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Pharmacology of Berberine and Its Metabolites, Is It the Natures Ozempic or Imatinib?

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Abstract

Background: Berberine, a naturally occurring alkaloid, is widely explored for several health benefits, including weight management and metabolic disorders. The major pharmacological action of berberine is reported to be by activation of AMP-activated protein kinase, while its other clinical outcomes are devoid of clear mechanism of action/s. Hence in this study a detailed pharmacology of berberine and its two major metabolites (berberrubine, and jatrorrhizine) in humans was evaluated using well established Insilco tools. **Materials and Methods**: The targets of berberine and its metabolites were identified in SwissTargetPrediction server and their affinity was assed using AutoDock vina 1.2.0. The binding pockets of the highest ligand receptor combinations was assessed using the PrankWeb: Ligand Binding Site Prediction tool. **Results**: Kinases, enzymes and family A GPCR's were identified as the top three target category of berberine and its metabolites. ROCK2, PIK3CD, KCNMA1, CSF1R, and KIT were observed to be the high affinity targets of berberine and its metabolites with affinity values of <4 uM. The affinity of berberine and its metabolites against all AMPKs and lipid/glucose regulator targets (LDLR, DDP4 and PCSK9) were >10 uM. The IC50 value of berberine and its metabolites against ROCK2 was the least (<1 uM), while their other high affinity targets (PIK3CD, KCNMA1, CSF1R and KIT) showed IC50 values <5 uM. **Conclusion**: The diverse range of protein targets and the observed novel high affinity targets (ROCK2, PIK3CD, KCNMA1, CSF1R and KIT) offer valuable insights into the potential mechanisms of action and therapeutic effects of berberine and its metabolites in various disease conditions, which warrants validation in suitable efficacy analysis studies.

Keywords: Berberine, Berberrubine, Jatrorrhizine, Cancer, Diabetes, Obesity, Mechanisms, Pharmacodynamics

Introduction

Berberine is a naturally occurring alkaloid compound found in various medicinal plants belonging to the Berberidaceae and Ranunculaceae families. Berberis species, such as *Berberis aristate, Coptis trifolia, Mahonia bealei*, and *Hydrastis canadensis*, have garnered significant attention in recent years due to its diverse pharmacological properties and therapeutic potential [1,2]. Some common natural sources of berberine along with their geographical locations, are outlined in **Table 1**. Berberine is commonly found in the roots and rhizomes of these plants, and it is extracted through various methods for use in traditional medicine and pharmaceutical applications [3,4]. The berberine content can vary depending on the plant's species, geographical location, and growing conditions, while the extraction methods and purification processes used play a crucial role in influencing the quality of berberine used for research and medicinal purposes [5]. In the pharmaceuticals and nutraceuticals era, this potent alkaloid has captured the interest of researchers and scientists worldwide, prompting rigorous investigations into its multifaceted pharmacological effects and potential applications in various disease conditions [6-10]. The molecular structure of berberine consists of a quaternary ammonium cation linked to a benzylisoquinoline

skeleton, conferring it with unique physicochemical properties and biological activities [11]. The distinctive chemical composition has been associated with a plethora of biological effects, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, and cardiovascular properties, among others.[1-4] These attributes have propelled berberine into the spotlight as a promising natural compound with potential therapeutic benefits for a wide range of medical conditions.

Table 1. Common natural source of berberine and their major geographical locations.

Plants	Geographical Location
Berberis vulgaris (European barberry)	Middle east, Europe
Berberis aristata (Indian barberry)	Asia
Berberis aquifolium (Mountain grape)	America
Berberis bealei (Oregon grape)	America
Berberis japonica (Beale's barberry)	Asia
Coptis chinensis (Chinese goldthread)	Asia
Coptis japonica (Japanese goldthread)	Asia
Coptis trifolia (Threeleaf goldthread)	Asia and America
Hydrastis canadensis (Goldenseal)	America
Mahonia aquifolium (Oregon grape)	America
Mahonia bealei (Beale's barberry)	Asia
Mahonia fortunei (Fortune's barberry)	Asia
Tinospora cordifolia (Heart-leaved moonseed)	Asia
Phellodendron amurense (Amur cork tree)	Asia

Berberine exerts its pharmacological effects through multiple mechanisms of action, making it a versatile and promising natural compound with potential therapeutic applications [12-14]. Some of the key mechanisms of action of berberine include: 1) Regulation of cellular signalling pathways: Berberine can modulate various cellular signalling pathways, including AMP-activated protein kinase (AMPK) [15] sirtuin1, and mitogen-activated protein kinase (MAPK) pathways [16]. Activation of AMPK helps regulate energy metabolism and glucose homeostasis, making berberine a potential candidate for managing metabolic disorders such as type 2 diabetes mellitus. 2) Interaction with enzymes: Berberine can interact with several enzymes, affecting their activity and function. For instance, it inhibits dipeptidyl peptidase-4 (DPP-4), an enzyme involved in the breakdown of incretin hormones, thus prolonging incretin hormone action and improving insulin secretion in management of diabetes mellitus [17]. 3) Anti-inflammatory activity: Berberine has been shown to possess anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines and reducing the activation of nuclear factor-kappa B (NF-κB) [18]. This anti-inflammatory action may contribute to its potential in treating various acute and chronic inflammatory conditions. 4) Antioxidant effects: Berberine has demonstrated antioxidant activity by neutralizing free radicals and reducing oxidative stress. This property can protect cells and tissues from damage caused by reactive oxygen species (ROS) and may have implications for various oxidative stress-related diseases [19,20]. 5) Antibacterial and antimicrobial activity: Berberine exhibits potent antibacterial and antimicrobial properties, making it effective against a wide range of pathogens, including bacteria, viruses, fungi, and parasites. It can inhibit bacterial growth and disrupt the integrity of microbial cell membranes [21,22]. 6) Modulation of gut microbiota: Berberine can modulate the composition and diversity of the gut microbiota. By promoting the growth of beneficial bacteria and inhibiting harmful species, berberine may contribute to gut health and overall well-being [23,24]. 7) Anticancer effects: Berberine has demonstrated anticancer activity by inducing cell cycle arrest, promoting apoptosis (programmed cell death), and inhibiting the growth and metastasis of cancer cells. It also exhibits anti-angiogenic properties, hindering the development of new blood vessels that support tumour growth [25,26]. 8) Cholesterol and lipid regulation: Berberine can lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which is involved in cholesterol synthesis. It also enhances the expression of LDL receptors, promoting the clearance of LDL cholesterol from the bloodstream [27]. 9) Neuroprotective effects: Berberine has been investigated for its potential neuroprotective properties. It can cross the blood brain barrier to modulate neurotransmitter systems and attenuate neuroinflammation, offering potential benefits in neurodegenerative diseases and cognitive disorders [28]. Despite these diverse mechanisms of actions, it is important to note that the exact mechanisms of action of berberine may vary depending on the specific cellular context and the target tissue or organ. Additionally, more research is needed to fully elucidate the comprehensive mechanisms underlying berberine's diverse pharmacological effects. Despite the increasing evidence supporting the pharmacological activities of berberine, several critical questions remain unanswered. The mechanisms governing its multitargeted effects, together with its pharmacokinetic properties, and the factors influencing its bioavailability are areas that warrant further exploration. Furthermore, while numerous clinical trials have demonstrated the prophylactic and therapeutic potential of berberine in certain disease conditions, additional robust clinical investigations are necessary to elucidate its safety and efficacy profile across different patient populations.

