRB, with pertinent details outlined in Table S1.

Figures:

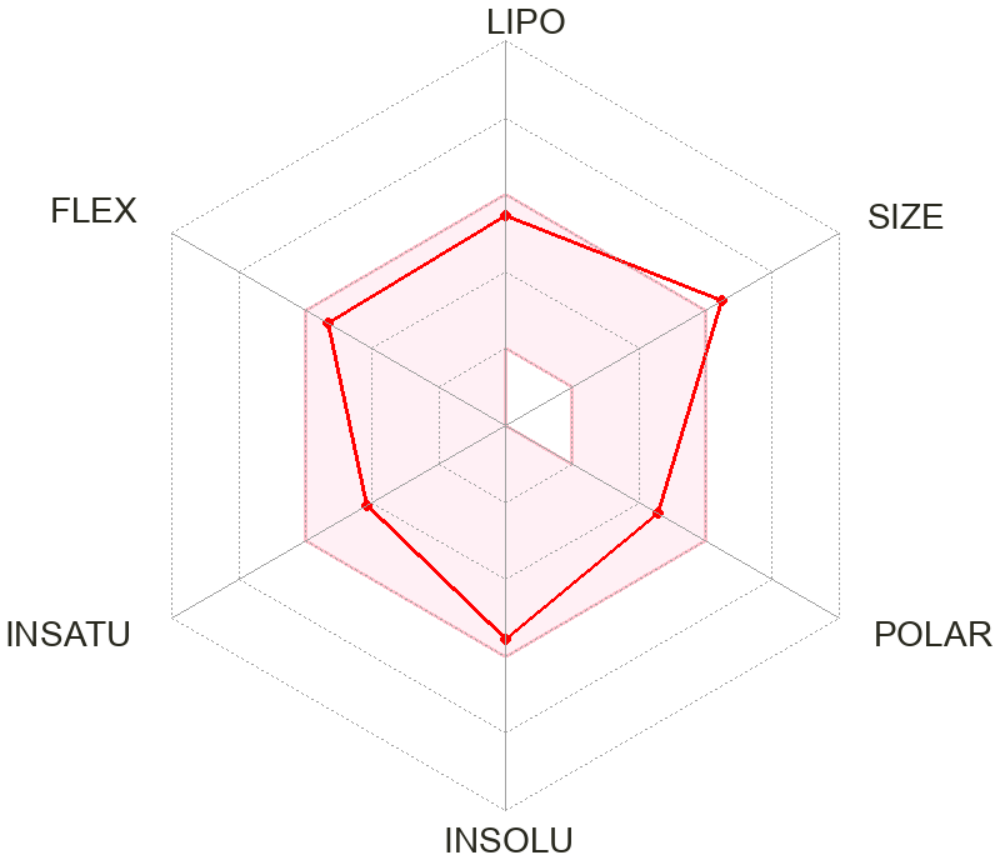
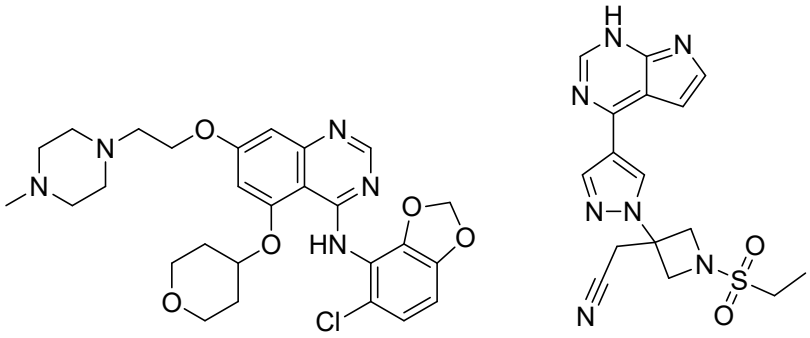


Fig. S1. The SRB ADME radar chart was made using the in silico SwissADME online software. Lipophilicity (LIPO) is determined as XLOGP3 = +4.06. Molecular weight (SIZE): 542.03 g/mol. Saturation (INSATU): fraction of carbons in sp^3 hybridization 0.48. Polarity (POLAR): TPSA 90.44 $Å^2$. Flexibility (FLEX): 8 rotatable bonds. Solubility (INSOLU): log S ≤ 7.40.



Saracatinib
Molecular Weight: 542.03

Baricitinib (IS)
Molecular Weight: 371.42

Fig. S2. The chemical structure of saracatinib and baricitinib (IS).

