Review

Pharmacokinetic Profiles of Orally Bioavailable Natural Compounds for Integration into Biomedical Cancer Drug Discovery Research

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Pharmacokinetic Profiles of Orally Bioavailable Natural Compounds for Integration into Biomedical Cancer Drug Discovery Research

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Abstract

Oral drug delivery enhances patient compliance with treatment, as it is a non-invasive method of drug administration and reduces the need for travel to healthcare facilities. One approach to developing oral medications involves researching natural compounds. Numerous of these compounds have shown promising in vitro results. However, the first-pass effect after oral delivery extensively metabolises many of them, leading to poor oral bioavailability. In this article, the author reviews numerous natural compounds and identifies several that demonstrate at least 30% oral bioavailability, with at least 2 hours of half-life ($t_{1/2}$). These compounds include (1) decursinol, (2) trigonelline, (3) phenethyl isothiocyanate (PEITC), (4) theobromine, (5) pterostilbene, (6) rocaglamide, (7) sinomenine, (8) sulforaphane, (9) piperine, (10) carnosic acid, (11) niazirin, (12) falcarinol, (13) acetylcorynoline, (14) gastrodin, (15) bergapten, (16) imperatorin, (17) betaine, (18) kurarinone, (19) d-limonene, and (20) usnic acid. The pharmacokinetic profiles of the compounds are listed in this article. By examining the pharmacokinetic profiles such as concentration maximum (Cmax) and $t_{1/2}$ of the shortlisted compounds, researchers can design concentration to be used in in vitro experiments and predict/establish dosing regimens for preclinical studies. The researchers can incorporate these 20 orally bioavailable compounds as a screening panel to select the most suitable compound for their study. When combining a natural compound with standard chemotherapy, it is essential for researchers to thoroughly investigate potential toxicity effects, as some natural compounds are known to act as CYP3A4 inhibitors, which may influence the metabolism and clearance of chemotherapy drugs. The author believes that even more orally bioavailable compounds remain undiscovered. This article can guide researchers in their quest for orally bioavailable natural compounds. The use of proper orally bioavailable natural compounds in biomedical research is likely to encourage pharmaceutical companies to develop these compounds into viable treatments.

Keywords: concentration maximum **(**Cmax); drug discovery; half-life ($t_{1/2}$); metabolism; natural compounds; oral bioavailability; pharmacokinetics

Introduction

Preclinical and clinical trials are currently investigating a variety of natural compounds for their potential uses in various medical fields, including cancer treatment [1]. However, the successful therapeutic use of these natural compounds is often hindered by pharmacokinetic issues, particularly bioavailability. Bioavailability is a critical factor in assessing a compound's therapeutic efficacy, as it determines the proportion of the administered dosage that enters the bloodstream in an active form [2]. Administering a compound or drug via the oral route offers several advantages, primarily its non-invasive nature and enhanced patient compliance, as it necessitates less supervision and monitoring by medical professionals [3,4]. Unlike intravenous drug administration, which can achieve 100% bioavailability, drug delivery through the oral route often results in significantly lower

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oral bioavailability (Figure 1). This is due to the challenges associated with drug absorption and elimination during the first-pass metabolism in the liver as well as the ionic state of the compound when it interacts with the surrounding pH [5]. Consequently, pharmaceutical companies frequently refrain from developing medications with an oral bioavailability of less than 30% [6].

In laboratory/biomedical drug discovery research, many natural compounds have shown promising in vitro results but did not have good oral bioavailability in vivo as shown by Gao et al. [6]. For example, curcumin, berberine, genistein, quercetin, compound K, resveratrol, epigallocatechin gallate, ellagic acid, coumarin, phenolic acid, naringenin, daidzein, rutin, caffeic acid, baicalin, rosmarinic acid, hesperetin, apigenin, alliin and many more exhibit poor oral bioavailability [6,7]. Despite this limitation, they are frequently used in biomedical drug discovery research in pure form, even though numerous publications have highlighted their limited oral bioavailability. For example, an article published in 2024 reported 10 mg/kg berberine was significantly reduced the volume of breast cancer in female nude mice [8]. The absolute bioavailability of berberine at a dosage of 100 mg/kg in rodents was found to be 0.68% and 0.36% [9,10].

