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Research Article

Integrated Computational Analysis of Physicochemical Features, Biological Properties, Kinase Target Prediction and Biotransformation Pathways in Drug Discovery

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Abstract

Background and Objective: Recently, many scientific teams have been able to elucidate the structural and functional aspects of highly complex macromolecules due to the significant contribution of highly sophisticated bioinformatics platforms, representing these days a primordial steps in drug design and development processes. **Materials and Methods:** In this context, this work aimed to investigate in depth some fundamental properties of four bio-compounds, using diverse computational approaches but also to reveal the distribution of these compounds across kinase families and their possible biotransformation pathways. **Results:** The degree of polarity of compound 1 was the most important among the four tested compounds, while compound 2 exhibited the best natural product-likeness property score (P=1). Concerning metabolism part, data revealed that almost all tested compounds could be considered as non-substrate and non-inhibitor for cytochrome P450 isoforms, but could be effective to target key kinases, especially the class of serine/threonine-protein kinase. The hepatoprotective effect of compound 3 was considerably high (Pa=0.902), while compound 4 could be suitable to inhibit testosterone 17 beta-dehydrogenase ($NADP^+$) (Pa=0.912). **Conclusion:** Both experimental and bioinformatics analysis are mandatory in this era to characterize the full potential of molecules, providing advanced research in modern biology.

Key words: Bio-compounds, bioinformatics platforms, biotransformation, fundamental properties, kinases

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INTRODUCTION

The prosperity of a society has always been linked to scientific and technological progress and scientists agree to say that all disciplines are complementary to each other to understand more our surrounding environment, to treat complex data but also to resolve and overcome many problems related to our daily life[1]. As an example, technology-based interventions allowed scientists to improve the nutritional quality of our food's daily consumption, especially in minerals and protein, which has been recently proven using plant-based milk analogs[2]. Moreover, this technological innovation allowed also the elaboration of more effective clinical protocols to face and overcome pandemic situation such as COVID-19[3], by providing more targeted approaches and prevention strategies.

Among these disciplines, bioinformatics is playing a crucial role due to the fact that these innovative methods can give a coherent and accurate prediction due to their accurate algorithms. As an example, simulations approaches allowed scientists to analyze a vast expanse of sequenced genomes, which helped geneticists to identify a potential molecular biomarkers, especially those related to metabolic and depressive disorders[4]. Bioinformatics allowed also the scientific community to create a common database repository platform in order to share crucial informations related to the physico-chemical, pharmacokinetics and molecular aspects of chemicals[5], providing an important gain of time, a constructive collaboration and thus serving the research community at the international level.

These innovative simulation techniques had also a positive impact on the sectors of applied microbiology and biotechnology by allowing scientists to better understand the bioremediation of soils by biological means, especially the various ways of biodegradation of chemicals, such as organic materials by microorganisms including bacteria, fungi or algae[6]. This has also allowed over time to develop sustainable management techniques to properly use macro-or microplastics from biodegradable plastics and thus to minimize the environmental impact of harmful products[7].

At the internal level, human gut host more than 100 trillion of microorganisms, in which most of them live in colon and ensure crucial metabolic functions via enzymatic systems[8]. For example, this flora allow a coherent metabolism process of xenobiotic, shifting their pharmacokinetic and pharmacodynamic characteristics in order to synthesis active metabolites[9,10], which allowed scientists to develop innovative and efficient techniques to eliminate toxic chemicals and thus to clean up industrial, agricultural or military sites[11]. Let's not forget the impact of bioinformatics on enzymology, in which this discipline offered an accurate modeling process of kinase-substrate relationships but also to analyze generated data in a reproducible and robust manner to better understand protein kinases regulatory network, which allowed clinicians to learn more about kinase signaling pathway in dendritic development associated with neuropsychiatric diseases[12,13].

Our previous research on the plant *Centaurea tougourensis* revealed the pharmacological potential of this species. Indeed, various *in vitro* and *in vivo* techniques were performed and allowed us to characterize the antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, anti-ulcer, neuroprotective, haemostatic, wound healing and antimicrobial capacities of the n-butanol and ethyl acetate fractions of this plant[14-18]. In addition, the various phytochemical screening, principally made by GC-MS and HPLC allowed our team to identify a large number of phyto-compounds, which could be responsible of these pharmacological effects[17,19]. However, the physico-chemical and biological aspects of most of these bio-compounds was not determined yet, which explain the necessity of this work.

In this study, we preferred to investigate various aspects of compounds using computational intelligence (CI) methods instead of regular traditional laboratory techniques, since the algorithmic system integrated in each used server allow to sift through enormous amounts of biological data[5]. In this context, several accredited bioinformatics tools used in this study such as SwissADME, AVCpred, DIGEP Pred as well as new introduced platforms such as KinScreen and BioTransformer 3.0, whose allowed the resolution of the structuro-functional aspects of many compounds[20-29], were used in this work in order to predict for the first time some physico-chemical features, natural product-likeness characteristics, pharmacokinetics, pharmacological aspect, antimicrobial property, gene expression profiles, kinase targets and biotransformation pathways of *Centaurea tougourensis* bioactive compounds to reveal the full potential of these phyto-compounds and thus to make the initial steps before pre-clinical research.

MATERIALS AND METHODS

Phyto-compounds origin: In the present work, some fundamental properties has been predicted and investigated in depth using 4 compounds that were previously determined by our team via GC-MS from the species *C. tougourensis* (voucher specimen: CT/2019/LPTPCMB)[19]. Indeed, the physicochemical, biological properties as well as possible biotransformation pathways of these compounds were studied. These compounds are, respectively; 2,5-Monoformal-l-rhamnitol (compound 1), Cholest-7-en-3,beta,5,alpha,-diol-6,alpha,-benzoate (compound 2), 7,8-epoxylanostan-11-ol, 3-acetoxy- (compound 3) and 1H-pyrrole-2,5-dione, 3-ethyl-4-methyl-(compound 4).

Computational study

Physicochemical and natural product-likeness characteristics: The intrinsic physical and chemical characteristics of each tested compound has been predicted via SwissADME web-service (http://www.swissadme.ch/, accessed on 11 January, 2023). For this purpose, 6 parameters were investigated and noting that the colored zone in the figure, indicate the suitable physicochemical space for oral bioavailability[20]. In addition, the natural product-likeness characteristics of each compound was estimated using a new machine learning approach integrated in NP-Scout system, developed by the Computational Drug Discovery and Design team from the University of Vienna (https://nerdd.univie.ac.at/npscout/, accessed on 13 January, 2023).

Pharmacokinetics profile: The ADMET properties is considered a crucial throughout drug design process and noted for the failure of approximately 60% of all phases of clinical research[21]. In this context, the pharmacokinetics and toxicity of each tested compound has been predicted using admetSAR 2.0 interface (http://lmmd.ecust.edu.cn/admetsar2, accessed on 19 January, 2023)[22]. Noting that other important ADMET parameters (CYP inhibitory promiscuity, UGT catalyzed, nephrotoxicity, reproductive toxicity, respiratory toxicity, mitochondrial toxicity, skin sensitisation, binding process with PPAR gamma and aromatase receptors) were recently added in the platform system, which allowed us to investigate these compounds it in depth to enrich the quality of this work.

Pharmacological properties: More than 4000 types of biological effects can be predicted with the online platform of PASS online[23]. In this context, the database of this server was used to allow the prediction of bioactivity score of the four studied compounds (http://way2drug.com/passonline/, accessed on 25 January 2023).