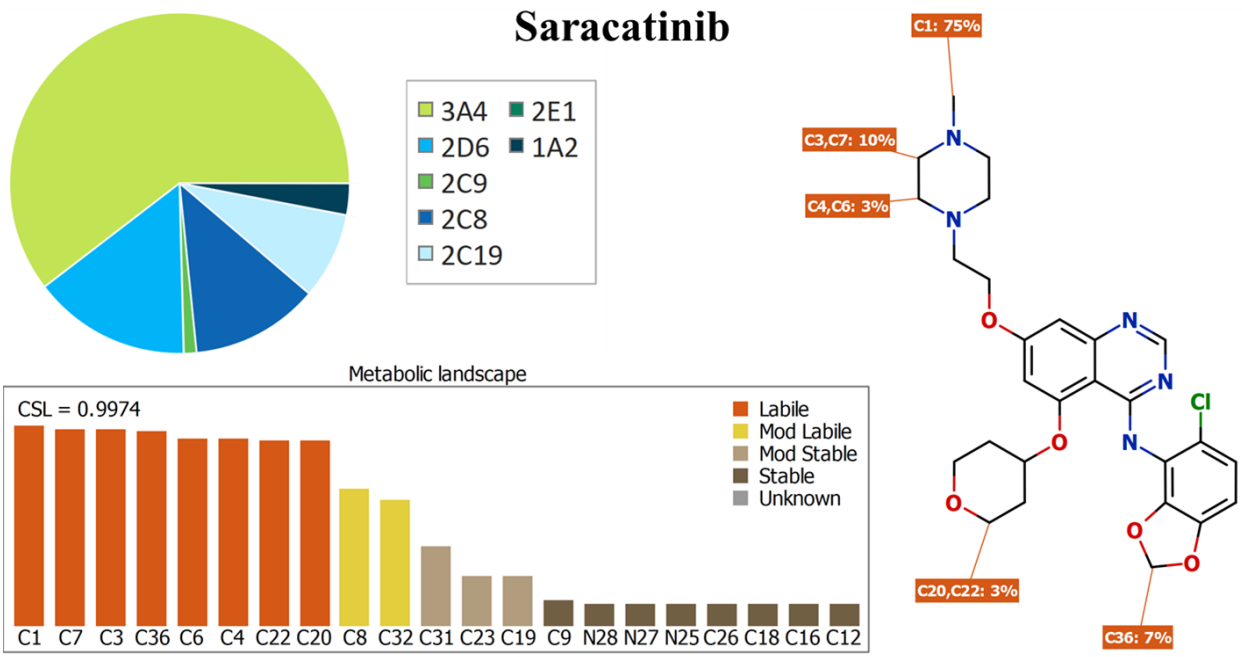


Fig. S3. The results from the StarDrop metabolism module, as depicted in the pie chart, indicate that SRB is primarily metabolized by CYP3A4, aligning with its recognized essential metabolic process. The CSL rating of 0.9974 suggests that SRB exhibits considerable vulnerability to procedures connected to metabolism. The outcomes were evaluated utilizing the WhichP450 module of the StarDrop program. The expected metabolism regioselectivity is solely ascribed to the major isoform, CYP3A4.