In recent years, advancements in systems biology and computational approaches have revolutionized drug discovery, enabling researchers to comprehend complex interactions within biological systems. Network analysis, a powerful

tool in this domain, has gained prominence as an effective method to unravel the intricacies of pharmacological actions and reveal the underlying mechanisms of active compounds like berberine. Network analysis offers a comprehensive and holistic perspective by representing biological entities, such as proteins, genes, or metabolites, as nodes and their interactions as edges in a network [29-31]. This approach not only elucidates individual components' roles but also emphasizes the importance of interconnectedness and crosstalk within cellular pathways. The integration of diverse omics data, including genomics, proteomics, and metabolomics, in conjunction with network analysis enables the construction of sophisticated networks that can capture the multifaceted interactions involved in berberine's pharmacological effects. In this study, a comprehensive examination of the pharmacology of berberine was performed using the Insilco tools, aiming to shed light on the molecular mechanisms underlying its multifaceted effects. The findings from this study will not only enhance our understanding of berberine's biological activities but also provide valuable insights that could contribute to the development of novel therapeutic strategies harnessing the potential of this interesting herbal alkaloid.

Materials and Methods

The isomeric SMILES sequence of berberine and its metabolites (berberrubine and jatrorrhizine) obtained from the PubChem database were inputted into the SwissTargetPrediction server to identify the targets specific to homo sapiens. As reported before, the affinity values of berberine and its metabolites with their respective targets were assessed using AutoDock vina 1.2.0 for other ligand-receptor combinations [30-32].

The top 10 target network of berberine was identified from the STITCH database (https://stitch-db.org), and the affinity of berberine and its metabolites with these targets was assessed using AutoDock vina 1.2.0. As AMPK is reported to be a major target of berberine, the network protein analysis of human AMPK was conducted as reported before using the STRING Database (https://string-db.org), and the affinity of berberine and its metabolites against all known forms of human AMPK identified in this network was evaluated using AutoDock vina 1.2.0. In addition, some selective lipid targets (GLP1R, ZGLP1, DPP4; Uniprot ID P43220, P0C6A0, P27487 respectively) and targets of berberine listed in the DrugBank (BIRC5 and qacR; Uniprot ID O15392, P0A0N5 respectively) were also assessed for affinity using AutoDock vina 1.2.0 [30-32].

The pharmacokinetic parameters of berberine and its metabolites were assessed using the SwissADME server. The targets with top five affinity values with berberine or its metabolites were further assessed using the PrankWeb: Ligand Binding Site Prediction tool (https://prankweb.cz/) to identify the major binding sites, and an inhouse algorithm reported previously was used to estimate the IC_{50} values of berberine and its metabolites against each of the top five targets.

Results

Berberine belongs to the class of benzylisoquinoline alkaloids, and its chemical structure can be divided into three main parts: the quaternary ammonium group, the benzylisoquinoline core, and the methoxy group (**Figure 1**). The berberine's quaternary ammonium group (-NR4+) contains four carbon atoms bonded to a central nitrogen atom. It confers a positive charge to the nitrogen atom, making berberine a water-soluble compound. The benzylisoquinoline core of berberine consists of two aromatic rings (A and B) connected by a bridgehead carbon (C). Ring A is a quaternary benzene ring, and ring B is a partially hydrogenated aromatic ring. The bridgehead carbon (C) is a unique feature of the benzylisoquinoline alkaloids, and in berberine, it plays a crucial role in the subsequent formation of its metabolites. At position C9 of the B-ring, berberine contains a methoxy group (OCH3). This functional group is important for understanding how berberine is converted to its metabolites. Berberine is metabolized in the body through various enzymatic reactions, leading to the formation of several metabolites. Two significant metabolites of berberine are berberrubine and jatrorrhizine (**Figure 1**). The conversion of berberine to berberrubine involves the oxidation of the methoxy group (OCH3) at position C9 of the B-ring. This oxidation reaction replaces the methoxy group with a hydroxy group (OH), resulting in the formation of berberrubine. This process is typically facilitated by enzymes in the liver, where most of the metabolism of berberine occurs. While jatrorrhizine is formed through a demethylation reaction, where the methoxy group (OCH3) at position C9 of the B-ring is removed. The demethylation process exposes a hydrogen atom, converting berberine into jatrorrhizine. Like the conversion to berberrubine, this reaction is also catalysed by specific enzymes in the body.

The distribution of different protein targets of berberine and its metabolites is shown in **Figure 1**. There were 29 kinases targeted by berberine and its metabolites. Kinases are enzymes that transfer phosphate groups to other proteins, regulating various cellular processes, including signal transduction, cell cycle control, and metabolism. Fourteen enzymes were targeted by berberine and its metabolites which are biological catalysts that facilitate biochemical reactions in cells. Twelve Family A G Protein-Coupled Receptors (GPCRs) were targeted by berberine and its metabolites. GPCRs are a large family of membrane receptors that play crucial roles in cell signalling and are involved in various physiological processes. Nine ion channels were targeted by berberine and its metabolites, which are integral membrane proteins that allow the passage of ions across cell membranes, controlling electrical signalling in cells. Eight cyclins were targeted by berberine and its metabolites, which are proteins that regulate the cell cycle. Seven types of membrane receptors were targeted by berberine and its metabolites, which are proteins located on the cell membrane that interacts with extracellular molecules to initiate cellular responses. Six oxidoreductases were targeted