Antiviral, antibacterial and antifungal properties prediction: In order to explore these parameters, several platforms were used as follow; AVCpred to predict the antiviral activity of our compounds against human viruses, based on ChEMBL database (http://crdd.osdd.net/servers/avcpred/, accessed on 3 March, 2023)[24], antiBac-Pred for the antibacterial capacity on more than 353 bacteria (http://www.way2drug.com/antibac/, accessed on 7 February, 2023)[25] and MICF for the antifungal effect on 38 fungi (http://www.way2drug.com/micF/, accessed on 9 March, 2023)[26]. Noting that these servers generate informations based on ChEMBL database.

Kinase targets prediction: Protein kinases contribute to the regulation of almost all aspects of cell function and represents a particular interest as therapeutic targets. However, kinase may also be responsible of pathological state and disease manifestation at the organismal level[27]. For this purpose, KinScreen, a new platform has been used to calculate the probability of our compounds to target various classes of kinases. This webserver allow also the visualization of results in the form of a mapped kinome tree (https://www.way2drug.com/KinScreen/, accessed on 12 February, 2023).

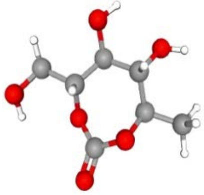
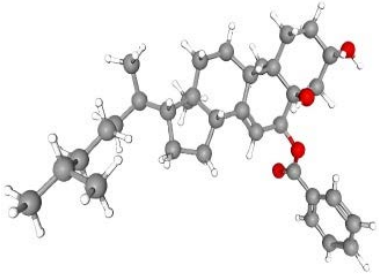
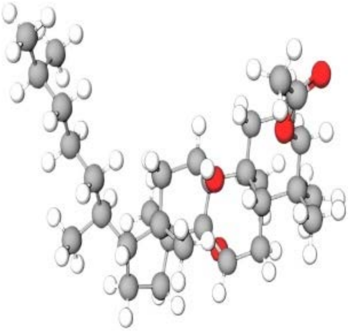
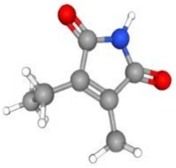
Gene expression profiles: The possible influence of our compounds to induce changes in gene expression profiles was investigated using DIGEP Pred web service (http://www.way2drug.com/ge/, accessed on 17 February, 2023) and thus to understand the upregulation or downregulation processes of selected genes[28].

Xenobiotics biotransformation (human gut microbial) pathways prediction: For this test, BioTransformer 3.0, a newly Canadian server was used to predict the plausible human gut microbial enzyme-catalysed reaction pathways of our compounds. This server use metabolic transformation module including both knowledge-based approach and machine learning based approach to displays all predicted reaction pathways of transformed compounds[29] (https://biotransformer.ca/, accessed on 25 February, 2023). Noting that this platform; use the same approach and accuracy as EAWAG Pathway Prediction system.

RESULTS AND DISCUSSION

Canonical format extraction: In order to correctly predict the properties of particular chemicals, the use of canonicalization algorithm is mandatory to generate the respective canonical SMILE of compound. Table 1 reports all details about the molecular formula, structure, PubChem Identifier as well as canonical smiles of each targeted compound.

Table 1: Structural characteristics of each selected compound

Extract	Compound	Molecular formula	3D structure	PubChem identifier	Canonical smiles
n-BuOH	1	$C_7H_{12}O_6$		552228	CC1C(C(C(OC(=O)O1)CO)O)O
	2	$C_{34}H_{50}O_4$		91703478	CC(C)CCCC(C)C1CCC2C1(CCC3C2=CC(C4(C3(CCC(C4)O)C)O)OC(=O)C5=CC=CC=C5)C
EA	3	$C_{32}H_{54}O_4$		541562	CC(C)CCCC(C)C1CCC2(C1(CC(C3C24C(O4)CC5C3(CCC(C5(C)C)OC(=O)C)C)O)C)C
	4	$C_7H_9NO_2$		29995	CCC1=C(C(=O)NC1=O)C

Physicochemical and natural product-likeness characteristics: The determination of physicochemical parameters is fundamental to characterize and optimize the structure of candidate compounds or to adjust their composition in order to obtain the desired functionality[30]. These properties help also to anticipate formulation process but also to measure the performance of specific compound in a reaction mixture[31]. As represented in Fig. 1, the proportion of unsaturation of compound 4 was considered the best, suggesting a higher binding affinity in cyclic hydrocarbons and thus giving addition reactions[32]. That's why compounds which possesses this property are qualified more reactive and are usually involved in many industrial processes, especially for the production of pesticides, paints, fuel[33], but also as a basis for drug design and synthesis processes[34].

However, in term of polarity, compound 1 exhibited better result and this parameter is crucial in pharmacology. Indeed, it was reported that polar drugs or their respective metabolites can be filtered and excreted in appreciable amounts by kidneys than non-polar drugs[35]. The fact that polarity confers to molecules the capacity to be absorbed across gut wall by transcytosis, thus may partially explain why highly polar oral drugs are often used as antibiotics for the treatment of gastrointestinal infections[36].

On the other hand, the insolubility, lipophilicity and flexibility of compounds 2 and 3 was considered the most important among the four tested compounds, which mean that these two compounds could potentially affect lipid-soluble drugs transport through lipid structures, in positive way[37], but also a considerable impact on binding and

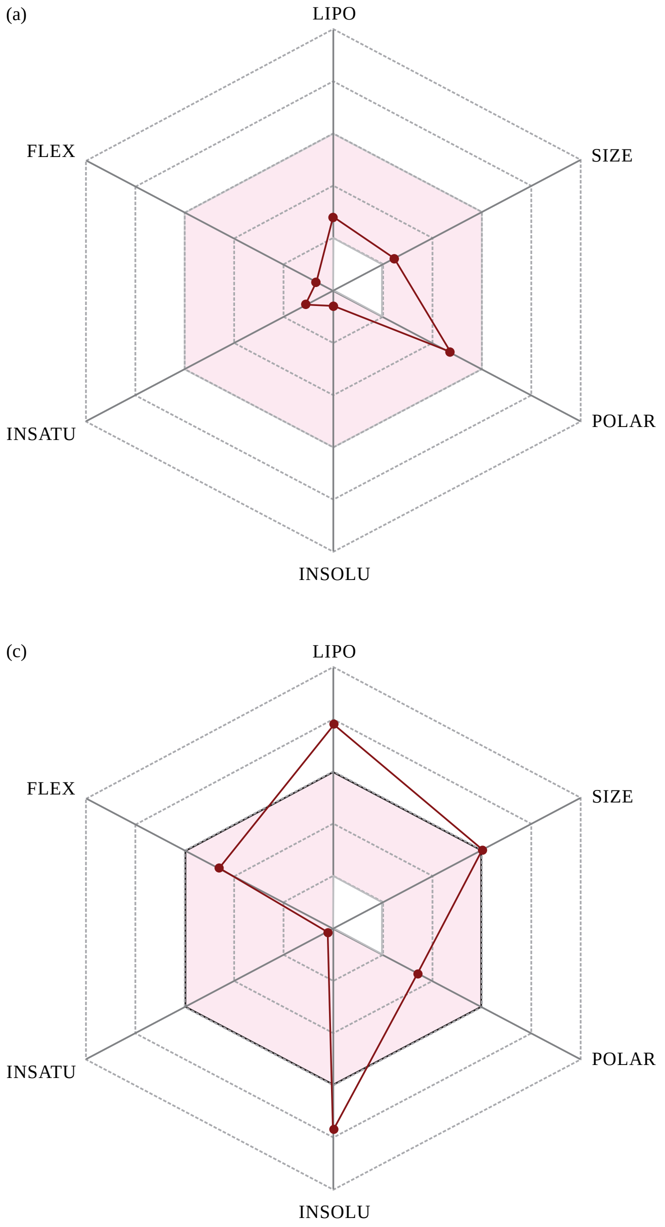
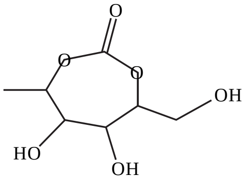
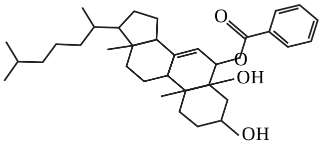
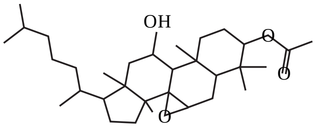


