



Article In Silico Investigation of Some Compounds from the N-Butanol Extract of Centaurea tougourensis Boiss. & Reut.

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Abstract: Bioinformatics as a newly emerging discipline is considered nowadays a reference to characterize the physicochemical and pharmacological properties of the actual biocompounds contained in plants, which has helped the pharmaceutical industry a lot in the drug development process. In this study, a bioinformatics approach known as in silico was performed to predict, for the first time, the physicochemical properties, ADMET profile, pharmacological capacities, cytotoxicity, and nervous system macromolecular targets, as well as the gene expression profiles, of four compounds recently identified from Centaurea tougourensis via the gas chromatographymass spectrometry (GC-MS) approach. Thus, four compounds were tested from the n-butanol (n-BuOH) extract of this plant, named, respectively, Acridin-9-amine, 1,2,3,4-tetrahydro-5,7-dimethyl-(compound 1), 3-[2,3-Dihydro-2,2-dimethylbenzofuran-7-yl]-5-methoxy-1,3,4-oxadiazol-2(3H)-one (compound 2), 9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione (compound 3), and 3-[3-Bromophenyl]-7chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-acridinedione (compound 4). The insilico investigation revealed that the four tested compounds could be a good candidate to regulate the expression of key genes and may also exert significant cytotoxic effects against several tumor celllines. In addition, these compounds could also be effective in the treatment of some diseases related to diabetes, skin pathologies, cardiovascular, and central nervous system disorders. The bioactive compounds of plant remain the best alternative in the context of the drug discovery and development process.

Keywords: ADMET profile; biocompounds; bioinformatics; *Centaurea tougourensis; in silico;* n-BuOH extract

1. Introduction

Bioinformatics, as a new emerging discipline, has evolved considerably over the past few decades and now represents an important field of study, allowing researchers to store, retrieve, organize, and analyze biological data [1]. Among these developments, a modern computational approach, named *in silico*, has allowed the prediction of the chemical and biological properties of thousands of natural and synthetic compounds, due to high



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). algorithms accuracy and constantly updated databases [2]. Indeed, this approach allowed the scientific community to gain considerable time and decrease the cost of studies related to laboratory experiments on animals.

The *in silico* approach also allowed the scientific community to understand and overcome several ambiguities related to the cerebral and cognitive aspects [3], but also to explain some mechanisms linked to blood–brain barrier permeability and hence answer some fundamental questions related to some pathologies that affect the brain, such as epileptic encephalopathy, Alzheimer's and Huntington's diseases [4], which gives unprecedented credibility to this methodology, since the nervous system is well known for its complexity. This recent methodology has also provided mandatory data for the scientific community, helping them to understand more some phenomena related to oxidative stress, inflammation, and even cancer [5,6].

Phytotherapy is a newly emerging discipline, considered nowadays a reference for the elaboration process of new drugs, due in part to its accessibility, affordability, and efficacy, especially in developing countries [7]. This natural resource has a long history and large scale of applications, used in the past by many civilizations as a quick remedy to heal wounds and treat various inflammatory conditions, including fevers, arthritis [8]. However, plants are also known for their relaxation property and their ability to reduce stress and anxiety [9]. This helps to explain why it is currently being used and recommended by health care professionals.

In many countries, *Centaurea* species are prepared as an infusion due to their relaxing property, helping people reduce stress and anxiety [10]. These plants may also improve the digestion process and possibly support enzymatic reactions in the liver [11]. The genus *Centaurea* is also known for its antioxidant, immunomodulatory, anti-inflammatory, antidiabetic, and antimicrobial potential [12–15]. Recent studies [16,17] showed that *Centaurea* species could be able to inhibit the proliferation of several tumor celllines, which opens the door to many therapeutic avenues. It was also reported that *Centaurea* species could be useful as insecticides [18].

This study aims to predict, for the first time via in silico approach, the physicochemical and biological properties of four compounds recently identified in the n-BuOH extract of *C. tougourensis* by GC–MS analysis.

2. Materials and Methods

2.1. The Plant Material

C. tougourensis was collected at the Belezma national park in the municipality of Fesdis (Algeria) (GPS coordinates: latitude 35.621975; longitude 6.241327) and was identified by experts in the field from the Agronomic department of the Batna-1 University (Algeria). A voucher specimen under the code (CT/2019/LPTPCMB) was deposited at the Laboratory of Improvement of the Phytosanitary Protection Techniques in Mountainous Agrosystems, Agronomy Department, Institute of Veterinary and Agricultural Sciences, University of Batna-1-, Batna, Algeria.