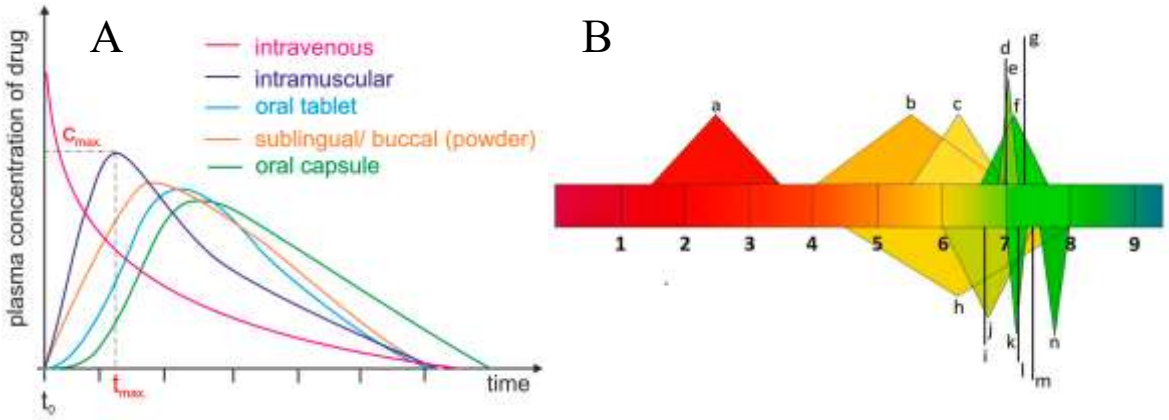

Figure 1. The plasma level time curves associated with different drug delivery methods (A), alongside the pH values of human bodily fluids (B). a) stomach; b) small intestine; c) large intestine; d) liver; e) muscle; f) uterus; g) testis; h) bladder; i) lungs; j) saliva; k) kidneys; l) brain, heart, and spleen; m) bone; n) pancreas. The ionic form of a drug, which varies with the pH of its surroundings, can influence its ability to permeate the membrane. The process of ionisation can significantly affect the absorption, distribution, metabolism, elimination, and associated toxicity of drugs in both animals and humans [5]. Drug absorption is more effective when the drug molecule is in its non-ionised form, as this form is significantly more lipophilic than its ionised counterpart [11]. The images were taken from Stielow et al. [5] which are covered by CC BY licence for unrestricted use.

The tumour size reduction was likely not associated with the pure form of berberine, but rather with the metabolites produced after the parent compound has been extensively metabolised [10]. It has been reported that 20 metabolites are produced in vivo following the metabolism of berberine in the body [12]. The author of this manuscript hypothesised that an increased dose of berberine would lead to a higher production of metabolites. If the anticancer activity is reliant on these metabolites rather than the well-studied parent compound (in vitro- 2D & 3D IC_{50} determination assays, migration & invasion assays, flow cytometry, western blots, testing on organoids/organ on chips, cell based 'omics' relates assays), it follows that a greater dose of berberine could result in more pronounced adverse effects and this is not limited to berberine alone. The safety profile of the metabolites has probably not been fully studied, although some natural compounds may perform well. Timofeeva et al. [12] noted that the precise mechanism underlying the antitumour effect of berberine remains unclear. Although the article reported numerous signalling pathways, these are primarily associated with berberine in cell culture conditions rather than its metabolites. This situation is further complicated by the presence of standard chemotherapy, as in some countries, it is not permitted to test investigational compounds alone on cancer patients when proven chemotherapy drugs are available. The interaction of these metabolites with standard chemotherapy or with the