Fig. 1(a-d): Radar plots describing the physicochemical characteristics of each tested compound, (a) Compound 1, (b) Compound 2, (c) Compound 3 and (d) Compound 4

LIPO: Lipophilicity, SIZE: Size, POLAR: Polarity, INSOLU: Insolubility, INSATU: Unsaturation and FLEX: Flexibility

distribution process of these drugs allowing them to reach the therapeutic site of action in optimal conditions[38]. Its also interesting to note that high flexibility leads to stronger binding affinity and thus guarantee a certain stability of the protein-ligand complex[39]. Data also revealed that among the tested compounds, three of them could possibly possess natural product-likeness property, in which compound 2 exhibited a perfect score (Table 2). This possibility could be directly linked to the structural and physico-chemical aspects of these compounds, since Nakashima *et al.*[30] revealed that these features have a clear impact on the concepts of drug-likeness and thus the expected pharmacological response of a chemical. Also, let's not forget that NP-likeness is considered nowadays a crucial criteria during the selection and designing process of novel drugs[5].

Table 2: Estimation of the Natural Product-Likeness property of the four selected compounds

	n-BuOH extract		EA extract	
Parameters/compounds	Compound 1	Compound 2	Compound 3	Compound 4
Prediction	Possible NP-likeness	Possible NP-likeness	Possible NP-likeness	None NP-likeness
NP class probability	0.99	1 (perfect score)	0.99	NA
Probability Map				NA

NA: Not applicable and NP-likeness: Natural product-likeness

ADMET profile: In pharmacology, pharmacokinetics properties play a crucial role in drug design and development processes but also to understand the safety and efficacy of a particular drug candidate[40]. As reported in Table 3, all tested compounds could be absorbed by human intestine but also permeable to blood brain barrier. These informations means that our compound could be helpful to increase the permeability of drugs to brain, which could be helpful to treat CNS Inflammation using a process called nanocarriers[41]. This also suggest that our compound may also be good candidate to regulate the transport of biological substances that are essential for brain function[42], but also to study gastrointestinal disease in depth, such as ulcerative colitis[43].

Data also revealed that almost all tested compounds may have a sufficient oral bioavailability in humans, except compound 2. This information is interesting and suggest that our compounds may easily enter systemic circulation, thereby accessing the site of action in order to exert the desired pharmacological effect[44]. Its important to underline that this parameter is considered nowadays a crucial factor in determining the fate of new drugs in clinical trials[45]. However, it seems that only compound 4 could be permeable to Caco-2 cells. Noting that this parameter is used nowadays to measures the rate of flux of a compound and thus to predict *in vivo* absorption of drugs[46].

In term of distribution, mitochondria was the principal targeted organelle for the four tested compounds, which means that these natural compounds may have the capacity to slowdown the excessive production of mitochondrial reactive oxygen species (ROS) by controlling/regulating the activity of mitochondrial Oxidative Phosphorylation (OXPHOS) system[47], which is very important, since this organelle is well known to generate approximately 90% of ROS[48] and it has been reported that an imbalance in redox status may increase the risk of developing cancer[49].

Concerning metabolism part, data revealed that almost all tested compounds could be considered as non-substrate and non-inhibitor for cytochrome P450 isoforms, suggesting that they will not interfere with drugs and other xenobiotics biotransformation process made by cytochrome P450 enzymes[50]. The four compounds exhibited also a low CYP inhibitory promiscuity, which is very logic since this parameter refer to the capacity for a compound to bind and decrease the activity of various CYP450 isoform enzymes[51]. Its also interesting to note that compounds 1, 2 and 3 may increase the catalytic function of Uridine Diphospho-Glucuronosyltransferase (UGT) since this category of enzyme is considered a major phase II drug-metabolizing enzymes[52], but play also a major role in metabolic and detoxification pathways[53].

In term of toxicity, the tested compounds seem to be non-hepatotoxic, while only compound 2 could be non-nephrotoxic, which means that this compound could be considered a potential candidate in the formulation process of nephroprotective drugs in order to limit renal tubular dysfunction and a cumulative impairment in kidney[54], since pathologies that affect this organ such as Acute Kidney Injury (AKI) concern 13.3% of the worldwide population. Noting that this compound exhibited the lowest rate of acute oral toxicity[55]. However, only compound 1 appear to be non-toxic for the reproductive system, while compounds 2 and 3 could be potentially toxic for mitochondria and respiratory system.

Its also interesting to note that all compounds could be non-carcinogenic but also non-mutagenic making them a suitable candidate for phase I clinical trials in order to test their possible cytotoxic effect against malignant cell lines[56]. Moreover, almost all tested compounds appears to be non-corrosive and non-irritant agents, which open new perspective in the elaboration process of natural eye care products, more eco-friendly, but also to reduce the adverse irritant effects of artificial constituents that compose the actual eye drops present in the market[57]. The fact that the four compounds are non-sensitive for skin, could make them less susceptible to activate allergic reaction linked to skin contact such as dermatitis, rhinitis or atopic eczema[58].

Table 3: Pharmacokinetics characteristics and toxicity of the selected compounds

	n-BuOH extract		EA extract	
Parameter/compound	Compound 1	Compound 2	Compound 3	Compound 4
Absorption				
Human oral bioavailability	HOB+	HOB-	HOB+	HOB+
Human intestinal absorption	HIA+	HIA+	HIA+	HIA+
Blood brain barrier	BBB+	BBB+	BBB+	BBB+
Caco-2 permeability	Caco2-	Caco2-	Caco2-	Caco2+
P-glycoprotein substrate	Non-substrate	Substrate	Substrate	Non-substrate
P-glycoprotein inhibitor	Non-inhibitor	Inhibitor	Inhibitor	Non-inhibitor
Distribution and metabolism				
Subcellular localization	Mitochondria	Mitochondria	Mitochondria	Mitochondria
CYP450 3A4 substrate	Non-substrate	Substrate	Substrate	Non-substrate
CYP450 2C9 substrate	Non-substrate	Non-substrate	Non-substrate	Substrate
CYP450 2D6 substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C9 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 1A2 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP inhibitory promiscuity	Low CYP inhibitory promiscuity	Low CYP inhibitory promiscuity	Low CYP inhibitory promiscuity	Low CYP inhibitory promiscuity
UGT catalyzed	Increase catalysis	Increase catalysis	Increase catalysis	Decrease catalysis
Excretion and toxicity				
Acute oral toxicity	Class IV	Class I	Class III	Class III
Hepatotoxicity	Non-hepatotoxic	Non-hepatotoxic	Non-hepatotoxic	Non-hepatotoxic
Nephrotoxicity	Nephrotoxic	Non-nephrotoxic	Nephrotoxic	Nephrotoxic
Reproductive toxicity	Non-reprotoxic	Reprotoxic	Reprotoxic	Reprotoxic
Respiratory toxicity	Non-toxic	Toxic	Toxic	Non-toxic
Mitochondrial toxicity	Non-toxic	Toxic	Toxic	Non-toxic
Carcinogenicity	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Ames mutagenesis	Non-mutagenic	Non-mutagenic	Non-mutagenic	Non-mutagenic
Eye corrosion	Non-corrosive	Non-corrosive	Non-corrosive	Non-corrosive
Eye irritation	Non-irritant	Non-irritant	Non-irritant	Irritant
Skin sensitisation	Non-sensitive	Non-sensitive	Non-sensitive	Non-sensitive
Honey bee toxicity	Non-toxic	Non-toxic	Non-toxic	Non-toxic
Crustacea aquatic toxicity	Non-toxic	Non-toxic	Non-toxic	Non-toxic
Fish aquatic toxicity	Non-toxic	Toxic	Toxic	Toxic
Estrogen receptor binding	Non-binding	Binding	Binding	Non-binding
Androgen receptor binding	Non-binding	Binding	Binding	Non-binding
Thyroid receptor binding	Non-binding	Binding	Binding	Non-binding
Glucocorticoid receptor binding	Non-binding	Binding	Binding	Non-binding
PPAR gamma binding	Non-binding	Binding	Binding	Non-binding
Aromatase binding	Non-binding	Binding	Binding	Non-binding
Biodegradation	Non-biodegradable	Non-biodegradable	Non-biodegradable	Biodegradable
ADMET predicted profile (regression)				
Water solubility (logS)	-0.134	-4.733	-4.531	-1.109
Plasma protein binding (%)	0.145	1.13	1.067	0.38
Acute oral toxicity (kg/mol)	0.883	3.566	1.847	2.557
Tetrahymena pyriformis (pIGC50, μg/L)	-1.117	1.214	0.846	-0.267