2.2. Preparation of Plant Extract

The aerial parts of *C. tougourensis* were dried in a dry, ventilated place away from the sun rays and then ground to obtain a fine powder. Maceration was carried out three times with 3L EtOH–H20 (70:30) at room temperature for 3 days followed by liquid–liquid extraction using solvents of gradual polarity (hexane, ethyl acetate, and n-butanol).

2.3. In Silico Study

2.3.1. Determination of Canonical SMILES

To predict the possible biological properties of four phytocompounds recently identified in the n-BuOH extract of *C. tougourensis*, two bioinformatics tools were used: Open Babel (version 2.0.2) [19] and InDraw webserver (http://in.indraw.integle.com/, accessed on 8 September 2021), respectively, to generate the canonical smile of each compound by inputting their respective structures. These four compounds were, respectively, Acridin-9-amine, 1,2,3,4-tetrahydro-5,7-dimethyl- (**compound 1**), 3-[2,3-Dihydro-2,2-dimethylbenzofuran-7-yl]-5-methoxy-1,3,4-oxadiazol-2(3H)-one (**compound 2**), 9,9-Dimethoxybicyclo[3.3.1]nona-2,4dione (**compound 3**), and 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)acridinedione (**compound 4**).

2.3.2. Physicochemical Properties

In this test, six physicochemical parameters of the four compounds were predicted using the online platform of SwissADME (http://www.swissadme.ch/, accessed on 13 September 2021). These tested properties were lipophility, size, polarity, insolubility, unsaturation, and flexibility [20].

2.3.3. ADMET Profile

The pharmacokinetics and toxicity properties of the four compounds were estimated using the new online platform of admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2, accessed on 15 September 2021) [21]. The possible influences of these compounds were tested on different parameters, principally, human intestinal absorption (HIA),human oral bioavailability (HOB),blood–brain barrier (BBB) penetration, Caco-2 cells permeability, subcellular localization, interaction with P-glycoprotein and Cytochrome P450 (CYP450), mutagenicity, carcinogenicity, eye corrosion, eye irritation, hepatotoxicity, binding to receptors of estrogen, androgen, thyroid hormones, glucocorticoid, toxicity to the honey bee, fish, and aquatic crustaceans, biodegradation, water-solubility, plasma protein binding, and acute oral toxicity, as well as the median growth inhibition concentration (pIGC50) of *Tetrahymena pyriformis*.

2.3.4. Pharmacological Properties

The four compounds' potential biological activities were predicted using the PASS online web server (http://way2drug.com/passonline/, accessed on 13 September 2021) [22]. This server is based on two probabilities, Pa and Pi, in which Pa represents the probability of the compound being active and Pi represents the probability of the compound being inactive, with values ranging from zero to one. The model type Pa > Pi was used by default because it is the most widely accepted model for studying activities.

2.3.5. Cytotoxicity Prediction

Based on the structural formula of each compound previously obtained [23], the possible cytotoxic effect of the four compounds on human tumor celllineswasinvestigated using CLC-Pred (Cell Line Cytotoxicity Predictor) web services (http://www.way2drug. com/cell-line/, accessed on 19 September 2021).

2.3.6. Gene Expression Profiles

The DIGEP Pred online server (http://www.way2drug.com/ge/, accessed on 5 October 2021) was used to make an insilico prediction of the four compounds' potential impacts on gene expression, which is primarily based on upregulation and downregulation processes retrieved from the Comparative Toxicogenomics Database (CTD) [24]. An upregulation is a cell's ability to increase the expression of one of its constituents in response to a stimulus, whereas a downregulation is the opposite [25].

2.3.7. Macromolecular Targets Prediction

SwissTargetPrediction was used to estimate the most probable macromolecular targets of a small molecule, assumed as bioactive. The prediction is founded on a combination of 2D and 3D similarity with a library of 370,000 known actives molecules on more than 3000 proteins from different species. In this study, the macromolecules of human nervous system were preferentially targeted in order to predict to neuroprotective effect of these four tested compounds [26].

3.1. Canonical SMILES Generation

Table 1 shows the corresponding molecular formula, structure, and canonical smile of each compound.