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liver enzyme like CYP3A4 which is one of the major cytochromes P450s [13] may have either beneficial or detrimental effects. For example, metabolites with a CYP3A4 inhibitory effect are likely to influence the metabolism of chemotherapy drugs that rely on this enzyme [13]. In this article, the author has reviewed the literature and surveyed numerous of natural compounds, identifying several with notable oral bioavailability and favourable pharmacokinetic profiles in their pure forms. This strategy was taken because many compounds are not orally bioavailable and/or have very short half-lives, which are repeatedly used in their pure form for oral delivery in animals. The primary objective of this article is to inform the scientific community about the availability of natural compounds that exhibit an oral bioavailability of at least 30% with acceptable half-life (mainly depending on the concentration maximum-Cmax). The proposed integration of these compounds into advanced biomedical cancer research, with and/or without the inclusion of standard chemotherapy, aims to enhance the quality of research and publications while effectively utilizing public funding in a way that benefits patients. Consequently, with good study designs, this integration could possibly improve the success rate of translational research, which relies on the effects of the parent compounds. This approach could potentially encourage pharmaceutical companies to develop parent natural compounds as viable treatments for cancer patients.

In this article, two main keywords used to shortlist the natural compounds listed in Table 1 are (1) oral bioavailability in animals, with the availability of human data being an added advantage, and (2) $t_{1/2}$. The oral bioavailability was set as at least 30%, and the $t_{1/2}$ of at least 2h. In Table 1, twenty natural compounds are listed, which include (1) decursinol, (2) trigonelline, (3) phenethyl isothiocyanate (PEITC), (4) theobromine, (5) pterostilbene, (6) rocaglamide, (7) sinomenine, (8) sulforaphane, (9) piperine, (10) carnosic acid, (11) niazirin, (12) falcarinol, (13) acetylcorynoline, (14) gastrodin, (15) bergapten, (16) imperatorin, (17) betaine, (18) kurarinone, (19) d-limonene, and (20) usnic acid. Following the shortlisting of these compounds, pharmacokinetics-related information has been tabulated in Table 1, encompassing four main parameters: (1) Cmax; (2) half-life ($t_{1/2}$); (3) bioavailability (F); and (4) volume of distribution (V_d). The Cmax represents the maximum concentration of a compound that can be achieved in the plasma following oral administration. The term $t_{1/2}$ refers to the half-life, or the time required for the concentration to decrease to half of its initial value. For instance, if a compound's Cmax is 10 μM, the duration it takes for this concentration to reduce to 5 μM is referred to as $t_{1/2}$. The V_d denotes the volume of distribution, indicating how well the compound is distributed from the plasma to the tissue compartments [14,15]. The original reported Cmax values were converted to micromolar (μM) using a molarity and concentration calculators, which helps readers in interpreting the information more easily [16]. Having a high Cmax is advantageous, even though the half-life is short (approximately 3 hours), as the drug can be administered orally two, three, or four times per day. This approach also depends on the IC_{50} values of the cancer cell lines. For instance, if a particular cancer cell line exhibits an IC_{50} of 2 μM for the decursinol compound, researchers may opt to administer 20 mg/kg of decursinol four times daily (15.8 μM to 7.9 μM_{2h}; 7.9 to 3.95 μM_{4h}; 3.95 to 1.975 μM_{6h}) or 37.5 mg/kg as twice daily (43.3 μM to 21.65 μM_{3h}; 21.65 μM to 10.83$_{6h}$; 10.83 μM to 5.41 μM_{9h}; 5.41 μM to 2.71 μM_{12h}) if toxicity is not a concern. The V_d was 2.5 L/kg is considered high. The compound can be distributed from the plasma to the tissue compartments without significant issues. This scenario applies to all other compounds.

Table 1. Natural compounds with acceptable oral bioavailability and their pharmacokinetic profiles.

No	Compound	Dose (mg/kg)	Cmax** (μM)	$t_{1/2}$ (h)	*F* (%)	V_d (L/Kg)	Animal	Ref
1	Decursinol	5	5.81	0.74	45.1		Male SD rats	[17]
		10	18.52	0.87	66.6	NA		
		20	15.8	2.07	81.0			
		37.5	43.3	~3*	NA	2.5	Male CD rats	[18]