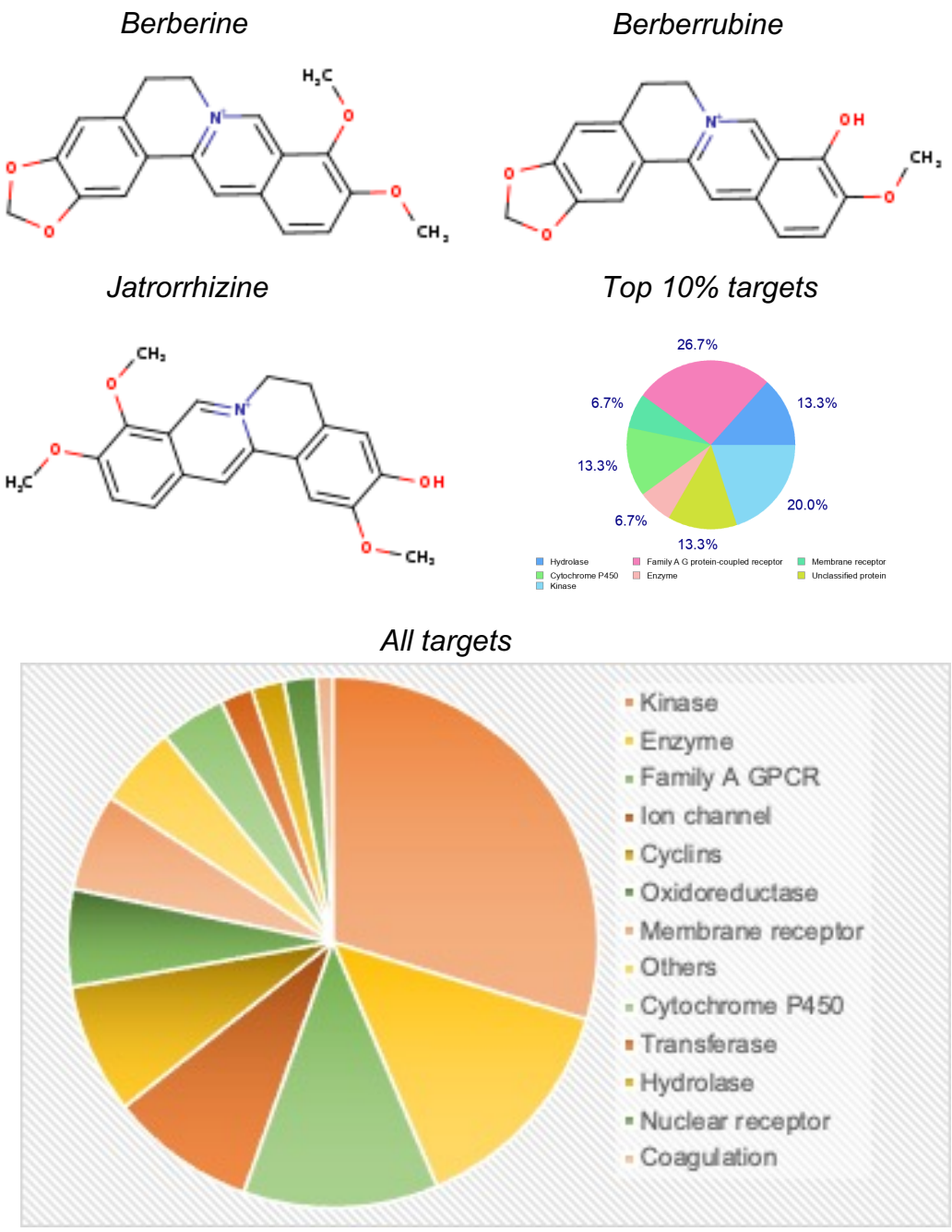


Figure 1. The Chemical structure of berberine, its two major metabolites, and their major target categories.

by berberine and its metabolites, which are a class of enzymes involved in redox reactions. Four cytochrome P450 enzymes were targeted by berberine and its metabolites, which are a superfamily of enzymes involved in the metabolism of various compounds, including drugs and toxins. Two transferases were targeted by berberine and its metabolites, which are a class of enzymes that transfer functional groups between molecules. Two compounds hydrolases were targeted by berberine and its metabolites, which are enzymes that catalyse hydrolysis reactions, breaking down compounds with the addition of water. Two nuclear receptors were targeted by berberine and its metabolites, which are a class of ligand-activated transcription factors that regulate gene expression. One protein involved in the coagulation cascade was targeted by berberine and its metabolites, which is critical to the process of blood clot formation. A diverse range of protein targets were targeted by berberine and its metabolites, with a significant preference towards kinases, enzymes and family

A GPCRs. Kinases, enzymes and family A GPCRs were the top three category of targets of berberine and its metabolites (**Figure 1**). Affinity analysis of berberine and its metabolites against each of its identified targets in humans suggested that ROCK2, PIK3CD, KCNMA1, CSF1R and KIT were the high affinity targets with affinity values of <4 uM (**Figure 2, Tables 2-4**). A complete list of all targets of berberine and its metabolites is shown in **Figure 2**, while the targets with affinity values <10 uM are shown in **Tables 2-4**. The affinity of berberine and its metabolites with their targets ranged from 20.98 uM to 450 uM (**Figure 2**).

Berberine

Target	Uniprot ID	Affinity (nM)
ROCK2	O75116	2336.37
PIK3CD	O00329	2812.39
CSF1R	P07333	2859.63
KIT	P10721	3065.3
PIK3CB	P42338	4127.4
MAP4K4	O95819	4613.45
PIK3CA	P42336	4858.43
PIK3CG	P48736	5506.48
PIM2	Q9P1W9	7496.72
CHEK1	O14757	8902.03
SLC1A3	P43003	8906.26
ROCK1	Q13464	9889.22
MAPK14	Q16539	10405.1
AURKB	Q96GD4	10420.64
CDK4	P11802	10510.07
AURKA	O14965	11178.66
GRM5	P41594	14488.2
CNR2	P34972	14872.18
IKBKB	O14920	14881.62
CDK2	P24941	15088.5
JAK2	O60674	15643.3
PGR	P06401	16197.53
RPS6KB1	P23443	16401.11
HTR2B	P41595	19783.4
PARP2	Q9UGN5	20226.05
CDK8	P49336	20660.63
MAPK10	P53779	21187.81
PIM1	P11309	21854.91
TRPM8	Q7Z2W7	25872.9
ADORA2A	P29274	26193.8
BCAT2	O15382	26246.34
PRF1	P14222	27129.19
PARP10	Q53GL7	28367.42
SRC	P12931	28378.82
GRIA1	P42261	28684.44
PRKACA	P17612	29348.48
LCK	P06239	29649.1
PIK3R1	P27986	29883.17
CDK9	P50750	30726.53
TYMS	P04818	31089.16
NTRK1	P04629	31853.22
PLK1	P53350	32534.41
MET	P08581	33137.44
GRK2	P25098	34793.81
ABL1	P00519	34971.21
MAOB	P27338	35121.51
TBXAS1	P24557	35763.43
CHRM1	P11229	35858.07
NR3C2	P08235	36428.91
QPCT	Q16769	36725.49
BCHE	P06276	37175.78
GABRG2	P18507	37893.91
GRK5	P34947	38403.31
CHEK2	O96017	38633.85
GABRB3	P28472	38811.31
NPY5R	Q15761	39230.18
GRK3	P35626	39623.98
CYP2D6	P10635	40077.93
GABRA5	P31644	45985.97
PTGS2	P35354	46499.22
GABRA3	P34903	47317.74
UBA2	Q9UBT2	47375.76
ACHE	P22303	48034.04
SCD	O00767	48112.52
TGM2	P21980	48341.7
CCNC	P24863	48760.1
CYP19A1	P11511	50715.37
ADORA3	P0DMS8	51408.09
CYP11B1	P15538	52431.25
CCNB1	P14635	52971.58
ICAM1	P05362	53440.26
CHRM4	P08173	55341.82
CYP11B2	P19099	56053.27
MKNK1	Q9BUB5	57631.28
IMPDH2	P12268	58001.24
H5D17B1	P14061	58666.44
PTPN1	P18031	63396.44
ADRA2C	P18825	68569.74
CDC42	P60953	70302.93
SIRT2	Q8IXJ6	72357.77
AOC3	Q16853	73373.07
DRD4	P21917	74215.13
MAPKAPK2	P49137	77777.14
SAE1	Q9UBE0	85849.87
SELE	P16581	93673.19
AGPAT2	O15120	97207.65
ADRA2B	P18089	101674.08
HTR3A	P46098	106012.83
DRD3	P35462	114126.97
RAC1	P63000	115679.21
HPGD	P15428	117806.66
XBP1	P17861	121456.54
SIGMAR1	Q99720	126666.87
CDK1	P06493	145970.6
PTGES	O14684	147507.69
F3	P13726	150092.73
ALOX5AP	P20292	160566.03
PNMT	P11086	185493.49
DHFR	P00374	203696.28
CBFB	Q13951	233014.79
RPS27	P42677	417390.7