Table 1. Generation of the canonical smile of the four tested compounds

Compound	Molecular Formula	Structure	Canonical SMILES		
Compound 1	$C_{15}H_{18}N_2$	CH ₃ CH ₃ CH ₃ CH ₃	CC1=CC(=C2C(=C1)C(=C3CCCCC3=N2)N)C		
Compound 2	$C_{13}H_{14}N_2O_4$	H ₃ C N H ₄ C	CC1(CC2=C(O1)C(=CC=C2)N3C(=O)OC(=N3)OC)C		
Compound 3	$C_{11}H_{16}O_4$	H _g C O	COC1(C2CCCC1C(=0)CC2=0)OC		
Compound 4	C ₁₉ H ₁₃ BrClNO ₃		C1C(CC(=O)C2=C1N(C3=C(C2=O)C=C(C=C3)Cl)O)C4 =CC(=CC=C4)Br		

3.2. Physicochemical Properties

As shown in Figure 1, the unsaturation and insolubility of **compound 4** were considered the best among the four tested compounds. The lipophilicity of this compound was also considerable, noting that **compound 1** showed almost the same result. This information is crucial since high lipophilicity means that these compounds could easily cross biological membranes to bind with the corresponding receptor in order to generate the desired pharmacological effect [27] but will also have a positive repercussion on the pharmacokinetics and metabolism process of these compounds.

The polarity of **compound 4** was the highest but in terms of flexibility; **compound 2** exhibited the best results. However, a comparable flexibility was observed in **compound 3**. These pieces of information are crucial, since a high polarity and flexibility properties may increase the oral bioavailability of the compound, helping it to reach the therapeutic site of action due to a high molecular dynamics phenomenon, which also contributes to a nonnegligible increase of renal clearance process, and this was proved using rats and molecular docking approach [28].



Figure 1. Prediction of the physicochemical properties of the four tested compounds. LIPO: lipophilicity, SIZE: size, POLAR: polarity, INSOLU: insolubility, INSATU: unsaturation, FLEX: flexibility.

3.3. ADMET Profile

The four compounds were evaluated for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile using AdmetSAR server. As shown in Table 2, the results concerning the absorption process suggest that all compounds may be permeable to blood–brain barrier (BBB) and most of them to Caco-2 cell. The results indicated that these compounds could also be absorbed by the human intestine with a possible human oral bioavailability, suggesting that the four compounds may reach their site of action, which is beneficial in clinical trials, especially in the drug development process [29].The permeability of these compounds to the BBB membrane can make them good candidates for the elaboration of more effective central nervous system drugs(CNS) [30], but not good for non-CNS drugs due to their possible side effects [31]. On the other hand, the data showed that the four compounds could not be substrates for the P-glycoprotein (P-gp), also excluding a possible inhibition of P-gp activity which could mean that the compounds will not interfere with the metabolism, half-lifetime, and clearance of drugs [32].

Parameters/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	Non-substrate	Non-substrate	Non-substrate
P-glycoprotein Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Distribution and Metabolism				
Subcellular localization	Lysosomes	Mitochondria	Mitochondria	Mitochondria
CYP450 3A4 substrate	Non-substrate	Substrate	Non-substrate	Substrate
CYP450 2C9 substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor
CYP450 2C9 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 inhibition	Non-inhibitor	Inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 1A2 inhibition	Inhibitor	Non-inhibitor	Non-inhibitor	Inhibitor
Excretion and Toxicity				
Acute Oral Toxicity	Class II	Class II	Class III	Class III
Hepatotoxicity	Hepatotoxic	Hepatotoxic	Non-hepatotoxic	Hepatotoxic
Carcinogenicity	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens
Ames mutagenesis	Mutagenic	Non-mutagenic	Non-mutagenic	Non-mutagenic
Eye corrosion	Non-corrosive	Non-corrosive	Non-corrosive	Non-corrosive
Eye irritation	Non-irritant	Non-irritant	Irritant	Non-irritant
Honey bee toxicity	Non-toxic	Toxic	Toxic	Non-toxic
Crustacea aquatic toxicity	Toxic	Toxic	Toxic	Toxic
Fish aquatic toxicity	Toxic	Toxic	Toxic	Toxic
Estrogen receptor binding	Binding	Binding	Binding	Binding
Androgen receptor binding	Binding	Non-binding	Non-binding	Binding
Thyroid receptor binding	Binding	Binding	Non-binding	Non-binding
Glucocorticoid receptor binding	Binding	Binding	Non-binding	Binding
Biodegradation	Non-biodegradable	Non-biodegradable	Non-biodegradable	Non-biodegradable
ADMET Predicted Profile (Regression)				
Water solubility (logS)	-3.038	-3.82	-3.388	-3.987
Plasma protein binding (%)	1.074	0.92	0.702	1.022
Acute Oral Toxicity (kg/mol)	2.246	3.176	2.994	2.831
Tetrahymena pyriformis (pIGC50, ug/L)	1.065	1.065	0.836	1.172

Table 2. Prediction of the ADMET properties and toxicity of the four tested compounds.