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2	Trigonelline	10	89.98	3.6	57.37	NA	Male SD rats	[19]
		10	78.59	NA	NA	0.778$_{V1}$/ 0.851$_{V2}$	Wistar rats	[20]
3	PEITC	0.5 (single)	1.95	2.62	77	1.53	Male Wistar rats	[21]
		1.0 (single)	2.37	2.78	61	1.75		
		5.0 (single)	4.71	3.19	23	2.15		
		0.5 (repeated)	2.47	2.55	83	1.50		
		1.0 (repeated)	3.96	1.86	86	1.05		
		5.0 (repeated)	13.10	1.62	43	0.88		
		40 mg (single)	2.19	1.75	NA	1.13	Humans	[22]
		40 mg (repeated)	3.01	1.88		0.78		
4	Theobromine	5	44.96	4.96	NA	0.716	Pregnant SD rats	[23]
		10	84.37	4.83		0.735		
		50	404.64	7.51		0.749		
		100	929.17	5.88		0.598		
		5	41.63	5.27	NA	0.685	Non-pregnant SD rats	[23]
		10	73.82	4.38		0.694		
		50	444.60	6.00		0.632		
		100	941.94	5.98		0.589		
		4$^{\#}$ (240 mg)	31.64	7.1	NA	0.62	Humans (nursing women)	[24]
		10	6.66$^{Day\ 15^{\wedge}}$	NA	NA	NA	Male rabbits	[25]
		100	58.45$^{Day\ 15^{\wedge}}$					
		6 solid	35.69	9	80.67	0.783	Human (Men)	[26]
		6 liquid	49.23	10.17	100	0.72		
		8.33 (500 mg)	52.18$^{1st\ day}$ 122.11$^{7th\ day}$	13.0 13.4	NA	NA	Human (Men)	[27]
5	Pterostilbene	56 $^{1\ dose}$	11.00	1.5	66.9	NA	Male CD rats	[28]
		56 $^{14\ doses}$	9.95	1.6	73.2			
		168 $^{1\ dose}$	30.75	1.9	94.2			
		168 $^{14\ doses}$	21.69	1.9	80.8			

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		14	39.41	1.7	11.9	4.9	Normal	
		28	85.06	1.46	13.9	3.76	C57/BL6mice	
		56	127.58	0.95	26.4	1.57		
								[29]
		14	67.89	0.73	20.7	1.16	C57/BL6 mice	
		28	108.08	1.69	21.2	1.40	with cancer	
		56	269.22	0.86	26.7	1.51		
		22.5	NA	NA	35	NA	Male Wistar rats	[30]
		15	0.29		<5.5			
		Fasting/suspension	0.92		15.9			
		15 Suspension	1.62	NA	59.2	NA	Male SD rats	[31]
		15 Solution	4.05		105.8			
		30 Solution	6.43		115.9			
		60 Solution						
		25 Single	2.96	NA	NA	NA	Male SD rats	[32]
		25 8 doses	2.44					
6	Rocaglamide	5[+]	~0.8	2.4	50	NA	NSG mice	[33]
7	Sinomenine	90	42.20	5.54	79.6	17.07	Male SD rats	[34]
		1.33 (80 mg)	0.750	9.4	NA	NA	Human	[35]
		30	15.89	4.88	NA	NA	Male Wistar	[36]
		60	35.16	5.77			rats	
		50	8.71	3.27	NA	NA	Rabbits	[37]
		25 male***	5.98	1.27	NA	13.90	Both sexes of	[38]
		25 female	8.91	1.78		7.6	SD rats	
8	Sulforaphane	0.5	0.262	62.2	82.4	95	Male Wistar	[39]
		1	0.474	50.5	51.8	77	rats	
		5	1.19	27.3	21.0	42		
		0.1	0.203	0.7	77.8		Male SD rats	[40]
		0.2	0.367	3.3	72.4	NA		
		0.5	0.807	5.33	251.4			
		26.6[$]	15.2[1.5h++]				Male	[41]
			5.6[6h++]	NA	NA	NA	F344/NHsd	
			0.5[24h++]				rats	
9	Piperine	35	5.43	2.26	NA	NA	Male SD rats	[42]
		20	9.92	~2.5[&]	NA	NA	Rats	[43]
		50	5.81	2.6	NA	NA	Male Wistar	[44]
							rats	
		54.1	15.04	4.1	NA	NA	Male SD rats	[45]
		10	3.44	1.22	NA	4.692	Male Wistar	[46]
							rats	