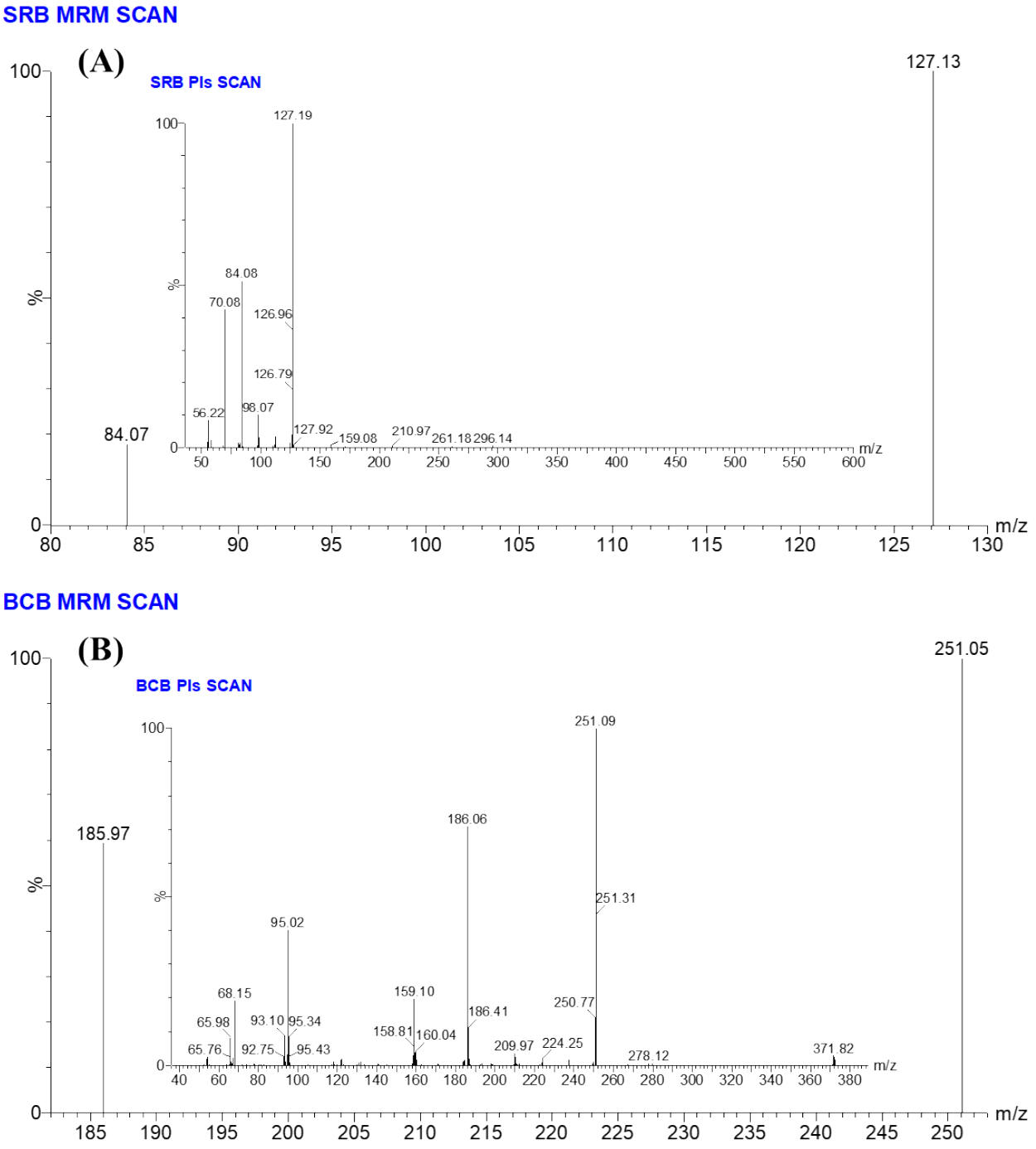


Fig. S4. MS spectra of SRB (**A**) and BCB as IS (**B**) attained through MRM analysis showing product ion MS spectrum for the target analyte (SRB) and the IS (BCB). The predicted dissociation patterns are displayed.

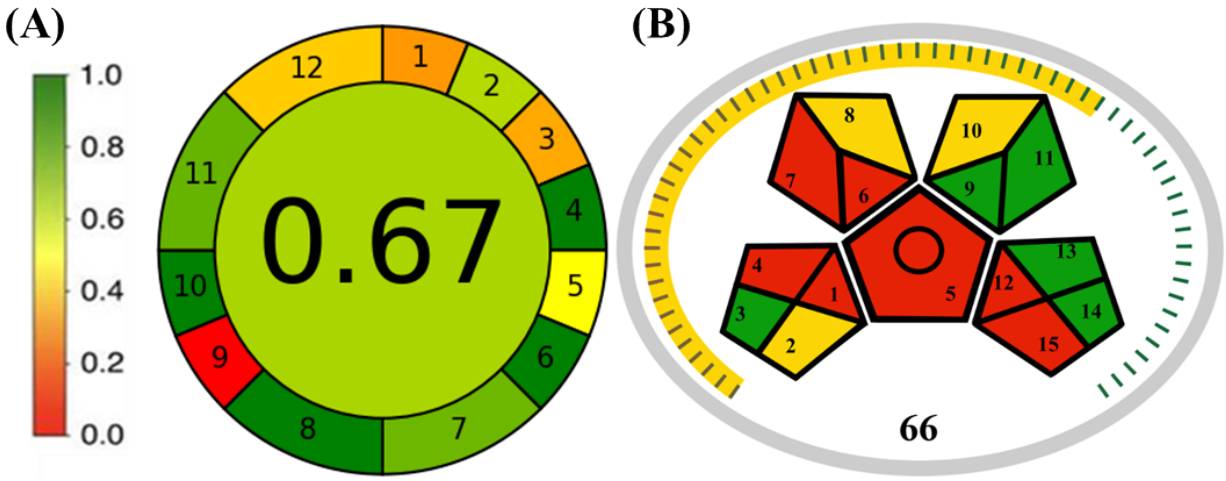


Fig. S5. The outcomes of the greenness assessment of the established UPLC-MS/MS approach revealing a value of 0.67 (AGREE) (**A**) and 66 (MoGAPI) (**B**) that revealed the good degree of greenness of the current UPLC-MS/MS method.