Data also suggested that compounds 2 and 3 may possess a high binding affinity with a large variety of receptors including estrogen, androgen, thyroid, glucocorticoid, PPAR gamma and aromatase making them a potential candidate for the elaboration of agonist drugs that may exert the same effect of original hormones of these receptors and this has been already proved. Indeed, several phyo-compounds have the capacity to regulate the reproduction and growth processes of herbivores by directly interacting with steroid hormone receptors[59]. Noting that only compound 4 could be potentially biodegradable.

In term of toxicity, results indicated that all compounds were non-toxic to honey bees or aquatic Crustacea. However, only compound 1 could be non-toxic to fish. The water solubility of compound 2 was considered the lowest (-4.733) among the tested compounds. However, this compound expressed the best plasma protein binding percentage (1.13%) followed very closely by compound 3 (1.067%) and

Table 4: Bioactivity score of the selected compounds

Extracts	Compound	Pa	Pi	Biological properties
n-BuOH	1	0.955	0.002	Sugar-phosphatase inhibitor
		0.851	0.008	Beta-adrenergic receptor kinase inhibitor
		0.788	0.005	Vasoprotector
	2	0.913	0.003	Anti-hypercholesterolemic
		0.774	0.004	Adenomatous polyposis treatment
		0.728	0.013	Respiratory analeptic
EA	3	0.902	0.002	Hepatoprotectant
		0.888	0.005	Respiratory analeptic
		0.795	0.005	Immunosuppressant
	4	0.912	0.005	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
		0.859	0.022	CYP2C12 substrate
		0.732	0.004	Erythropoiesis stimulant

Pa: Probability of activity and Pi: Probability of inactivity

this information is crucial, since a high degree of plasma protein binding (PPB) means that our compounds may serve as good model to predict the pharmacokinetics parameters of highly protein-bound drugs and thus the efficacy of drug candidates[60].

The acute oral toxicity of compound 1 was considered the lowest (0.883 kg/mol), which suggest that orally, a single-dose administration of this compound will not generate adverse effect or fatal toxic reactions linked to poisoning phenomenon[61,62]. Its also interesting to underline that the best median population growth inhibition of *Tetrahymena pyriformis* was expressed by compound 2 (1.214 μg/L), since this ciliated protozoa is able to invade internal organs, skin and muscle in all fish species and to cause a high mortality rate, especially in guppies[62].

Pharmacological properties: Table 4 indicated that compound 1 could potentially inhibit sugar-phosphatase activity but may also suppress the activity of Beta-Adrenergic Receptor Kinase (βARK) with respective values of Pa=0.955 and Pa=0.851. It has been demonstrated that the inhibition of sugar-phosphatase activity may considerably prevent the transition from insulin resistance to overt diabetes, but also to preserve the histological integrity of Langerhans islets[63]. On the other hand, the inhibition of βARK can considerably improve cardiac function by preserving beta-adrenergic receptor signaling pathways, but also to prevent cardiomyopathy induced by calsequestrin overexpression[64]. Noting that compound 1 could also be useful as vasoprotective agent (Pa=0.788).

Concerning compound 2, it seems that this compound may exert a considerable anti-hypercholesterolemic effect (Pa=0.913) and this information is very important since hypercholesterolemia can considerably increase the risk of coronary artery disease and thus affecting the performance of cardiovascular and cerebrovascular systems[65]. Data also revealed that compound 2 could be effective to treat adenomatous polyposis (Pa=0.774), which is crucial since adenomatous polyposis is a rare, inherited form of pre-cancer that can promote the development of colorectal cancers if not treated in time[66]. Compound 2 could be also a potent respiratory analeptic agent (Pa=0.728), but it seems that compound 3 exerted a more significant analeptic effect (Pa=0.888).

The hepatoprotective effect of compound 3 was considerably high (Pa=0.902), but its also interesting to underline that this compound may possess an important immunosuppressive capacity (Pa=0.795), making it a suitable candidate to develop a more sophisticated drugs in order to prevent cell damage and inflammation symptoms linked to the inappropriate activation of autoimmune system[67].

Compound 4 could be suitable to inhibit testosterone 17beta-dehydrogenase ($NADP^+$) (Pa=0.912), which could be helpful to regulate androgenic hormones production since an excessive concentration of sex steroids is associated with several disorders and pathologies such as acne, hirsutism and even prostate cancer[68]. This compound could be also considered an appropriate substrate for Cytochrome P450 2C12 (CYP2C12) (Pa=0.859), underlining the crucial role played by these isoforms in the metabolism process of drugs and other xenobiotics[69]. Noting that compound 4 may stimulate with a significant way erythropoiesis (Pa=0.732) and this could be helpful to develop erythropoietin-stimulating medications, especially for people suffering from anemia[70] and thus to cover the lack of red blood cells, normally produced by bone marrow.

Antiviral, antibacterial and antifungal properties prediction: Antibiotics and anti-infective drugs have been a real leap forward for modern medicine, whether to treat diseases or prevent infections. In this context, this work investigated the antimicrobial properties against certain pathogenic species of bacteria, fungi and virus. Indeed, as shown in Table 5, it seems that only compound 1 and 2 could potentially be active against HIV and HCV viruses, but with a potency slightly more pronounced for compound 2, which is

Table 5: Antimicrobial properties of the tested compounds

Extracts	Compound	Antiviral effect	p-value	Antibacterial effect	p-value	Antifungal effect	p-value
n-BuOH	1	HIV	0.589	*Yersinia pestis*	0.860	*Rhizopus oryzae*	0.645
		HCV	0.301	*Clostridium ramosum*	0.783	*Absidia corymbifera*	0.603
		HBV	0.234	Resistant *Mycobacterium ulcerans*	0.737	*Trichophyton mentagrophytes*	0.534
	2	HIV	0.671	*Porphyromonas gingivalis*	0.289	*Rhizopus oryzae*	0.308
		HCV	0.356	*Staphylococcus simulans*	0.264	*Absidia corymbifera*	0.241
		HHV	0.272	*Streptococcus mutans*	0.248	*Saccharomyces cerevisiae*	0.219
EA	3	NA-C	/	*Staphylococcus lugdunensis*	0.565	*Candida albicans*	0.237
				Lactobacillus plantarum	0.548	*Clavispora lusitaniae*	0.129
				Bacteroides stercoris	0.352	*Rhizopus oryzae*	0.126
	4	NA-C	/	*Bacteroides stercoris*	0.636	*Saccharomyces cerevisiae*	0.391
				Yersinia pestis	0.604	*Epidermophyton floccosum*	0.155
				Actinomyces meyeri	0.544	NA	/

P: Probability for the query molecule assumed as bioactive on virus, bacteria and fungi, HIV: Human Immunodeficiency Virus, HCV: Hepatitis C Virus, HBV: Hepatitis B Virus, HHV: Human herpes virus, NA: Not available and NA-C: Non-active compound

very important, since statistics clearly indicated that the number of persons affected by these two types of viruses dramatically increased the recent years, especially in developing countries[71].