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		20	3.77	1.27	23.2	5.078	Swiss albino mice	[47]
		20 mg 1 dose	1.02	13.3	NA	NA	Human	[48]
		20 mg 7 doses	2.10	15.8	NA	NA		
10	Carnosic acid^^	64.3	105.29 39.11 24th h	NA	40.1	NA	Male SD rats	[49]
		90	127.91 27.07 24th h	16<	65.09	3.23	Male SD rats	[50]
11	Niazirin	5	1.363	3.36	46.78	28.98		
		20	4.308	3.15	52.61	25.20	Male SD rats	[51]
		40	9.236	2.74	48.28	22.91		
12	Falcarinol	20	~6.55	5.89	50.4	NA	CD-1 mice	[58]
13	Acetylcoryno line	20	470.1 ng/ml\	2.7	58.9	NA	mice	[59]
14	Gastrodin	200 mg	5.184	6.06	NA	NA	Human	[62]
		100	156.63	1.13	NA	NA	SD rats	[63]
		40	75.80	2.81	40.8	NA	Male Wistar rats	[64]
		40	80.34	4.8	NA	1.57	Dog	[65]
15	Bergapten	5	3.975	6-7*	80.1	NA		
		10	6.462	6-8*	94	NA	Male SD rats	[67]
		15	6.046	7-9*	69.5	NA		
16	Imperatorin	6.25	0.137	1.46	3.85	6.617		
		12.5	2.124	6.01	33.51	10.237	SD rats	[68]
		25	3.922	7.901	34.76	16.017		
17	Betaine	50 (3 g/60kg)	939	14.38	NA	1.324	Human	[75]
		1 g	284	25.92				
		3 g	599	19.95	NA	NA	Human	[76]
		6 g	1015	12.98				
18	Kurarinone	20	0.206	4.56	38.19	NA	Dog	[78]
19	d-limonene	200	82.948	5.62	43	NA	Male SD rats	[79]
20	Usnic acid+^	20	94.389	18.9	77.8	NA	Rabbit	[80]

^=not Cmax but concentration 24h after last dose; #=manually calculated based on 60 Kg/adult; *=manually calculated to approximate value; **=calculated with molarity and concentration calculators [16]; += Maximum tolerated dose (MTD) in mice was 1.2 mg/kg but for unknown reason the researchers used 5 mg/kg which could be toxic if given for several days; ***=Sinomenine hydrochloride & LD_{50} value differences between male and female SD rats; ++=not Cmax but the concentration of sulforaphane at 1.5 h, 6th h and 24th h after administered with 26.6 mg/kg sulforaphane; $=Converted 150μmol/kg manually to mg/kg; &= manually calculated from the figure; <=substantial standard deviation; ^^=oral lethal dose (LD_{50}) was 7100 mg/kg [52]; *F*=Oral bioavailability; \= The conversion to μM could not be performed due to the unavailability of the molecular weight; +^= could not access the original article.

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When a natural compound is intended to be administered alongside standard chemotherapy, in vivo dose optimisation is essential due to the potential for toxicity to arise. Given that the compounds listed in Table 1 undergo only minor first-pass metabolism in the liver, most of the compound will enter the circulation, allowing for in vitro studies to be designed prior to conducting in vivo experiments. The selected concentration of the compound must align with its pharmacokinetics. The availability of human pharmacokinetics is advantageous and should be leveraged, as the drug will ultimately be used in humans. For instance, the Cmax of theobromine was observed to be 122.11 μM following a daily dose of 500 mg, with a $t_{1/2}$ of 13.4 hours in humans. If the IC_{50} of theobromine is 30 μM, in vitro studies can focus on this concentration for subsequent experiments and can be designed for once-daily dosing in vivo. In vitro, the 30 μM concentration may be combined with standard chemotherapy. For example, in the case of nasopharyngeal carcinoma, the standard chemotherapy regimen consists of cisplatin and gemcitabine. The methodology for designing the experiment is clearly outlined in Manoharan [53].