In terms of distribution, the mitochondria appear to be the preferred organelles for **compounds 2**, **3** and **4**, but not **compound 1**, for which distribution seems to be more oriented to lysosomes. This allows the emergence of the hypothesis that almost all compounds could be involved in the energy metabolism process of the cells [33], helping aerobic organisms to produce ATP by accelerating oxidative phosphorylation phenomenon [34].

Concerning the metabolism part, all tested compounds may be considered as nonsubstrate for cytochrome P450 2C9, 2D6 isoforms, while **compounds 2** and **4** could be potential substrates for CYP450 3A4. It means that the influence of the compounds on the activity of CYP450 depends on the type of isoforms tested. It was also observed that almost all compounds cannot inhibit CYP450 3A4, 2C9, 2C19, and 2D6 isoforms, which suggest that our compounds may not interfere with the drug biotransformation made by CYP450 isoforms [35].

The results concerning the Ames mutagenicity suggest that compound 1 may be the only mutagenic compound, but none of the four compounds were considered carcinogenic. The lowest rate of acute oral toxicity was observed by **compounds 1** and **2**, while this parameter was very high for compounds 3 and 4. Almost all tested compounds seem to be hepatotoxic except compound 3. The four compounds appear to be non-corrosive to eyes, but concerning eye irritation, only compound 1 could be considered as an irritant agent, which suggests that these compounds could be good candidates for the elaboration of artificial tears for dry eyes by limiting the possible corrosive and irritant effects of other constituents of eye drops [36]. The toxicity prediction revealed that all compounds expressed high toxicity on fish and Crustacea, while **compounds 1** and **4** were considered nontoxic to honey bees. This could mean that these compounds may be helpful for the development process of new drugs that may effectively limit the reproduction procedure of some harmful variety of fish such as pufferfish, considered deadly for humans due to their potent venomous effect generated by tetrodotoxin (TTX) [37] neurotoxin. These four tested compounds could be also effective against Xanthidaes, which is a family of Crustacea well known for their venomous effect, generally fatal for humans due to the presence of two neurotoxins, namely, saxitoxin and tetrodotoxin [38].

The data also revealed that **compounds 1**, **2**, and **4** may have a high interaction with receptors of estrogen, androgen, thyroid hormones, and glucocorticoid, which suggests that these compounds may mimic the activity of the original hormones of these receptors [39]: this may be a key for the elaboration of new drugs with a great affinity for receptors already mentioned [40], or even to facilitate the interaction of drugs with these receptors via a synergistic effect [41].

The water solubility of **compound 4** was considered the lowest (-3.987) among the tested compounds, while **compound 1** exhibited the best plasma-protein-binding percentage (1.074%). This information is very important since water solubility is considered as a key parameter to achieve desired concentration of drug in systemic circulation in order to generate the desired pharmacological response [42]. In this case, the low aqueous solubility of **compound 4** could be a major obstacle in the development of highly potent pharmaceutics, since poorly water-soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration [43]. On the other hand, **compound 1** may positively influence the pharmacokinetic properties and pharmacodynamics of drugs since the diffusion of drugs in tissues depends on the binding to plasma proteins [44]. The acute oral toxicity of **compound 2** was considered the highest (3.176 kg/mol), while the median population growth inhibition of Tetrahymena pyriformis was expressed by compound 4 $(1.172 \ \mu g/L)$ since the toxicity of this unicellular protozoa is well known and mostly found in polluted water, which partially explains why scientists use it as a toxic endpoint during experiments [45]. Noting that, acute oral toxicity is considered as a crucial parameter to determine the short-term adverse effects of a drug when administered in a single dose [46].