Let's not forget that HIV primarily targets immune system and weakens body's defenses against many infections, certain types of cancer and even central nervous system since this virus can easily cross blood brain barrier[72]. On the other hand, HCV, the most severe form Hepatitis, increases the risk of developing liver cirrhosis or hepatocellular carcinoma[73]. A recent study also revealed the implication of these two virus in adverse pregnancy and neonatal complications[74]. However, data revealed that only compound 1 may eventually prevent the proliferation of HBV virus, while compound 2 of HHV virus, but with a non-significant way, which suggest that these two compounds should be associated with more active agents to increase their antiviral effects.

It's also interesting to underline that all tested compounds may possess an antibacterial effect. Indeed, compound 1 exhibited a remarkable antibacterial effect against *Yersinia pestis* with a respective values of (p = 0.860), while a moderate effect was expressed by compound 4 on this bacterial strain (p = 0.604). These informations are crucial since this virulent pathogen considerably increase the risk to develop severe pneumonia or enlarged and painful adenopathies with high fever, often evolving to sepsis[75]. A significant antibacterial effect was also exerted by compound 1 on *Clostridium ramosum* (p = 0.783) and resistant *Mycobacterium ulcerans* (p = 0.737). It has been reported that an infection of intervertebral disc called spondylodiscitis could be caused by *Clostridium ramosum*[76], while resistant *Mycobacterium ulcerans* could lead to tissue necrosis, generating in long term Bairnsdale ulcer or Buruli ulcer pathologies[77].

Concerning compound 3, its seems that this compound could possibly exert a moderate antibacterial effect on *Staphylococcus lugdunensis* (p = 0.565) and *Lactobacillus plantarum* (p = 0.548), which could be interesting since these two pathogenic bacteria are responsible of infective endocarditis[78] and meningoencephalitis[79], respectively. A non negligible antiproliferative effect was also exhibited by compound 4 on *Bacteroides stercoris* (p = 0.636) and *Actinomyces meyeri* (p = 0.544), underlining the fact that *Actinomyces meyeri* has a direct role in complicating dental infection but also the main cause of chronic productive cough in smoker people[80]. Noting that the antibacterial effect of compound 2 was considered the lowest among the four tested compounds.

Concerning the antifungal capacity, it seems that compound 1 exhibited the best effect among the tested compounds. Indeed, this compound could be potentially active against *Rhizopus oryzae* (p = 0.645) by possibly blocking host-pathogen molecular factors that contribute to the pathogenesis conditions of these this fungus species, such as CotH fungal protein and GRP78 cell receptor[81]. Noting that a noticeable antifungal effect may also be exerted by compound 2 on *Rhizopus oryzae*, but with a low incidence (p = 0.308). A modest antifungal effect was also exerted by compound 1 on both *Absidia corymbifera* (p = 0.603) and *Trichophyton mentagrophytes* (p = 0.534), which is important since these fungal species can affect both humans and animals and are principally responsible of post-traumatic corneal keratitis and dermatophytosis, respectively[82,83].

Data also revealed that a possible antifungal effect may also be exerted by compound 4 against *Saccharomyces cerevisiae* (p = 0.391), underlying the fungal susceptibility to develop several pathologies such as septic emboli, but also on immunosuppressed condition including sepsis and COVID-19 infections[84,85]. Noting that compound 2 and 3 could, respectively inhibit the proliferation process of *Absidia corymbifera* and *Candida albicans*, but with a non-significant way.

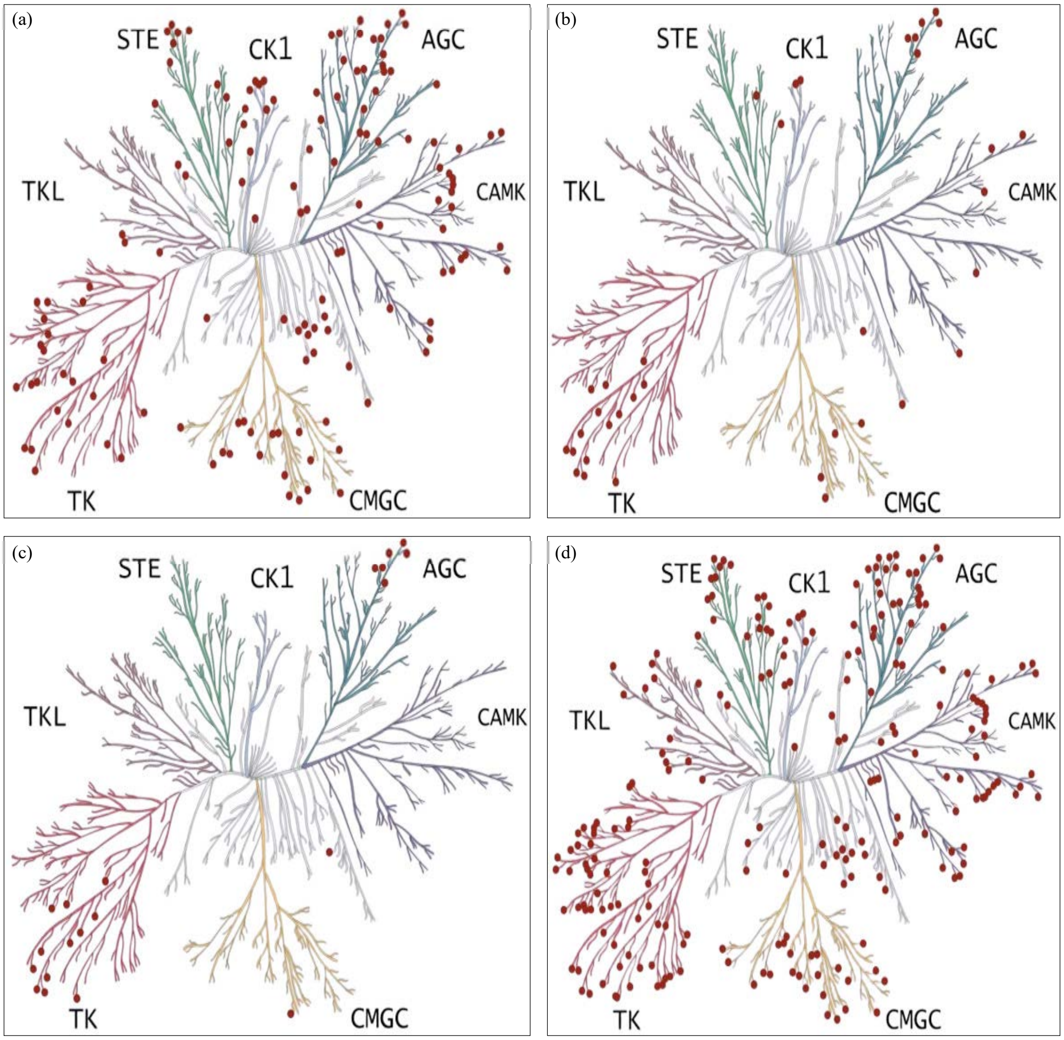


Fig. 2(a-d): Mapped kinome tree visualization of the possible distribution of our compounds across kinase families, (a) Data of compound 1, (b) Data of compound 2, (c) Data of compound 3 and (d) Data of compound 4

AGC: Containing PKA, PKG, PKC families; CAMK: Calcium/calmodulin-dependent protein kinase; CK1: Casein kinase 1; CMGC: Containing CDK, MAPK, GSK3, CLK families; STE: Homologs of yeast Sterile 7, Sterile 11, Sterile 20 kinases; TK: Tyrosine kinase and TKL: Tyrosine kinase-like

Kinase targets prediction: In this study, the probability for our compound to target some important classes of kinases was performed and as shown in Fig. 2, its seems that compound 1 and 4 has the best distribution profile across kinase families, while the members of TK (tyrosine kinase), AGC (containing PKA, PKG and PKC families) and possibly CMGC (containing CDK, MAPK, GSK3 and CLK families) could potentially be targeted by compound 2 and 3.