Another approach, if the IC_{50} value is not determined, is for researchers to utilise Cmax and $t_{1/2}$ values to establish a working concentration for in vitro studies. For instance, if the researchers intend to design a dosing frequency for trigonelline to be administered twice daily, the in vitro concentration can be approximately 7 μM. This is derived from the following calculations: 89.98 μM to 44.99 $\mu M_{3.6h}$; 44.99 μM to 22.50 $\mu M_{7.2h}$; 22.50 μM to 11.25 $\mu M_{10.8h}$; and 11.25 μM to 5.63 $\mu M_{14.4h}$. This is despite the IC50 value (if determined) indicating a value greater than 7 μM, such as 30 μM. Typically, this method can be applied in combination studies alongside standard chemotherapy. Additionally, if theobromine, trigonelline or other compounds are intended for use as either a neoadjuvant or adjuvant therapy, this process is clearly outlined in Manoharan [53], as the current article serves as an extended version of Manoharan [53]. It is recommended that readers refer to Manoharan [53], which is a preprint version that has already been accepted for publication in *Methods and Protocols* (manuscript ID: mps-3965433). The author discusses how to design biomedical cancer research to enhance translational success. When planning to combine a natural compound with standard chemotherapy in mouse models, the study should be designed to closely resemble the treatment protocols experienced by patients in hospitals. Since the compounds listed in Table 1 are intended for oral delivery, they should be administered orally and not through alternative routes. A systematic review has revealed that several studies have administered pyrimethamine (a repurposed non-natural compound but with good oral bioavailability and safety profiles), which is designed for oral delivery, via other routes such as intraperitoneal injection [54]. If the standard chemotherapy is approved for intravenous delivery, it must be administered intravenously and not via the intraperitoneal route. Furthermore, the frequency of drug administration must align with the approved guidelines for human use. For the treatment of nasopharyngeal carcinoma, cisplatin is administered on Day 1, while gemcitabine is given on both Day 1 and Day 8 of each cycle, with each cycle lasting 21 days. The same protocol should be employed in mouse models, although differences in pharmacokinetics related to species may arise. The process of designing trials that require the harmonisation of data from different species and their pharmacokinetic profiles is quite challenging [53].

During the search for orally bioavailable compounds, the author identified **fisetin** as one of them; however, it was not listed in Table 1. This omission occurred despite its 31.7% oral bioavailability at a 200 mg/kg dose in ICR male mice (Cmax = 37.032 μM; $t_{1/2}$ = 0.97 h) [55]. The Cmax data for orally administered unformulated fisetin at 1000 mg in humans only reached 35 nM/0.035 μM [56]. Currently, fisetin is available as a dietary formulation, tested in humans at a dosage of 20 mg/kg/day for up to two consecutive months [57], which is equivalent to 1200 mg for a 60 kg adult. **Alisol B 23-acetate**, another natural compound, exhibited a Cmax of 19.213 μM and 44.16% oral bioavailability when administered at a dose of 6 mg/kg, but it had a half-life ($t_{1/2}$) of 0.98 h [60]. Despite a good oral bioavailability of 65% after 10 mg/kg oral administration in rats, **arbutin** experienced a short half-life of 0.42 h [61]. **Piplartine** demonstrated an oral bioavailability of 76.4% (5 mg/kg-Cmax of 2.787 μM), but its half-life was 1.42 h [66]. Single oral delivery of 10 mg/kg **yohimbine** in human

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produced mean oral bioavailability of 33% but the $t_{1/2}$ was 0.58h [69]. **Chebulinic acid** has been reported to exhibit an oral bioavailability of 37.56% following the administration of 100 mg/kg of the compound in male SD rats [72]. However, the compound has a molecular weight of 956.680 g/mol, which is considered relatively large for drug development [73]. Biological space is limited, whereas chemical space is infinite. Given this limitation in biological space, the compound being studied is anticipated to be a small molecule with a molecular weight cut off 500 [73]. Although a cut-off of 500 is preferred, docetaxel, which is derived from a natural product and has a molecular weight of 807.89 Dalton (or in g/mol), is currently used as an intravenous chemotherapy drug for cancer patients [74]. **Arnicolide C**, **arnicolide D**, **brevilin A**, **microhelenin C** from *Centipeda minima* extract are predicted to have good oral bioavailability [77].