Tables:

Table S1. The ADME features of SRB were assessed applying the freely online available SwissADME program.

Physicochemical parameters		**Water Solubility**	
Formula	$C_{27}H_{32}ClN_5O_5$	Solubility	1.56e-03 mg/ml ; 2.87e-06 mol/l
Heavy atoms num.	38	Log S (ESOL)	-5.54
Molecular weight	542.03 g/mol	Class	Moderately soluble
Rotatable bonds num.	8	Solubility	1.18e-03 mg/ml ; 2.17e-06 mol/l
Arom. heavy atoms num	16	Log S (Ali)	-5.66
Fraction Csp3	0.48	Class	Moderately soluble
		Solubility	2.18e-05 mg/ml ; 4.02e-08 mol/l
Num. H-bond donors	1	Log S (SILICOS-IT)	-7.40
Num. H-bond acceptors	9	Class	Poorly soluble
TPSA	90.44 Å²	**Medicinal Chemistry**	
Molar Refractivity	151.69	PAINS	0 alert
Lipophilicity		Brenk	0 alert
Log Po/w (XLOGP3)	4.06	Leadlikeness	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Log Po/w (SILICOS-IT)	3.43	Synthetic accessibility	4.22
Log Po/w (iLOGP)	4.21	**Pharmacokinetics**	
Log Po/w (WLOGP)	3.18	P-gp substrate	Yes
Log Po/w (MLOGP)	1.67	GI absorption	High
Consensus Log Po/w	3.31	Permeant to BBB	No
Druglikeness		Inhibition of CYP2D6	Yes
Lipinski	Yes; 1 violation: MW>500	Inhibition of CYP1A2	No
Ghose	No; 2 violations: MW>480, MR>130	Inhibition of CYP3A4	Yes
Egan	Yes	Inhibition of CYP2C19	Yes
Muegge	Yes	Inhibition of CYP2C9	Yes
Veber	Yes	Skin permeation (Log Kp)	-6.72 cm/s
The score of bioavailability	0.55		

Table S2. MRM mass spectrometric parameters for SRB and BCB (IS) as optimized by the IntelStart software.

Compound	Formula/Mass	Transition	Parent m/z	Cone Voltage	Daughters	Collision Energy	Ion Mode
SRB MRM	$C_{27}H_{32}ClN_5O_5$	1	542	54	127	26	ES+
		2	542	54	84	54	ES+
BCB MRM	$C_{16}H_{17}N_7O_2S$	1	372	48	251	28	ES+
		2	372	48	186	36	ES+

Table S3. The greenness results of the UPLC-MS/MS method.

Criteria	Score	Weight
1. Utilize direct analytical techniques to obviate the necessity for the preparation of samples.	0.3	2
2. The goals are to attain a minimal sample size and a limited quantity of samples.	0.65	2
3. On-site observations are advised whenever feasible.	0.33	2
4. The amalgamation of analytical techniques and operating guidelines yields energy conservation and reduced reagent usage.	1.0	2
5. One should choose automated and miniaturized methods.	0.5	2
6. It is recommended to avoid derivatization.	1.0	2
7. It is crucial to prevent the emergence of significant analytical surplus and ensure that appropriate measures are implemented for its oversight.	0.79	4
8. Methods that simultaneously analyze many analytes or parameters are favored over those that evaluate a single analyte in succession.	1.0	4
9. Energy consumption must be reduced.	0.0	2
10. It is advisable to categorize reagents sourced from natural resources.	1.0	2
11. It is imperative to eradicate or substitute dangerous reagents.	0.8	4
12. The safety regulations for operators require enhancement.	0.4	4

References

1. M. W. Attwa, A. S. Abdelhameed and A. A. Kadi, *Medicina*, 2024, **60**, 1914.
2. M. W. Attwa, A. S. Abdelhameed, N. A. Alsaif, A. A. Kadi and H. AlRabiah, *RSC Advances*, 2022, **12**, 20387-20394.
3. I. Muegge, *Medicinal Research Reviews*, 2003, **23**, 302-321.
4. M. Motiwale, H. Verma, O. Silakari and B. Sapra, *Computational Drug Delivery: Molecular Simulation for Pharmaceutical Formulation*, 2024, 39.