3.4. Pharmacological Properties

The possible biological score of each compound was investigated using the PASS online web server. A study conducted by Druzhilovskiy et al. [47] demonstrated the predictive effectiveness of PASS online of 32 from 33 compounds, which explains the choice of this server. As shown in Table 3, **compound 1** could be useful as calcium channel (voltage-sensitive) activator but also as nootropic agent, with respective values of Pa = 0.703, Pa = 0.679. It is well known that voltage-gated calcium channels are mandatory for the initiation of many physiological events such as the secretion of hormones or the release of neurotransmitters, by maintaining a coherent synaptic transmission [48]. A nootropic effect

could mean that **compound 1** can boost brain performance, especially memory capacity, an important parameter of the learning process [49].

Compound	Pa	Pi	Biological Activities
	0.703	0.005	Calcium channel (voltage-sensitive) activator
Compound 1	0.679	0.047	Nootropic
	0.594	0.04	Antidyskinetic
	0.965	0.003	Antidiabetic (type 2)
Compound 2	0.906	0	Lipase inhibitor
	0.39	0.007	Retinoprotector
	0.864	0.013	Phobic disorders treatment
Compound 3	0.806	0.004	Cardiovascular analeptic
	0.73	0.036	Antieczematic
	0.926	0	Polarization stimulant
Compound 4	0.754	0.003	Antiprotozoal
-	0.426	0.04	(Plasmodium) Chemosensitizer

Table 3. Bioactivity prediction of the four tested compounds.

Pa: Probability of activity. Pi: Probability of inactivity.

It is also interesting to underline that **compound 2** could be a potential antidiabetic agent, especially against Type 2 diabetes (Pa = 0.965), to ameliorate pancreatic beta cells function in order to reduce the abnormal elevation of blood glucose level and ameliorate insulin sensitivity [50]. This compound may also exert a great inhibition on lipase activity (Pa = 0.906), which may help scientists to develop new drugs to treat obesity since an excessive activity of lipase will significantly increase gastrointestinal absorption of fats and, in the long term, an abnormal increase in weight [51]. Compound 3 could be also a good candidate for the treatment of phobic disorders (Pa = 0.864), making it a good candidate in psychotherapy and in other cognitive behavioral therapy [52]. **Compound 3** may also be effective against eczema (Pa = 0.730) but also be a great cardiovascular analeptic agent (Pa = 0.806) in order to boost heart activity to increase the blood pumping process in order to ensure an optimal distribution of oxygen and nutrients to all parts of the body [53]. This may also help scientists to develop a more effective treatment, especially for people suffering from valvular heart disease, cardiomyopathy, and heart failure [54]. These data could explain the antidiabetic and neuroprotective activities of the n-BuOH extract of C. tougourensis reported in the previous study [55,56].

The data also revealed that **compound 4** may exert an important stimulation on membrane polarization (Pa = 0.926), which is mandatory to maintain an asymmetric organization of proteins and lipids in the plasma membrane [57]. This compound could also exert great antiprotozoal activity against *plasmodium* (Pa = 0.754), since the proliferation of this parasite is responsible for malaria, a disease widely present in the tropics regions of Africa and Southeast Asia [58].

3.5. Cytotoxicity Prediction

Plants have demonstrated their ability to inhibit the proliferation of several tumor cell lines over time due to their abundance of bioactive compounds, particularly flavonoids, terpenoids, and saponins, which have a potent cytotoxic effect [59,60]. These classes of secondary metabolites have already been identified in *C. tougourensis* [61]. The ability of each compound to induce cytotoxicity on tumor cell line was investigated using CLC-Pred server and, as shown in Table 4, data clearly indicated that the four tested compounds expressed a moderate cytotoxic effect on the majority of cell lines evaluated in the present study. Indeed, three compounds exerted a non-negligible cytotoxic effect against melanoma, in which **compound 2** (Pa = 0.496) and **compound 3** (Pa = 0.430) were active against (M19-MEL) cell line, while **compound 4** (Pa = 0.548) was active against (SK-MEL-28) cell line. These details are crucial because melanoma is the most dangerous type of skin cancer [62],

and the ozone layer's depletion makes people more vulnerable to harmful UVB sun rays [63]. Note that the actual data reported in this study could partially explain the remarkable photoprotective effect of the n-BuOH extract of *C. tougourensis* reported in the previous study [64].

Table 4. Cytotoxicity probabilities of the four tested compounds on some tumor celllines.