In this article, the author emphasises that when a natural compound undergoes extensive metabolism and differs from its parent compound in circulation, researchers should conduct direct testing in a small batch of preclinical models instead of relying solely on in vitro testing to derive conclusion. This recommendation stems from the likelihood that in vitro results may not accurately reflect the compound's activity in animal models. For example, the in vitro western blot data relating to the compound indole-3-carbinol (I3C) may not represent its in vivo effects, as I3C is extensively metabolised in the acidic environment of the stomach, resulting in the production of diindolylmethane (DIM) following oral administration [70], unless a co-culture model is used. For example, if 1 μL of I3C stock is required for addition to a cell culture plate containing 2D cells with media at pH 7.4, the researcher might consider adding 1 μL of this stock to several microlitres of media adjusted to pH 2 preferably with the presence of stomach enzyme like pepsin, incubating it at 37 °C, and subsequently adding it to the cell culture plate [71]. This is just a recommendation, and optimisation is certainly necessary, particularly to determine the duration required for I3C to be metabolised and to confirm whether DIM is the actual main metabolite. Gómez-García et al. [82] have described the simulated digestion processes of the mouth, stomach, and gut, which are valuable for in vitro experiments. Natural compounds that undergo less metabolism after oral delivery can be properly evaluated through in vitro tests.

The similar scenario for in silico investigation involving natural compounds. Current molecular docking analyses focus solely on the parent compounds. For instance, if researchers utilize I3C as a ligand to inhibit BCL3, this method examines only the interaction between I3C and BCL3. However, when administered to animals or humans, I3C is metabolized to DIM. Evaluating a prodrug without considering its metabolites is inappropriate and potentially hazardous. Even if researchers can model the prodrug and its known metabolites, numerous other unknown metabolites may be overlooked in in silico experiments. It has been demonstrated that many metabolites are identified following the extensive metabolism of apigenin [83]. While it is possible to model these metabolites against BCL3 or any other targets, this approach becomes complicated, especially when considering toxicological profiles if more than one drug is present. Additionally, regulatory requirements in certain countries require that an investigational drug cannot be tested on humans if a proven chemotherapy drug is available [53]. For nasopharyngeal carcinoma, the standard chemotherapy consists of cisplatin and gemcitabine. The situation becomes even more complicated when considering the interactions of this three-drug combination and their related metabolites, which not only interact with BCL3 but may also engage with numerous other targets. This complexity makes it extremely challenging, if not impossible, to model these interactions through in silico approaches. Furthermore, it is uncertain what metabolites will be produced by less-studied or new compounds and drug combinations, which adds another layer of complexity. It is undeniable that in silico methods have contributed to drug development. For example, baricitinib, a drug for rheumatoid arthritis, was identified through an in-silico approach and later repurposed for the treatment of COVID-19. A meta-analysis revealed five phase 3 clinical trials with low risk of bias, involving a total of 3,944 participants, which demonstrated that the drug statistically significantly reduced mortality in hospitalized COVID-19 patients, with 0% heterogeneity [84]. It is important to note that in silico lead optimization typically targets binding energies between -12 and -16 kcal/mol, corresponding to a nanomolar to picomolar binding affinity

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range [85]. This approach aims to prevent misleading data from affecting the scientific community or general readers. Moreover, it enables the wise allocation of taxpayers' money towards high-quality scientific research. It is recognised that not all research institutions or universities have animal facilities, especially those capable of housing immunodeficient animals. In such cases, researchers should pursue collaboration with other institutes that can support these studies.