Berberrubine

Target	Uniprot ID	Affinity (nM)
KCNMA1	Q12791	3322.95
ROCK2	O75116	5384.83
PIK3CG	P48736	6358.9
CDK4	P11802	7510.44
CHEK1	O14757	9125.66
TERT	O14746	14131.41
GRIN2B	Q13224	15143.88
TYK2	P29597	16833.16
GRIK1	P39086	17354.58
SLC22A12	Q96S37	17970.65
RAF1	P04049	18210.83
GRM5	P41594	19439.19
ABCB1	P08183	19567.05
GRIK2	Q13002	20138.52
HTR2B	P41595	21282.52
HSP90B1	P14625	21696.11
CNR2	P34972	22397.26
CA9	Q16790	22990.98
PTK2B	Q14289	23269.55
IKBKB	O14920	29725.25
GRM4	Q14833	30841.72
LCK	P06239	31096.4
ALK	Q9UM73	31766.22
AVPR1A	P37288	32392.23
CDK2	P24941	34302.37
ESR1	P03372	34531.82
MMP2	P08253	34817.77
ERCC5	P28715	36417.59
CHRM1	P11229	37323.01
ADORA2A	P29274	37545.9
CYP2D6	P10635	38457.66
MAOB	P27338	38475.95
SRC	P12931	39778.77
CTSB	P07858	39791.52
MMP3	P08254	40458.64
HPD	P32754	44819.14
BCHE	P06276	45617.91
GCGR	P47871	46363.26
CCND1	P24385	46575.35
CHEK2	O96017	49709.98
ACHE	P22303	49853.07
CA12	O43570	49996.58
EZR	P15311	50823.68
CTSD	P07339	51423.27
CYP19A1	P11511	52388.32
MTNR1B	P49286	52821.27
UBA2	Q9UBT2	53084.18
MCL1	Q07820	53661.92
OPRK1	P41145	53704.66
AKR1B1	P15121	55635.53
LIG1	P18858	57335.59
IDH1	O75874	58466.64
MMP9	P14780	58558.45
SIRT1	Q96EB6	62366.37
CCNB1	P14635	63524.51
MMP12	P39900	63832.14
PTPN1	P18031	65145.83
WEE1	P30291	65893.55
BRD4	O60885	66170.06
HSP90AA1	P07900	66856.09
TNKS2	Q9H2K2	67723.59
VEGFA	P15692	67827.25
TNNI3	P19429	68169.41
DRD4	P21917	68557.25
HSP90AB1	P08238	69616.46
MTNR1A	P48039	73745.07
TNKS	O95271	74938.72
MPI	P34949	75656.32
H5D11B1	P28845	76568.96
CCR3	P51677	76686.58
ADRA2C	P18825	76751.36
MMP13	P45452	79282.54
CNOT7	Q9UIV1	80734.24
AVPR2	P30518	82207.65
CDC42	P60953	82448.36
H5D17B3	P37058	86258.04
SAE1	Q9UBE0	92529.25
CCNA1	P78396	92550.99
ADRA2B	P18089	92676.47
CCNE2	O96020	95740
CDK1	P06493	99901.01
FEN1	P39748	100388.39
DNASE1L3	Q13609	102204.16
CCNA2	P20248	104533.59
SIGMAR1	Q99720	125220.33
CA2	P00918	133798.58
DRD3	P35462	135872.7
CTSL	P07711	135955.6
ADRB2	P07550	136450.22
IL2	P60568	137046.14
ADRB1	P08588	140892.58
CCNE1	P24864	147417.18
PTGES	O14684	147798.85
CA6	P23280	153047.55
XBP1	P17861	157500.9
EGLN1	Q9GZT9	164566.34
DHODH	Q02127	165374.64
RAC1	P63000	173317.15
CA1	P00915	196756.4
CA4	P22748	199124.51
TNNT2	P45379	229870.42
EGLN3	Q9H6Z9	242335.78
TNNC1	P63316	267647.5
GLO1	Q04760	290237.35
BCL2L1	Q07817	290237.35

Jatrorrhizine

Target	Uniprot ID	Affinity (nM)
ROCK2	O75116	2098.92
DYRK1A	Q13627	4608.68
CSF1R	P07333	4641.66
KIT	P10721	5126.02
PI4KB	Q9UBF8	8860.07
CDK2	P24941	9644.38
PIK3CD	O00329	9784.86
CDK4	P11802	10763.25
FLT3	P36888	10985.1
DYRK1B	Q9Y463	11069.12
CLK4	Q9HAZ1	11093.84
PIK3CG	P48736	11361.79
PLK4	O00444	11501.33
RET	P07949	12168.75
PIK3CB	P42338	14418.13
SCN4A	P35499	14456.85
SLC1A3	P43003	15407.76
AURKA	O14965	16061.63
BRAF	P15056	16494.73
RAF1	P04049	17990.85
CLK1	P49759	19647.16
MAP2K1	Q02750	21087.32
AURKB	Q96GD4	23422.37
CLK2	P49760	23512.86
MAPK8	P45983	24364.73
TTK	P33981	24561.8
RPS6KA3	P51812	28234.55
PIM1	P11309	28493.97
EPHB4	P54760	29184.4
PRF1	P14222	29436.32
CDK8	P49336	31277.75
FGFR1	P11362	32023.33
OGT	O15294	32354.12
IRAK4	Q9NWZ3	33172.39
TRPM8	Q7Z2W7	34703.16
P2RX7	Q99572	35066.11
HTR2B	P41595	36267.88
PAK4	O96013	36447.63
PLK1	P53350	40198.95
GRIA1	P42261	40743.09
MMP3	P08254	41199.14
LCK	P06239	41390.84
CSNK1D	P48730	41410.57
CPT1A	P50416	43629.91
LIMK1	P53667	46131.1
CDK3	Q00526	46649.56
ALK	Q9UM73	46999.05
CHRNA7	P36544	49468.23
PSEN1	P49768	49878.04
PSEN2	P49810	49878.04
MMP2	P08253	52026.16
BCHE	P06276	53148.91
CYP2D6	P10635	54399.3
HCK	P08631	57182.51
UBA2	Q9UBT2	57718.73
CDC25B	P30305	58585.33
AKR1B1	P15121	63765.45
MKNK1	Q9BUB5	64687.39
ACHE	P22303	66791.96
NCSTN	Q92542	68075.25
MMP8	P22894	71826.25
WEE1	P30291	72944.38
PFKFB3	Q16875	73839.54
PARP10	Q53GL7	73839.54
CHEK2	O96017	75998.08
ADORA3	P0DMS8	79143.04
CHRM1	P11229	79523.07
PTK6	Q13882	80132.75
MAPKAPK2	P49137	80328.39
MMP9	P14780	80752.24
H5D17B2	P37059	89365.71
MAPK1	P28482	89648.45
HDAC10	Q969S8	90942.71
APH1B	Q8WW43	101709.31
H5D17B1	P14061	102735.08
PTPN1	P18031	103354.96
H5D17B3	P37058	104741.19
NEK2	P51955	105250.39
MMP1	P03956	106707.02
CCNC	P24863	109920.95
NR1D1	P20393	119873.77
TBXA2R	P21731	120347.93
HTR3A	P46098	121137.31
CCNA2	P20248	121666.56
MCL1	Q07820	122982.62
DRD4	P21917	124206.98
ADRA2C	P18825	125064.17
CCNA1	P78396	125590.37
CDC42	P60953	133699.6
LIMK2	P53671	139585.33
PLK3	Q9H4B4	139875.59
CDC25A	P30304	154933.9
APH1A	Q96BI3	158312.11
HPGD	P15428	163875.59
RAC1	P63000	173917.61
CA2	P00918	177081.81
TNF	P01375	182607.85
SAE1	Q9UBE0	183054.49
CCNE1	P24864	186359.24
ADRA2B	P18089	194871.65
SIGMAR1	Q99720	205603.49
F3	P13726	208192.8
PSENEN	Q9NZ42	225304.74
PNMT	P11086	238904.13
MIF	P14174	449912.59