Compound	CellLine	CellLine Full Name	Tissue	Tumor Type	Pa	Pi
Compound 1	Jurkat SK-MES-1 MDA-MB-453	Acute leukemic T-cells Squamous cell lung carcinoma Breast adenocarcinoma	Blood Lung Breast	Leukemia Carcinoma Adenocarcinoma	0.456 0.411 0.351	0.008 0.016 0.204
Compound 2	SF-295 A498 M19-MEL	Glioblastoma Renal carcinoma Melanoma	Brain Kidney Skin	Glioblastoma Carcinoma Melanoma	0.593 0.552 0.496	0.01 0.016 0.016
Compound 3	Hs 683 HOP-18 M19-MEL	Oligodendroglioma Non-small cell lung carcinoma Melanoma	Brain Lung Skin	Glioma Carcinoma Melanoma	0.559 0.505 0.43	0.037 0.01 0.028
Compound 4	SK-MEL-28 DU-145 A549	MelanomaProstate carcinoma Lung carcinoma	Skin Prostate Lung	Melanoma Carcinoma Carcinoma	$0.548 \\ 0.469 \\ 0.456$	0.013 0.025 0.074

Pa: Probability of activity. Pi: Probability of inactivity.

Compound 1 exhibited a modest cytotoxic effect against acute leukemic T-cells (Jurkat) cell line (Pa = 0.456), which may show promise in the treatment of diseases related to hematopoiesis since, in a leukemia situation, bone marrow is replaced with tumors, which prevents the regular producing of B or T lymphocytes and may cause long-term anemia [65,66]. Note that a negligible effect (Pa = 0.351) was exerted by **compound 1** against breast adenocarcinoma (MDA-MB-453) cell line.

Compound 3 exerted a modest cytotoxic effect against oligodendroglioma (Hs 683) and non-small-cell lung carcinoma (HOP-18) cell lines with a respective activity values of Pa = 0.559 and Pa = 0.505. Despite the fact that non-small-cell lung carcinoma is classified as the tenth most common cancer in the world [67], its number has increased considerably over the last years, for example by 25% between 2014 and 2016 in Canada [68]. Recent statistics reported that this type of cancer accounts for 80% to 85% of all lung cancers [69], in which women have the highest rates for this type of cancer [70]. These facts highlight the critical need for a new treatment option for this type of cancer. It is worth noting that **compound 2** was active on brain tissue, particularly against the glioblastoma (SF-295) cell line, with a cytotoxic effect of Pa = 0.593.

3.6. Gene Expression Profiles

As shown in Table 5, the results suggest that **compound 1** may exert a moderate upregulation process on the mRNA expression of FOXO1 and ADIPOQ genes with respective values of Pa = 0.708 and Pa = 0.596. These two genes are mainly involved in the anti-diabetic process [71,72]. Indeed, the expression of the FOXO1 gene, which is mainly orchestrated by MAP kinase pathway, will significantly contribute to the maintenance of energy homeostasis, especially those in relation to carbohydrate metabolism, but may also strengthen insulin sensitivity, glucose uptake, and also promote the formation of adipose tissue and skeletal muscle [72-74]. This gene also seems to protect the keratinocytes of skin and mucous membranes by enhancing immune system functions when promoting the maturation and production of lymphocytes, especially type B, as well as the activation of macrophages, neutrophils, and dendritic cells [75]. The ADIPOQ gene encodes a protein called adiponectin, and it was reported that low plasma levels of this protein may lead to an abnormal increase of insulin resistance, as well as lipid metabolism imbalance [76]. The expression of this gene could also stimulate the sensitivity of insulin to the variation of blood sugar level, and thus to prevent the development of possible diabetes [77]. **Compound 1** may also moderately (Pa = 0.597) stimulate the expression of LST1 gene, which may possibly modulate immune responses, especially against rubella [78,79]. On the

other hand, **compound 1** showed a moderate downregulation process on the expression of CCR6 (Pa = 0.573), DHFR (Pa = 0.474), and BCAS3 (Pa = 0.451) genes, and this information is very important since the overexpression of these genes is associated with several illnesses; CCR6 with psoriasis, systemic sclerosis, rheumatoid arthritis, lupus nephritis [80], while BCAS3 may alter neural tissue development [81] and DHFR with cerebral folate deficiency and megaloblastic anemia that lead in the long term to the development of severe neurologic diseases, especially brain tumors [82].