Selecting a natural compound with a favourable pharmacokinetic profile is crucial. Researchers have the responsibility to enhance the success rate of translational studies. The current failure rate in translating preclinical results into actionable translational research is 92% [86]. Over the past 30 years, 95% of attempts to develop new cancer drugs have failed, and only 30% of the drugs that are approved extend patients' lives by more than 2.5 months [87]. The likelihood of approval (LOA) for new cancer medications stands at only 5.1%, compared to the overall average of 9.6%. Among cancer treatments, haematology has the highest success rate at 26.1%. Additionally, hematologic cancer drug initiatives have a 50% greater chance of receiving approval than programs focused on solid tumours [88,89]. Begley and Ellis, [90] in their comment in Nature, highlighted that one significant reason for the failures observed in clinical studies is the quality of the preclinical data. Quality of preclinical data related to the quality of the preclinical study designs [91]. If cisplatin plus gemcitabine is administered intravenously in a hospital setting, the same route should be utilized in animal studies. Although administering drugs intravenously in animals poses technical challenges, this difficulty cannot justify substituting the intravenous route with the intraperitoneal route, which is rarely used in clinical practice. Intravenous and intraperitoneal routes are distinct methods of administration. This issue frequently arises in preclinical studies, ultimately compromising the quality of the overall results [53]. Many preclinical studies have not adhered to established guidelines. Adhering to these guidelines will offer clearer direction in preclinical research, particularly regarding the transparent conduct of work and the generation and reporting of results [53]. All preclinical studies must adhere to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, established in 2010. These guidelines aim to assist preclinical scientists in enhancing study designs by incorporating critical parameters such as appropriate sample sizes, randomization, blinding techniques, and transparent reporting of results from animal studies [92]. Drug testing on animals is still regarded as the gold standard, even though this method may exhibit variability in outcomes due to differences between species [93,94]. It is also noted that test findings vary even when rats of the same strain are bought from various vendors [93]. Although reproducibility in animal studies is also an issue to a certain extent where reproducibility level drops under 50% is considered as reproducibility crisis [95], it is important that, with good study designs, toxicology profiles are established in animal models before advancing to human trials. This process not only provides confidence in moving forward with clinical trials but also encourages participation from human subjects, particularly in the early phases of clinical trials, which are crucial for drug development.

The researchers can incorporate the 20 orally bioavailable compounds listed in Table 1 as a screening panel to select the most suitable compound for their respective cancer research. It is important to note that rocaglamide is expected to produce an IC_{50} in the nanomolar range, while the other compounds are likely to be in the micromolar range. This distinction is crucial, as rocaglamide may emerge as the most effective compound if these 20 natural compounds are used. In Table 1, the author indicates that the dose of rocaglamide used for the pharmacokinetic study in NSG mice was 5 mg/kg, whereas the maximum tolerated dose (MTD) was only 1.2 mg/kg. It is unclear why the researchers from the original article opted for a dose of 5 mg/kg, which could pose toxicity risks with repeated dosing, despite the established MTD of 1.2 mg/kg. At 5 mg/kg, rocaglamide maintained a steady concentration of 20-50 nM, which is appropriate for once-daily dosing. However, given that the MTD was 1.2 mg/kg, the author questions whether a similar concentration can be achieved in circulation. It could potentially be four times lower than reported, possibly falling into the single digit nanomolar range. Researchers should be mindful of this dosing discrepancy to inform their work. It is advisable to use single digit nanomolar concentration when conducting in vitro tests, despite the IC_{50} being 50 nM. For instance, when testing rocaglamide alone or in combination with standard

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chemotherapy, researchers may consider using rocaglamide in the range of 5 μM to 8 μM. It is advisable to establish a specific MTD value when using rocaglamide for testing in mice. This recommendation arises from the fact that, in the same study, the researchers used a 20% DMSO solution in saline for the pharmacokinetic analysis. However, for the subsequent experiments, they used the MTD value, with rocaglamide being dissolved in a 30% hydroxypropyl-β-cyclodextrin (HPβCD) solution. This indicates a lack of consistency in the study design [33]. The oral administration of a free compound (rocaglamide + DMSO + saline) and one with a specific delivery system (rocaglamide + HPβCD) could yield different results. The percentage of HPβCD will also influence the outcome [81]. The author of this manuscript has considerable experience in administering rocaglamide orally in NSG mice.

In conclusion, there are several significant orally bioavailable parent natural compounds among the thousands of natural compounds that are either non-orally bioavailable or extensively metabolised by first-pass metabolism. This article highlights several of these compounds, and the author believes that there may be many other valuable compounds concealed within the vast array of natural substances. The author invites the scientific community working on natural compounds to expand Table 1 by including additional meaningful orally bioavailable natural compounds. This expansion aims to assist more researchers in conducting more meaningful research. Unlike extensively metabolised compounds, in vitro data generated from bioactive parent natural compounds perhaps can be translated into preclinical animal studies, as these compounds are not only less metabolised but also orally bioavailable. This article has the potential to save researchers time in their quest for appreciable orally bioavailable natural compounds.

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