In more specific way, Table 6 revealed that compound 1 could potentially target and regulate the activity of Dual specificity Mitogen-Activated Protein Kinase Kinase 7 (MAP2K7) with a significant confidence score of p = 0.780, which is important since any dysfunction in the function of this enzyme may increase the risk to develop psychiatric conditions like schizophrenia[86]. On the other hand, recent pre-clinical study revealed that MAP2K7 inhibition could considerably attenuate certain sever form of leukemia, such as T-Cell Acute Lymphoblastic Leukemia (T-ALL) by inhibiting MAP2K7-JNK pathway, increasing at the same time the efficacy of some anti-leukemia drugs like dexamethasone[87].

Furthermore, the four compounds could be active on various classes of Serine/Threonine-Protein Kinases (STPKs). As an example, compound 1 on MST4, while compound 2 and 3 on NEK6 and finally compound 4 on NIM1 and TLK1, respectively. Despite the crucial physiological role of these categories of kinases, it has been reported that an

Table 6: Tabular form results of the main compounds classes of kinases targeted by our compounds

Extracts	Compound	Name	UniProt ID	ChEMBL ID	Confidence score
n-BuOH	1	Dual specificity mitogen-activated protein kinase kinase 7	O14733	CHEMBL3530	0.780
		Serine/threonine-protein kinase MST4	Q9P289	CHEMBL5941	0.720
		Serine/threonine-protein kinase haspin	Q8TF76	CHEMBL1075163	0.700
	2	Ephrin type-A receptor 4	P54764	CHEMBL3988	0.930
		Ephrin type-A receptor 8	P29322	CHEMBL4134	0.910
		Serine/threonine-protein kinase NEK6	Q9HC98	CHEMBL4309	0.660
EA	3	Ephrin type-A receptor 4	P54764	CHEMBL3988	0.920
		Ephrin type-A receptor 8	P29322	CHEMBL4134	0.900
		Serine/threonine-protein kinase NEK6	Q9HC98	CHEMBL4309	0.710
	4	Serine/threonine-protein kinase NIM1	Q8IY84	CHEMBL3542	0.760
		Serine/threonine-protein kinase tousled-like 1	Q9UKI8	CHEMBL5388	0.720
		Myosin light chain kinase smooth muscle	Q15746	CHEMBL2428	0.710

Table 7: Probable effect of the selected compounds on mRNA expression profile of some genes

Extracts	Compound	Genes (upregulation)	Pa	Pi	Genes (downregulation)	Pa	Pi
n-BuOH	1	SMPD1	0.892	0.013	ADAM19	0.906	0.006
		TCF12	0.863	0.014	APTX	0.876	0.008
		PGM2	0.857	0.007	ABCA13	0.863	0.008
	2	KCNH2	0.868	0.031	MSH5	0.868	0.031
		PLLP	0.868	0.031	TREX1	0.868	0.031
		FOXO1	0.865	0.045	RPS19	0.867	0.037
EA	3	KCNH2	0.828	0.040	MSH5	0.828	0.040
		PLLP	0.828	0.040	TREX1	0.828	0.040
		CHRNE	0.803	0.054	RPS19	0.822	0.056
	4	POR	0.846	0.049	NSF	0.861	0.022
		CXCL11	0.820	0.022	ALDH18A1	0.825	0.036
		C10ORF118	0.804	0.015	SLC15A1	0.761	0.036

Pa: Probability of activity and Pi: Probability of inactivity

overexpression of these STPKs may generate in long term several pathologies including breast and prostate cancers but also promotes reactive astrogliosis and exacerbates brain lesions[88-90], which explain why these kinases are considered by many clinicians as potential oncogene. The regulation of the activity of may also bring new strategies to increase plants disease resistance and elaborate more effective fungicides[91].

Moreover, target report revealed that compound 2 and 3 exhibited the best confidence score on both ephrin type-A receptors 4 and 8, which is crucial since these two members of receptors tyrosine kinase maintain epithelial tissue homeostasis and an optimal immune response[92,93]. Noting that compound 4 may target with a non-negligible way Myosin Light Chain Kinase (MLCK) smooth muscle, underlining the physiological role of this kinase to ensure a proper contraction of smooth muscle by maintaining a coherent actin-myosin interaction process[94]. At this stage, all computational approaches performed in this study could be considered complementary to each other and provided a preliminary qualitative investigation. Molecular docking and dynamics simulations were not investigated in the present study, since the algorithms of this axis execute quantitative predictions and thus not suitable for the aspect of this work. However, in the future, it is fundamental and crucial to perform docking approach on these compounds in order to determine the molecular complex stability, binding affinity energy score, categories of participating bonds as well as to determine if ligand-receptor interaction follows the agonist or antagonist model[5] and thus to validate the results of the present work.

Confidence score represent the difference between the probability for compound of interest to inhibit particular kinase and probability to not to inhibit this kinase.

Gene expression profiles: As reported in Table 7, an important upregulation and downregulation processes has been exerted by our phyto-compounds on the mRNA expression of key genes. Indeed, compound 1 may significantly increase the mRNA expression of SMPD1, TCF12 and PGM2 genes with a respective values of Pa=0.892, Pa=0.863 and Pa=0.857. These genes ensure crucial functions, as an example, SMPD1 gene can modulates stress-induced apoptosis[95] and participate in the export of cholesterol from the intra-endolysosomal membranes by allowing the conversion of sphingomyelin to ceramide[96].

Moreover, TCF12 gene could be involved in the early initiation of neuronal differentiation but also in the development of mesodiencephalic dopaminergic (mdDA) neurons in murine midbrain[97], while PGM2 gene may greatly facilitate carbohydrate metabolic process[98]. Concerning downregulation process, data indicated that compound 1 may considerably decrease the mRNA expression of ADAM19 (Pa=0.906), APTX (Pa=876) and ABCA13 (Pa=0.863) genes, respectively. The overexpression of these genes has been linked with several pathologies such as tumors, inflammatory diseases, psychiatric disorders and ataxia with oculomotor apraxia type 1[99-101].

Data also revealed that compound 2 and 3 could potentially upregulate the mRNA expression of KCNH2 gene (Pa=0.868 and Pa=0.828), respectively. It has been reported that this gene provide instructions for making potassium channels and thus confer to cells the capacity to generate and transmit electrical signals[102]. These two compounds may also enhance the mRNA expression of PLLP gene but it seems that only compound 2 could be active on FOXO1 gene with a possible probability of Pa=0.865. Shulgin *et al.*[103] indicated that PLLP gene could be involved in ion transport events but also in the regulation of Notch signaling pathway and thus controlling cell proliferation, differentiation and cell death processes[104]. Its also interesting to underline that FOXO1 gene is essential for muscle energy metabolism to support muscular contractions[105], but could be also essential for adaptive immune functions by affecting lymphocyte development and cytokine expression, which could be helpful to treat immune/oxidative stress-related diseases[106].