Figure 2. Affinity values of berberine and its two major metabolites with all their targets in humans identified in SwissTargetPrediction server. The affinity values are arranged in ascending order and colour-coded (green with the highest affinity and red with lowest affinity values).

Table 2. High-affinity targets of berberine.

Target	Gene	Uniprot ID	Affinity (nM)	Target Class
Rho-associated protein kinase 2	ROCK2	O75116	2336.4	Kinase
PI3-kinase p110-delta subunit	PIK3CD	O00329	2812.4	Enzyme
Macrophage CSF receptor	CSF1R	P07333	2859.6	Receptor
Stem cell growth factor receptor	KIT	P10721	3065.3	Receptor
PI3-kinase p110-beta subunit	PIK3CB	P42338	4127.4	Enzyme
Mitogen-activated protein kinase 4	MAP4K4	O95819	4613.5	Kinase
PI3-kinase p110-catalytic	PIK3CA	P42336	4858.4	Enzyme
PI3-kinase p110-gamma subunit	PIK3CG	P48736	5506.5	Enzyme
STPK PIM2	PIM2	Q9P1W9	7496.7	Kinase
STPK Chk1	CHEK1	O14757	8902.0	Kinase
Excitatory amino acid transporter 1	SLC1A3	P43003	8906.3	Ion channel
Rho-associated protein kinase 1	ROCK1	Q13464	9889.2	Kinase
MAP kinase p38 alpha	MAPK14	Q16539	10405.1	Kinase
STPK Aurora-B	AURKB	Q96GD4	10420.6	Kinase
Cyclin-dependent kinase 4	CDK4	P11802	10510.1	Kinase

Table 3. High-affinity targets of berberrubine.

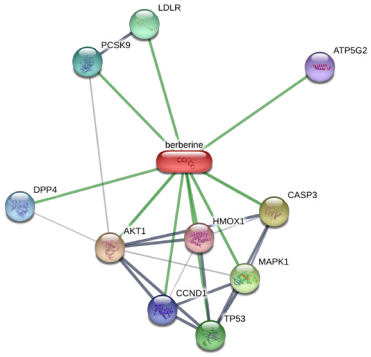
Target	Gene	Uniprot ID	Affinity (nM)	Target Class
Calcium-activated potassium channel subunit alpha-1	KCNMA1	Q12791	3322.95	Ion channel
Rho-associated protein kinase 2	ROCK2	O75116	5384.83	Kinase
PI3-kinase p110-gamma subunit	PIK3CG	P48736	6358.9	Enzyme
Cyclin-dependent kinase 4	CDK4	P11802	7510.44	Cyclins
STPK Chk1	CHEK1	O14757	9125.66	Kinase

Table 4. High-affinity targets of jatrorrhizine.

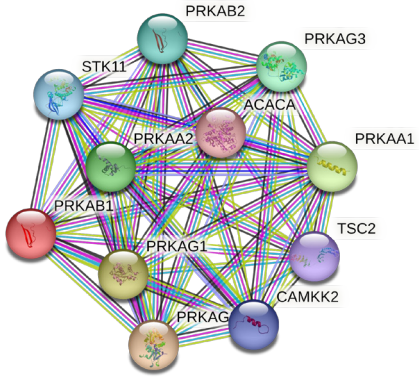
Target	Gene	Uniprot ID	Affinity (nM)	Target Class
Rho-associated protein kinase 2	ROCK2	O75116	2098.92	Kinase
Dual-specificity tyrosine-phosphorylation regulated kinase 1A	DYRK1A	Q13627	4608.68	Kinase
MCSF receptor	CSF1R	P07333	4641.66	Receptor
Stem cell growth factor receptor	KIT	P10721	5126.02	Receptor
PI4-kinase beta subunit	PI4KB	Q9UBF8	8860.07	Kinase
Cyclin-dependent kinase 2	CDK2	P24941	9644.38	Cyclins
PI3-kinase p110-delta subunit	PIK3CD	O00329	9784.86	Kinase
Cyclin-dependent kinase 4	CDK4	P11802	10763.25	Kinase
Tyrosine-protein kinase receptor	FLT3	P36888	10985.1	Kinase

The top ten targets of berberine and its metabolites identified in the STITCH database were also subjected to affinity analysis. Among these, the top three targets were those associated with lipid regulation, i.e., LDLR, DDP4, and PCSK9 (**Figure 3**). However, all these targets showed affinity values >10 uM. Additionally, the affinity of berberine against GLP1 (Uniprot ID P0C6A0) and its receptor (Uniprot ID P43220) was observed to be 75.29 uM and 45.44 uM respectively. BIRC5 (Uniprot ID O15392) and qacR (Uniprot ID P0A0N5) are also reported to be targets of berberine in the drug bank database, and their affinity was observed to be 39.08 uM and 23.94 uM respectively. Inhibition of AMPK has been reported to be the major mechanism of action by which berberine is reported to exhibit its pharmacodynamic effects. Hence, in this study, all known forms of human AMPK were assessed for their affinity with berberine, which ranged from 12 to 104 uM (**Figure 3**). Among the human AMPKs, PRKAA2 showed the highest affinity of 12.03 uM while PRKAB2 had the least affinity of 104.12 uM (**Figure 3**).

A detailed ADME analysis of berberine and its metabolites was performed using the SwissADME database, and the relevant Physicochemical Properties, Lipophilicity, solubility, Pharmacokinetics, and Druglikeness parameters are summarised in **Table 5** and **Figure 4**. The binding affinity of berberine and its metabolites with their high-affinity targets was performed using AutoDock vina to identify the binding pockets and IC_{50} values, summarised in **Figure 4**. The least IC_{50} value of berberine and its metabolites was for ROCK2 (<1 uM). The details of the best binding pocket for each of the high-affinity ligand-receptor combinations are summarised in **Table 6**.