Compound	Genes (Upregulation)	Ра	Pi	Genes (Downregulation)	Ра	Pi
Compound 1	FOXO1 LST1	0.708 0.597 0.506	0.135 0.146 0.042	CCR6 DHFR	0.573 0.474 0.451	0.151 0.222 0.076
Compound 2	CYP3A4 CYP2B6 BRMS1	0.908 0.778 0.639	0.043 0.005 0.018 0.065	BGLAP MSH5 TREX1	0.431 0.627 0.502 0.502	0.047 0.134 0.134
Compound 3	PBK RACGAP1 BRMS1	0.812 0.781 0.779	0.052 0.071 0.022	PLCG2 SUV39H2 TREX1	0.735 0.732 0.725	0.038 0.075 0.065
Compound 4	REL TRIOBP ADAMTS9	$0.557 \\ 0.411 \\ 0.4$	0.115 0.167 0.234	FABP4 TAGLN NCOA1	0.567 0.395 0.335	0.075 0.177 0.253

Table 5. Probable effects of the four tested compounds on the expression of some genes: mRNA-based prediction result.

Pa: Probability of activity. Pi: Probability of inactivity.

Among the four tested compounds, results revealed that **compound 2** could be a potential molecule to upregulate the mRNA expression of CYP3A4 (Pa = 0.908) and CYP2B6 (Pa = 0.778) genes. These two genes are members of the cytochrome P450 superfamily of enzymes and play important roles. A study carried outby Chen et al. [83] indicates that CYP3A4 can detoxify bile acids, which gives a promising expectation for the treatment of cholestasis. CYP2B6 is mainly expressed in the liver, and it was reported that this enzyme can regulate the mRNA splicing and expression processes to form protein [84]. Clinically, data collected about CYP2B6 could be considered relevant for the treatment of HIV-infected patients [85]. A moderate downregulation process was exerted by **compound 2** on them-RNA expression of BGLAP (Pa = 0.627), MSH5 (Pa = 0.502), and TREX1 (Pa = 0.502) genes. The overexpression of the BGLAP gene is associated with the development of pancreatic ductal adenocarcinoma and chronic pancreatitis, and several assays, such as quantitative RT-PCR, have already proven it [86]. The mutation of the MSH5 gene could be responsible for premature ovarian failure [87], while mutations in the TREX1 gene are strongly linked with Aicardi–Goutières syndrome, which is a severe encephalopathy [88].

The results also indicated that **compound 3** may possibly enhance the mRNA expression of PBK (Pa = 0.812), RACGAP1 (Pa = 0.781), and BRMS1 (Pa = 0.779) genes. These genes are very important in human physiology. Indeed, the protein encoded by the PBK gene serves as novel target for development of new cancer immunotherapy and diagnostic biomarker, especially to treat human bladder cancer [89], while the RACGAP1 gene is considered as an important controller of cellular phenomena related to hematopoietic cells, especially their growth and differentiation [90]. A recent study indicated that the BRMS1 gene is a potent metastasis suppressor, especially against breast cancer, via the regulation of NF- κ B and epidermal growth factor receptor (EGFR)-signaling pathways [91]. Note that **compound 2** showed also a non-negligible upregulation effect on the expression of the BRMS1 gene (Pa = 0.639). On the other hand, **compound 3** showed almost the same probability to downregulate the expression of PLCG2 (Pa = 0.735), SUV39H2 (Pa = 0.732), and TREX1 (Pa = 0.725) genes. A recent study suggests that mutations in the PLCG2 gene

can generate an autoimmune pathology condition characterized by an abnormal inflammation throughout the body and the incapacity of the body to correctly fight infections [92]. An overexpression of this gene is associated with osteosarcoma, considered as the most common primary bone cancer in children [93]. It was also reported that mutations in the TREX1 gene could generate an autosomal-dominant disorder called retinal vasculopathy with cerebral leukodystrophy (RVCL) [94].