On the other hand, compound 2 and 3 could effectively decrease the mRNA expression of MSH5 but the best effect has been exhibited by compound 2 (Pa=0.868) and lets not forget that a mutation in this gene could be responsible of primary ovarian insufficiency[107] but also reproductive malfunctions affecting both genders[108]. Noting that the same downregulation process could be exerted by these compounds on TREX1 and RPS19 genes. Its has been reported that TREX1 mutation may generate autoimmune and cerebroretinal vasculopathy syndromes[109], while another study made on Korean patients confirmed that a mutation in RPS19 gene could possibly lead to the development of Diamond-Blackfan anemia[110]. Noting that this pathology is inherited in up to 45% of cases.

Compound 4 may significantly upregulate the mRNA expression of POR (Pa=0.846) gene, which is very important since this gene provides instructions for making cytochrome P450 oxidoreductase, considered a crucial enzyme for multiple metabolic processes[111] and nowadays an important factor to determine drug metabolism and efficacy. In addition, this compound could also be effective to increase the mRNA expression of CXCL11 (Pa=0.820) and C10ORF118 (Pa=0.804) genes. Previous works have reported the potential of these two genes in regulating various physiological processes. Indeed, Hirota *et al.*[112] revealed that CXCL11 gene promote the migration of trophoblast cells but also the proliferation of endometrial stromal cells and thus supporting implantation and early embryo development. Concerning C10ORF118 gene, it seems that the expression of this gene may help controlling the proliferation of cancer cells via a possible regulation of hyaluronan synthesis, since this glycosaminoglycan is well known to influence tumor proliferation, migration and neoangiogenesis and this has been proved using several breast tumor cell lines[113].

A potent downregulation process has been exerted by compound 4 on the NSF (Pa=0.861) and ALDH18A1 (Pa=0.825) genes. It has been reported that heterozygous mutations on NSF gene could cause an irreversible early infantile epileptic encephalopathy[114], while an overexpression of ALDH18A1 gene could promote mice neuroblastoma growth[115] and even retinopathy[116]. In addition, a non-negligible decrease in the mRNA expression of SLC15A1 gene may also exerted by this compound (Pa=0.761), underlining the possible implication of this gene in several pathologies such as bladder cancer[117], but also to increase the risk of dyslipidemia and thus of cardiovascular deseases[118].

Biotransformation pathways prediction: Human gut microbiota constitute one of the most complex and fascinating community, in which their diversity significantly contribute to the maintain of metabolic hemostasis. Indeed, this internal microbial ecosystem guarantee a proper biotransformation process of nutrients and drugs[119], to supports the generation of active metabolites, known as microbiome metabolites, considered primordial to maintain a coherent function of immune system but also to generate Butyrate, a fatty acid representing a vital energy source for human colonocytes[120,121].

As shown in Fig. 3, the biotransformation prediction result revealed that three compounds could potentially be transformed by human gut microbial flora following various metabolic pathways of degradation. Indeed, carboxylesterase could potentially catalyze the hydrolysis of carboxylic acid ester of compound 2 and 3 to generate four different metabolites as indicated in Table 8. This information is very interesting since Wang *et al.*[122] reported that human gut microbiota, especially genera *Escherichia* and *Lactobacillus* may possess the capacity to accumulate and transform dangerous heavy metals such as arsenic via its functional carboxyl groups following a process called Biosorption in order to reduce it to a less toxic form called arsenate (As^{5+}) and thus to neutralize its toxicity[122].

Its also interesting to underline that two other metabolites may possibly be obtained from the previous cited compounds by two distinct glucuronidation processes, the first one called 3-OH-glucuronidation of sterol and the second one was Alkyl-OH-glucuronidation (reactions 3 and 7), respectively. These reactions were catalyzed by the same enzyme named bacterial UDP-glucuronosyltransferase, an important member of intestinal epithelial barrier and considered crucial for detoxification pathways in humans[123] and the phenolic nature of our compounds could possibly