		Affinity (nM)		
Targets	**Uniprot ID**	**Berberine**	**Berberrubine**	**Jatrorrhizine**
LDLR	P01130	12617.65	18591.6	16698.97
DPP4	P27487	20932.53	28194.43	35918.87
PCSK9	Q8NBP7	37655.26	45510	55357.62
CCND1	P24385	43041.3	46575.35	53531.87
AKT1	P31749	47817.24	70035.28	64787.18
MAPK1	P28482	50583.8	62575.71	89648.45
TPRA1	Q86W33	73251.2	68565.23	121634.97
CASP3	P42574	84495.32	82487.93	69195.66
SELE	P16581	93673.19	102832.3	154353.89
TP53	K7PPA8	114056.07	131145.97	127489.03
HMOX1	P09601	142216.75	136358.19	172339.23
ATP5G2	Q06055	228812.33	233355.17	358726.97



AMPK's	Uniprot ID	Affinity (nM)
PRKAA2	P54646	12026.89
PRKAA1	Q13131	23120.41
PRKAG2	Q9UGJ0	51666.29
PRKAG3	Q9UGI9	56324.97
PRKAG1	P54619	61260.01
PRKAB1	Q9Y478	70212.06
PRKAB2	O43741	104115.79

Figure 3. Affinity values of berberine and its two major metabolites with their target networks (Network image) identified in the STITCH database. Bottom panel shows the networks of human AMP-activated protein kinases (AMPK) identified in STRING database along with the affinity values of berberine against all forms of AMPK in humans. The affinity values are arranged in ascending order and colour coded (green with the highest affinity and red with the lowest affinity values).

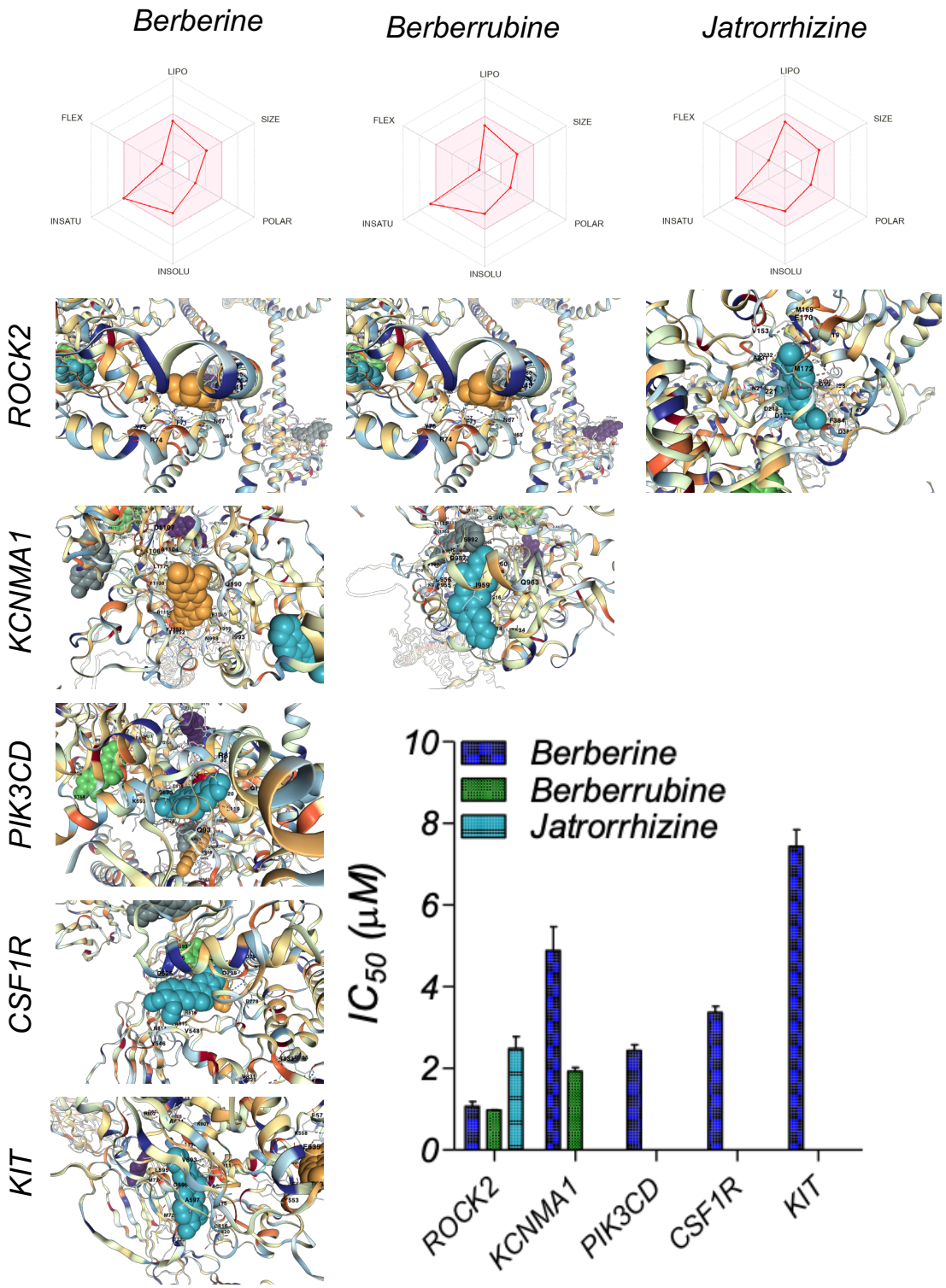


Figure 4: Pharmacokinetic parameters of berberine and its two major metabolites. The binding pockets of high-affinity ligand-receptor combinations of berberine and its two major metabolites with their respective targets is shown along with its IC_{50} values (bar graph).

Table 5. Physicochemical Properties, Lipophilicity, Solubility, Pharmacokinetics, and Druglikeness parameters of berberine and its two major metabolites.

	Berberine	**Berberrubine**	**Jatrorrhizine**
Mol Wt	336.36	322.33	338.38
Heavy atoms	25	24	25
Aromatic heavy atoms	16	16	16
Fraction Csp3	0.25	0.21	0.25
Rotatable bonds	2	1	3
H-bond acceptors	4	4	4
H-bond donors	0	1	1
Molar Refractivity	94.87	90.41	97.33
TPSA (Surface area)	40.8	51.8	51.8
Log_P	3.74	3.21	3.43
Consensus Log P	2.53	2.24	2.31
Log_S	-4.16	-4.05	-4.19
Solubility (mg/ml)	2.30E-02	2.85E-02	2.20E-02
Solubility (mol/l)	6.85E-05	8.85E-05	6.49E-05
Class	Moderately soluble	Moderately soluble	Moderately soluble
Log_{Sw}	-5.92	-5.23	-5.72
GI absorption	High (~97%)	High (~97%)	High (~97%)
BBB permeant	Yes	Yes	Yes
Pgp substrate	Yes	Yes	Yes
CYP1A2 inhibitor	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	Yes	Yes	Yes
CYP3A4 inhibitor	Yes	Yes	Yes
log_{Kp} (cm/s)	-5.78	-5.93	-5.94
Lipinski violations	0	0	0
Ghose violations	0	0	0
Veber violations	0	0	0
Egan violations	0	0	0
Muegge violations	0	0	0
Bioavailability Score	0.55	0.55	0.55
PAINS #alerts	0	0	0
Brenk alerts	1	1	1
Lead likeness violations	1	0	0
Synthetic Accessibility	3.14	3.01	3.06
Plasma Protein Binding	58.54	58.54	58.54

Table 6. The binding pockets of high-affinity ligand-receptor combinations of berberine and its two major metabolites.