In this study, **compound 4** showed a modest upregulation on the mRNA expression process of REL (Pa = 0.557), TRIOBP (Pa = 0.411), and ADAMTS9 (Pa = 0.400) genes. The protein encoded by the REL gene called c-REL plays a key role in hemopoietic cells growth, differentiation, and function, especially those of lymphocytes B and T, which may help scientists treating certain autoimmune diseases such as psoriasis, arthritis, and celiac disease [95,96]. On the other hand, the TRIOBP gene is mandatory to regulate cell spreading and contraction by directly binding and stabilizing filamentous F-actin [97], while the ADAMTS9 gene helps transport a variety of secretion from the endoplasmic reticulum into Golgi apparatus via its own protease-independent function [98]. A moderate downregulation process was also exerted by compound 4 on the mRNA expression of FABP4 (Pa = 0.567), while non-negligible values were recorded for TAGLN (Pa = 0.395) and NCOA1 (Pa = 0.335) genes. Note that the FABP4 gene activity is associated with the development of coronary restenosis [99] and liposarcoma of bone diseases [100], while the overexpression of the TAGLN gene is associated with the development of non-small-cell lung cancer, which is considered nowadays as the main cause of tumor mortality in the world [101]. Finally, a study showed that there could be a link between a mutation in NCOA1 gene and the development of rhabdomyosarcoma [102], which is a type of tumor that affects more neonatal and young children.

3.7. Macromolecular Targets Prediction

As shown in Table 6, **compound 1** seems to be the most active on the nervous system by targeting key enzymes named acetylcholinesterase (Pa = 0.292) and butyrylcholinesterase (Pa = 0.219) in a non-negligible way, which suggests that this compound could actively participate in the regulation of the activity of these two enzymes known for their important roles in cholinergic transmission by facilitating the hydrolysis of acetylcholine (ACh) neurotransmitter [103]. On the other hand, **compound 1** may also participate with other compounds ina synergistic way to limit the activity of these enzymes, since an excessive activity of acetylcholinesterase may lead in the long term to neurodegenerative illnesses such as Alzheimer's and Parkinson's diseases [104]. A key receptor called muscarinic acetylcholine receptor (M1) could be also targeted by this compound (Pa = 0.175), and several studies [105,106] reported the crucial role of this receptor during learning and memory processes, but it could also be a promising therapeutic target for the improvement of cognitive decline in Alzheimer's disease (AD).

Both beta amyloid A4 protein and serine/threonine protein kinase AKT may be targeted by **compound 2** but in anonsignificant manner (Pa = 0.112), while **compound 3** seems to be inactive on nervous system molecules. However, it is important to underline that **compound 2** may be considered to have noticeable contribution in the elaboration process of anti-Alzheimer's drugs, since Beta-amyloid protein is the main component of amyloid plaques, a protein aggregate found in neurons in certain neurodegenerative diseases [107]. This protein would notably decrease communication between neurons due tofilamentous aggregation in the extracellular space [108]. On the other hand, **compound 2** may promote neuronal survival of the cerebellum through serine-threonine protein kinase Akt, and researchers underlined that this enzyme cangenerate this effect via a possible activation of insulin-like growth factor 1 (IGF-1)-signaling pathway [109].

Furthermore, the probability of **compound 4** to target matrix metalloproteinase 16, matrix metalloproteinase 12, and nitric-oxide synthase in the brain is negligible (Pa = 0.097). Note that matrix metalloproteinase 16 is responsible for neural crest cell migration and the emergence of multiple cell lineages in the developing avian embryo [110]. However,

matrix metalloproteinase 12 activity may significantly compromise the integrity of the blood–brain barrier after ischemic stroke in rats [111]. Finally, nitric-oxide synthase is mandatory to catalyze the production of nitric oxide (NO) considered as a major component of the signaling pathways that operate between cerebral blood vessels, neurons, and glial cells [112].

Compound	Target	Target Class	Probability
	Acetylcholinesterase	Hydrolase	0.292
Compound 1	Butyrylcholinesterase	Hydrolase	0.219
	Muscarinic acetylcholine receptor M1	Family A G protein-coupled receptor	0.175
Compound 2	Beta amyloid A4 protein Serine/threonine	Membrane receptor	0.112
	protein kinase AKT	Kinase	0.112
Compound 3	NA	/	/
	Matrix metalloproteinase 12	Protease	0.097
Compound 4	Matrix metalloproteinase 16	Protease	0.097
	Nitric-oxide synthase, brain	Enzyme	0.097

Table 6. Nervous system macromolecular targets prediction of the four tested compounds.

Probability for the query molecule—assumed as bioactive—to have this protein as target. NA: Non-active.

4. Conclusions

Various insilico tests conducted in this study revealed that certain compounds recently identified in the n-BuOH extract of this plant could be effective in the treatment of diabetes, cardiovascular disease, and central nervous system disorders. These compounds may also be used to regulate the expression of certain genes in order to better prevent and treat diseases related to immunity, cancer, and diabetes. These compounds may also have potent cytotoxic effects on several tumor cell lines.

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