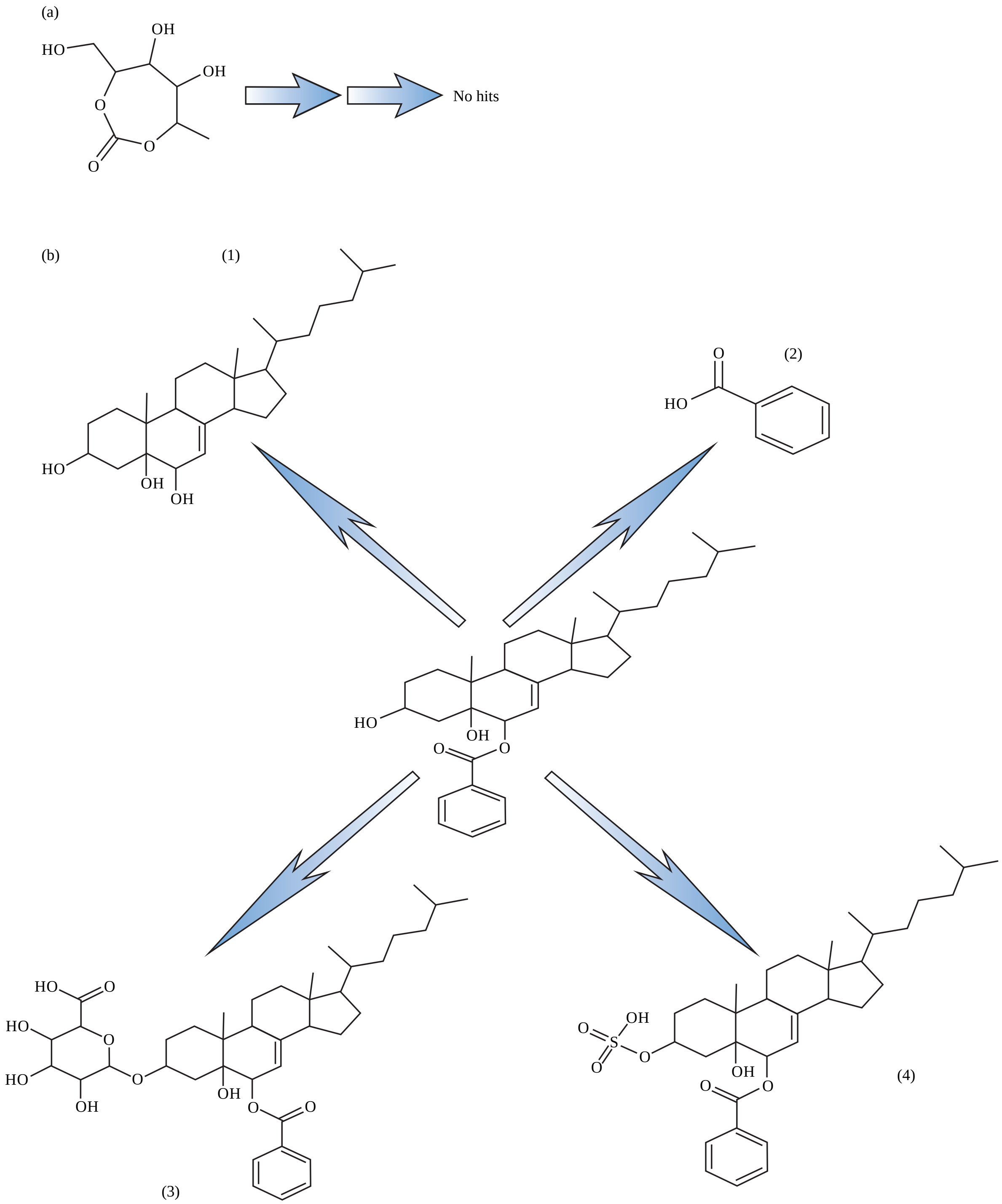


Fig. 3(a-d): Continue

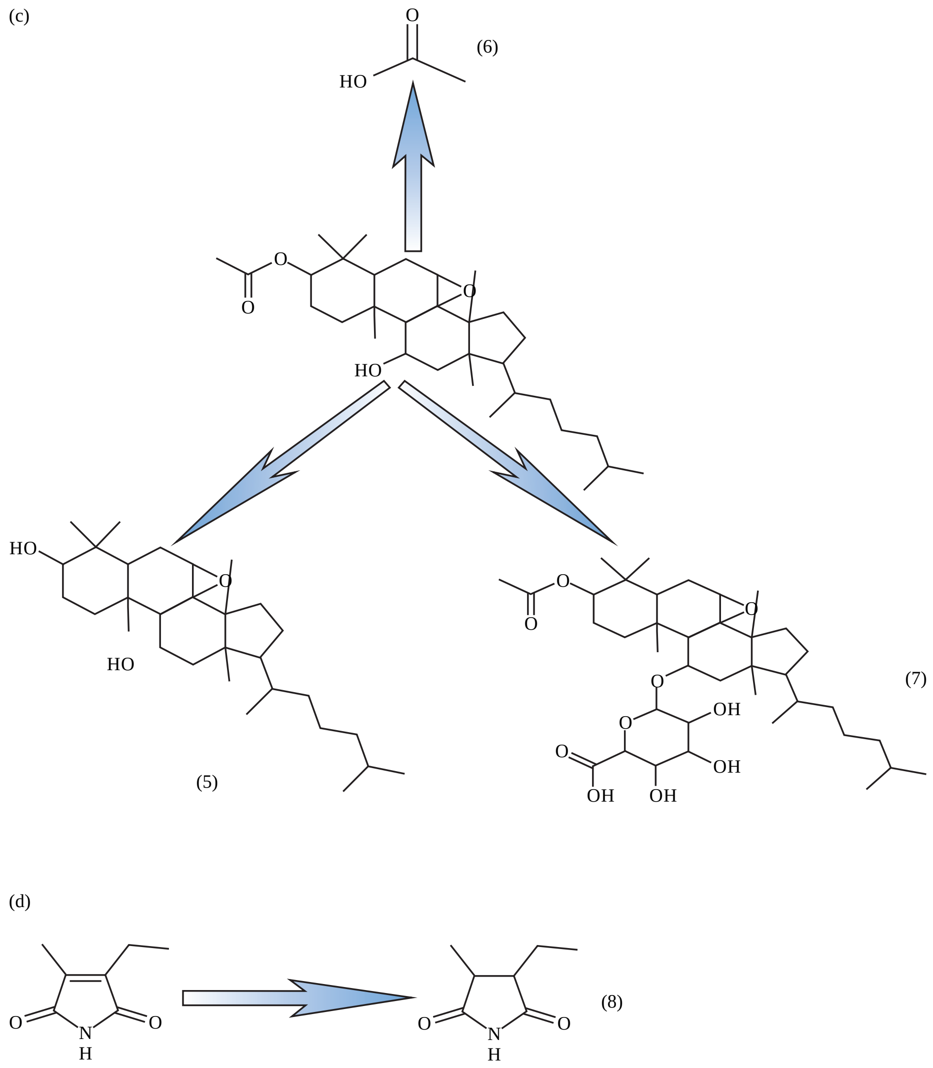


Fig. 3(a-d): Pathway prediction result of the microbial biotransformation process of the tested compounds, (a) Compound 1, (b) Compound 2, (c) Compound 3 and (d) Compound 4

Table 8: Biotransformation reaction details of the tested compounds

Tested compound	Reaction No.	Chemical name	Chemical formula	Reaction type	Catalytic enzyme
Compound 1	NA	NA	NA	NA	NA
Compound 2	1	(3β,5α,6β)-Cholest-7-ene-3,5,6-triol	$C_{27}H_{46}O_3$	Hydrolysis of carboxylic acid ester	Carboxylesterase
	2	Benzoic Acid	$C_7H_6O_2$	Hydrolysis of carboxylic acid ester	Carboxylesterase
	3	(3β,16β)-3,17-Dihydroxy-22-oxocholest-5-en-16-yl 2-O-(4-methoxybenzoyl)-β-D-xylopyranoside	$C_{40}H_{58}O_{10}$	3-OH-Glucuronidation of sterol Bacterial UDP-glucuronosyltransferase	
	4	(5α,7α,24R)-3-Oxo-24-(sulfooxy)cholestan-7-yl benzoate	$C_{34}H_{50}O_7S$	3-OH-Sulfation of sterol	Sulfotransferase
Compound 3	5	20,25-Epoxydammarane-3,12-diol	$C_{30}H_{52}O_3$	Hydrolysis of carboxylic acid ester	Carboxylesterase
	6	Acetic acid	$C_2H_4O_2$	Hydrolysis of carboxylic acid ester	Carboxylesterase
	7	6,16,25-Trihydroxy-20,24-epoxy-9,19-cyclolanostan-3-yl 2-O-acetyl-6-deoxyhexopyranoside	$C_{38}H_{62}O_{10}$	Alkyl-OH-glucuronidation	Bacterial UDP-glucuronosyltransferase
Compound 4	8	3-Ethyl-4-methylpyrrolidine-2,5-dione	$C_7H_{11}NO_2$	Reduction of alpha,beta-unsaturated carbon-carbon double bond adjacent to electron withdrawing group	NADPH dehydrogenase

NA: Not available data

explain the implication of this enzyme, since Wu *et al.*[123] and Isvoran *et al.*[124] indicated that UDP-glucuronosyltransferase target and promote in a more specific way the metabolism of phenolic-nature compounds with an extensive glucuronidation phenomenon in order to obtain the adequate structural modification needed to properly control oral bioavailability of drugs.

Sulfotransferases are considered a crucial phase II drug-metabolizing enzymes[125] and at the clinical level, several approaches underlined the intervention of this enzyme in drug-drug interactions but also in bio-transformation process of hydrophobic drugs to improve their pharmacological effect or simplify their excretion process[125,126]. In our study, data revealed that sulfotransferase may act on compound 2 by exerting a reaction of 3-OH-sulfation to obtain (5α, 7α, 24R)-3-Oxo-24-(sulfooxy)cholestan-7-yl benzoate. Concerning compound 4, it seems that the enzymatic mechanism of NADPH dehydrogenase could possibly be adopted by gut microbes to obtain 3-ethyl-4-methylpyrrolidine-2,5-dione via a reduction of alpha, beta-unsaturated carbon-carbon double bond adjacent to electron withdrawing group of the compound. Noting that recently, a new approach involving both experimental and computational approaches made by Yan *et al.*[126] allowed the identification of 17 unique novel biotransformations pathways, mainly orchestrated by human gut microbiome, using metagenomics datasets, which prove once again the accuracy and fundamental role of bioinformatics in biotransformation pathways discovery.

CONCLUSION

In summary, this work revealed that the four tested compounds may possesses a stable pharmacokinetics property, a non-negligible pharmacological effect but also an exceptional impact on the expression profile of key genes and thus, the molecular aspect could be considered the most important and promising aspect of this study. In addition, the 2nd compound could be considered the suitable candidate for the next pre-clinical and clinical investigations since the insolubility, lipophilicity, flexibility as well as the natural product-likeness capacities of this compound were considered the most appropriate. In addition, various classes of kinases were preferentially targeted by the 2nd compound and let's not forget that the enzymatic catalysis of this compound may generate four new metabolites, which could be used as a valuable strategy to synthetize molecules, similar to parent drug and thus make an initial step in drug discovery programme.

SIGNIFICANCE STATEMENT

However, due to the limitations of this study, it was not possible to experimentally validate the accuracy of the various results related to this theoretical predictions. That's why, wet lab experiments should be performed to validate the findings of this preliminary work, but also to properly investigate the physico-chemical/biological characteristics of other phyto-compounds previously identified in *Centaurea tougourensis*.

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