Ligand-Receptor	Pocket ID	Vina score	Volume (Å3)	Center (x, y, z)	Docking size (x,y,z)	Pocket Sequence
Berberine ROCK2	C2	-8.6	1358	-42, 8, 3	22, 22, 22	LEU53 LEU57 ASN65 ASN67 ILE68 PHE71 ARG74 TYR75 PHE403 LEU449 LEU452 LEU456 GLU459 MET460 LYS463
Berberine PIK3CD	C4	-8.9	2633	37, 16, 0	34, 22, 35	ARG87 GLN93 GLN116 LEU119 LEU120 TRP649 ARG652 MET683 GLY686 GLU687 LEU689 SER690 LYS693 THR818 GLY819 ASP820 THR822
Berberine CSF1R	C1	-8.1	4899	-19, 25, 5	22, 32, 28	TYR546 VAL548 PHE593 ASP625 GLU626 ASP778 ASN783 ASP796 GLY798 LEU799 ALA800 ARG801 ILE803 TYR809 ASN814 ALA815 ARG816
Berberine KIT	C1	-7.7	2822	-13, -14, -7	22, 22, 35	LEU595 GLY596 ALA597 VAL603 ALA621 ASP677 GLU720 TYR721 MET722 MET724 ARG796 LEU799 PHE811 ALA814 ARG815 ASP816 ASP820
Berberine KCNMA1	C2	-7.7	1484	-11, -7, 31	28, 22, 22	GLN566 GLN990 ILE993 ASN998 ILE999 PRO1000 CYS1034 THR1036 ALA1104 LEU1106 ASP1107 TYR1138 THR1152 LYS1153 ARG1154 LEU1171
Berberrubine KCNMA1	C4	-8.5	1123	-13, 2, 40	22, 22, 22	ALA834 ARG913 ALA914 ASN916 ASN918 LEU919 PHE955 ASP956 ASP957 ILE959 GLY960 GLN963 SER992 ILE993
Berberrubine ROCK2	C2	-8.6	1358	-42, 8, 3	22, 22, 22	LEU53 LEU57 ASN65 ASN67 ILE68 PHE71 ARG74 TYR75 PHE403 LEU449 LEU452 LEU456 GLU459 MET460 LYS463
Jatrorrhizine ROCK2	C3	-8.1	1321	-6, -12, 5	30, 22, 32	ILE98 GLY99 ARG100 VAL106 ALA119 LYS121 VAL153 MET169 GLU170 MET172 ASP176 ASP218 ASN219 LEU221 ALA231 ASP232 PHE384 ASP385 ILE387
Jatrorrhizine DYRK1	C2	-9.3	1593	-12, -4, 6	22, 22, 22	ILE165 PHE170 VAL173 ALA186 LYS188 GLU203 VAL222 PHE238 MET240 LEU241 SER242 TYR243 ASN244 ASP247 GLU291 LEU294 VAL306 ASP307 VAL753 VAL755

Discussion

The results from this study indicate that berberine and its metabolites have a diverse range of protein targets, and the top three categories of targets are kinases, enzymes, and Family A G Protein-Coupled Receptors (GPCRs). This aligns with existing literature on the pharmacology of berberine and its metabolites [2,24]. The significant targeting of kinases by berberine and its metabolites aligns with previous research highlighting the importance of kinase inhibition as a major mechanism of action for berberine. Kinases play crucial roles in signal transduction pathways, and their inhibition can lead to various physiological effects, including anti-inflammatory [33], anticancer [34], and antidiabetic [35] properties. Among the kinases AMPK (AMP-activated protein kinase) is extensively reported to be the major target of berberine pharmacodynamics, as AMPK activation is considered a major mechanism by which berberine exerts its beneficial effects [36,37]. AMPK activation leads to increased glucose uptake, improved insulin sensitivity, and enhanced fatty acid oxidation, contributing to its potential antidiabetic and metabolic effects. However, the affinity analysis of berberine against various forms of human AMPK showed values ranging from 12 to 104 uM, which was much higher than the affinity of berberine and its metabolites against its high-affinity targets (ROCK2, PIK3CD, KCNMA1, CSF1R, and KIT), for which the affinity values were <4 uM. PRKAA2 showed the highest affinity of 12.03 uM, while the affinity with the rest of the AMPK's was >20 uM. Hence it is reasonable to assume that the activation of PRKAA2 regulates the influence of berberine on AMPK physiology, although the dose of berberine required to achieve this will be high. PRKAA2, which is highly expressed in kidneys, heart, and skeletal muscles, plays a crucial role in coordinating cellular responses to fluctuations in energy levels, making it a central player in maintaining cellular energy homeostasis and metabolic regulation. Its functions have implications for various metabolic diseases and potential therapeutic interventions for metabolic disorders, such as type 2 diabetes mellitus, obesity, and cardiovascular diseases [38,39].

Berberine is also reported to target various enzymes involved in cellular processes. For example, it has been reported to inhibit the enzyme dipeptidyl peptidase-4 (DPP4) [17], which plays a role in glucose regulation, and this could contribute to its antidiabetic effects. The regulation of other related targets may also contribute to the diverse therapeutic potential of berberine in metabolic and lipid disorders. Hence it wasn't surprising to find the top targets (LDLR, DPP4, and PCSK9) of berberine in the STITCH database were all associated with lipid regulation, which aligns with berberine's known effects on lipid metabolism [27,40]. However all these targets showed affinity values >10 uM, which is achievable only at higher doses of berberine. It may be likely that berberine underdoes significant bioconcentration (accumulation in tissue niche), which may account for its therapeutic benefits through influencing these high affinity targets. However, this remains to be validated in suitable in vivo studies. Despite low bioavailability, berberine is reported to have high tissue distribution. Following oral administration, the levels of berberine and its active metabolites in various organs are reported to be higher than in the bloodstream [2]. The organ distribution of berberine is specifically reported for the liver (hepatic metabolism), kidneys (renal excretion), skeletal muscle, lungs, brain, heart, and pancreas. In addition berberine is also reported to remain stable in adipose tissue for over 48-hours, which suggests a depot effect in fat/lipid tissue, influencing the pharmacokinetics and pharmacodynamics of berberine [2]. The observed organ distribution of berberine highlights its remarkable ability to reach various target sites throughout the body, potentially influencing multiple physiological processes [2]. Understanding the specific distribution patterns of berberine in different organs provides valuable insights into its pharmacological effects and lays the groundwork for optimizing its therapeutic applications in the treatment of various diseases. Further studies investigating the mechanisms and factors influencing the organ distribution of berberine are crucial for harnessing its full therapeutic potential and enhancing our understanding of its pharmacokinetic profile.

In contrast to the known and widely reported targets of berberine, in this study ROCK2, PIK3CD, KCNMA1, CSF1R, and KIT were identified as high affinity (<4 uM) targets of berberine and its metabolites. The targetability of these targets was further validated in this study by revealing the specific binding pockets of berberine or its metabolites on these targets at therapeutically feasible concentrations. ROCK2's involvement in actin cytoskeleton regulation and cell contractility makes it an essential player in various cellular activities, including cell migration, adhesion, proliferation, and tissue development. Dysregulation of ROCK2 has been associated with several diseases, making it a potential target for therapeutic interventions in conditions such as cancer, cardiovascular diseases, and neurological disorders [41-44]. The major pathological role of ROCK2 is its involvement in promoting various aspects of cancer progression and metastasis [42,43]. Aberrant activation or overexpression of ROCK2 has been associated with cancer development and is linked to several pathological processes in cancer cells. Hence, by inhibiting ROCK2, berberine has a vital role as an anticancer therapeutic. The major pathological role of PIK3CD is its association with immunodeficiency disorders caused by mutations in the PIK3CD gene [45,46]. These disorders are collectively known as "PIK3CD-related primary immunodeficiency" or "Activated PI3K Delta Syndrome" (APDS) [47,48]. APDS is characterized by dysregulation of the PI3K signaling pathway, leading to aberrant immune cell function and impaired immune responses. There are two types of APDS, APDS1 and APDS2, depending on the specific genetic mutation involved. The potential of berberine and its metabolites to inhibit PIK3CD with high affinity offers a viable option in treating APDS for which currently very limited precision medicine options are available.

KCNMA1, a high affinity target of berberine and its metabolites, is also known as BKCa (Big Potassium Calcium-Activated Channel), and is associated with various cardiovascular and neurological disorders [49,50]. KCNMA1 encodes the alpha-subunit of the BKCa channel, a large-conductance, calcium-activated potassium channel. Dysregulation or mutations in the KCNMA1 gene can lead to cardiovascular disorders such as hypertension, arrhythmias, and vasospasms and following neurological disorders, epilepsy, Parkinson's disease, and dementia [51]. Also, overexpression of BKCa channels has been associated with increased invasion and metastasis in certain types of cancer [52-54]. In addition abnormal BKCa channel function is also reported to be associated with gastrointestinal motility disorders and urinary incontinence. It remains to be established how berberine and its metabolites can be therapeutically beneficial for these clinical conditions directly associated with abnormal BKCa channel function.

CSF1R is a receptor tyrosine kinase that binds to Colony-Stimulating Factor 1 (CSF-1 or M-CSF), a cytokine that regulates the differentiation, survival, and function of macrophages and other myeloid cells. It is primarily associated with dysregulated immune responses and abnormal cell proliferation in various diseases. Dysregulation of CSF1R signalling has been implicated in various cancers, particularly those of myeloid cell origin [55,56]. Overexpression or activation of CSF1R in cancer cells or in the tumour microenvironment can lead to increased recruitment, proliferation, and survival of tumour-associated macrophages (TAMs) [57]. TAMs play a critical role in promoting tumour progression by creating an immunosuppressive microenvironment, promoting angiogenesis, and facilitating tumour invasion and metastasis. Targeting CSF1R signalling has emerged as a potential therapeutic strategy for cancer treatment and paraphs berberine will prove to be a valuable therapeutic as an anticancer agent due to its high affinity against CSF1R. CSF1R signalling is involved in the regulation of macrophage and monocyte functions, which play a central role in the immune response to infection and inflammation. Dysregulated CSF1R signalling can lead to excessive or aberrant activation of macrophages, contributing to chronic inflammatory conditions such as rheumatoid arthritis, atherosclerosis, unstable plaques, and inflammatory bowel diseases [58,59]. However, CSF1R is also involved in several physiological processes including differentiation and function of osteoclasts, microglia, macrophages and monocytes. Given the diverse roles of CSF1R in regulating immune responses, cell proliferation, and tissue homeostasis, its dysregulation can have far-reaching effects on various physiological processes. Understanding the pathological roles of CSF1R is crucial for the development of targeted therapies aimed at modulating its signalling pathways for therapeutic benefit in specific diseases.

KIT is a receptor tyrosine kinase that binds to stem cell factor (SCF), also known as KIT ligand. It plays essential role in regulating cell survival, proliferation, and differentiation of various cell types. The major pathological role of KIT is its association with several diseases, particularly cancer and certain haematological and gastrointestinal disorders [60,61]. Gastrointestinal Stromal Tumors (GIST) are the most common mesenchymal tumours of the gastrointestinal tract. The majority of GISTs have activating mutations in the KIT gene, leading to constitutive activation of the KIT receptor [62]. This abnormal activation drives uncontrolled cell proliferation and tumour growth. Although KIT inhibitors, such as imatinib [63], have shown significant clinical benefit for patients with GISTs, there remains a merit in the co-use of imatinib with berberine to achieve therapeutic synergy. Systemic mastocytosis is a rare disorder characterized by an abnormal accumulation of mast cells in various tissues and organs. The majority of cases of systemic mastocytosis have activating mutations in the KIT gene, leading to increased proliferation and survival of mast cells [64]. This results in the release of various mediators, causing symptoms such as skin rashes, itching, flushing, and potentially life-threatening allergic reactions. A similar response is also observed in cytokine release syndrome [65], and the role of KIT in this remains unknown. Nevertheless the higher affinity of berberine against KIT offers a value therapeutic option, which warrants to be investigated. Acute Myeloid Leukemia (AML), some melanomas and seminomas are reported to involve mutations in the KIT gene [66,67]. These mutations are associated with poor prognosis and resistance to standard chemotherapy. Targeting these KIT mutations with specific inhibitors is an area of ongoing research and berberine could be potentially useful.

The ADME analysis provides insight into the drug-like properties of berberine and its metabolites. Understanding these properties is crucial for assessing their potential for drug development. The reported physicochemical properties, solubility, and pharmacokinetic parameters are important factors in determining the drug-likeness of a compound, which were consistent with several studies reporting drug-likeness characteristics of berberine and its metabolites [68-70]. The drug-likeness of berberine and its metabolites is further validated by the binding affinity analysis and IC_{50} values which provide assurance about the strength of interaction between berberine and its metabolites with their high-affinity targets at therapeutically feasible concentrations.

In summary, this study while aligning well with existing literature on the pharmacology of berberine and its metabolites provides a novel insight into its potential mechanism of actions. The diverse range of protein targets and the observed high affinity targets (ROCK2, PIK3CD, KCNMA1, CSF1R and KIT) offer valuable insights into the potential mechanisms of action and therapeutic effects of berberine and its metabolites in various disease conditions, which warrants validation in suitable efficacy analysis studies. However, considering kinases being the major target category and cancer being the major therapeutic category of berberine, it appears that berberine is more of a natures Imatinib rather than Ozempic.

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