Research Article

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Estimating molecular properties, drug-likeness, cardiotoxic risk, liability profile, and molecular docking study to characterize binding process of key phyto-compounds against serotonin 5-HT2A receptor

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Abstract: Nowadays, the physiopathological and molecular mechanisms of multiple diseases have been identified, thus helping scientists to provide a clear answer, especially to those ambiguities related to chronic illnesses. This has been accomplished in part through the contribution of a key discipline known as bioinformatics. In this study, the bioinformatics approach was applied on four compounds identified in *Centaurea tougourensis*, using two axes of research: an in silico study to predict the molecular characteristics, medicinal chemistry attributes as well as the possible cardiotoxicity and adverse liability profile of these compounds. In this context, four compounds were selected and named, 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 1*H*-pyrrole-2,5-dione, 3-ethyl-4-methyl- (compound 4). The second part looked into molecular docking, which objective was to evaluate the possible binding affinity between these compounds and the serotonin 5-hydroxytryptamine 2A (5-HT2A) receptor. Results indicated that compounds 1 and 4 were respecting Pfizer and giant Glaxo-SmithKline rules, while compounds 2 and 3 exhibited an optimal medicinal chemistry evolution 18 score. The structural and molecular features of almost all tested compounds could be considered optimal, indicating that these phyto-compounds may possess drug-likeness capacity. However, only compounds 1 and 4 could be considered non-cardiotoxic, but with a level of confidence more pronounced for compound 1 (80%). In addition, these

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four biocompounds could preferentially interact with G protein-coupled receptor, ion channel, transporters, and nuclear receptors. However, the heat map was less pronounced for compound 2. Data also indicated that these four compounds could possibly interact with serotonin 5-HT2A receptor, but in an antagonistic way. This research proved once again that plants could be crucial precursors of pharmaceutical substances, which could be helpful to enrich the international pharmacopoeia.

Keywords: cardiotoxicity, liability profile, molecular docking, serotonin 5-HT2a receptor, phyto-compounds, drug-likeness

1 Introduction

The twenty-first-century era has been marked by multiple scientific progress and discoveries that affected the evolution of almost all disciplines. This has contributed to the emergence of modern science and the establishment of well-functioning socio-economic systems [1], considered nowadays a perfect indicator of human progress. In this context, bioinformatics as an interdisciplinary field has incorporated computer science technology and biology expertise, to serve the scientific community, thus providing them with more sophisticated approach to manage and process large sets of complex biological data [2] – for example, to understand the evolutionary aspects of molecular biology, especially the concept of genome-wide gene expression profile, which helped scientists to define species' gene networks [3], and besides to identify nucleotide variants that may contribute to a particular disease phenotype [4], which is crucial, since we are continuously subjected to mutations.

Scientists agree that the in silico and molecular docking approaches complement each other and represent the main axis of bioinformatics. The best illustration is the drug discovery process, which is a step-by-step process that requires a lot of preclinical and clinical studies [5]. Indeed, the accurate algorithms integrated in the branch of bioinformatics known as "structural bioinformatics" allowed the scientific community to search for drug–drug similarities, but also to identify potential drug candidates based on their structural features, while also predicting their toxicity and possible signaling pathway perturbations processes [6], representing nowadays a significant saving in time and cost gain time. The molecular modeling approach known as molecular docking also provided a new vision to optimize and describe new methods of ligand-binding site based on energy transfer process [7]. Serotonin, as a key neurotransmitter, plays numerous functions, in particular the regulation of behavior, mood, anxiety, and even learning process. However, an excessive serotonin activity may be linked to pathophysiological processes, such as neuromuscular dysfunction and psychotic depression [8], that is why, it is crucial to identify potent antagonists of serotonin 5-hydroxytryptamine 2A (5-HT2A) receptor in order to develop more effective antipsychotic drugs.

This discipline also provided a more targeted approach and a suitable platform to assist biologists in the development process of minimally invasive biomarkers, in order to detect cancer, especially some types of cancers, such as breast and cervical cancers, that need to be diagnosed in early stage to avoid future complications [8]. Concerning the cardiovascular system, these computational approaches allowed us to understand in depth the molecular complexities of human heart failure, in particular, the pathophysiological aspect of myocardial infarction, coronary artery, and rheumatic heart diseases [9]. This information is important since recent statistics clearly indicated that cardiovascular diseases are the leading cause of global mortality, claiming 17.9 million lives each year [10]. The high precision offered by bioinformatics tools had also a crucial impact on cardiology, by allowing the accurate quantification of cardiac mass and volumes, likewise the treatment of histopathological bioimaging data related to the heart with an automated and efficient processing way, which is nowadays referred as "Cardioinformatics" [11,12].

This work brings novel possibilities and accredited computational techniques to highlight for the first time unique and relevant characteristics of four biocompounds at the atomic, molecular, and organismal levels. In this context, recent approaches were performed to estimate the probable drug-likeness features based on molecular and medicinal chemistry attributes. The cardiotoxic risk of these phyto-compounds was also revealed and their intermolecular reaction stabilities and energies also estimated with serotonin 5-HT2A receptor and other transporters and key receptors as well.

2 Materials and methods

2.1 Origin of the phytoconstituents

Four compounds previously identified by our team via gas chromatography/mass spectrometry from *C. tougourensis* (voucher code: CT/2019/LPTPCMB) [13] have been selected and investigated for their possible drug-likeness, cardiotoxic risk, liability profile as well as their binding affinity using novel computational approaches. In this context,

four compounds [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 1*H*-pyrrole-2,5-dione, 3-ethyl-4-methyl- (compound 4)] were selected from two organic fractions of this plant.

2.2 *In silico* part drugs and reagents

2.2.1 Canonical SMILES generation

The generation of Canonical SMILES is considered a primordial step and represents a unique and specific line notation for each studied molecule. PubChem database was used to perform this approach. The molecular formula, three-dimensional (3D) structure, and identifier ID of each tested compound were also reported in this study.

2.2.2 Molecular properties and medicinal chemistry parameters prediction

The estimation of the molecular and medicinal parameters of each compound was performed using the molecular database of ADMETlab 2.0 (https://admetmesh.scbdd.com/, accessed on 14 January 2023). In summary, 12 molecular properties and 8 medicinal chemistry parameters were investigated, which allow us to estimate the drug-likeness capacity of the selected phyto-compounds [14].

2.2.3 Cardiotoxicity risk prediction

In order to perform this approach, Pred-hERG, a machine learning technology, was used. This server allows the prediction of the possible cardiotoxic effect of tested compounds, but also its potency as well as probability maps to visualize the fragment contribution of each compound, which could potentially be responsible for cardiotoxicity (http://predherg.labmol.com.br/, accessed on 18 January 2023) [15].

2.2.4 Toxic liability prediction

ToxProfiler, a newly web-based platform, was used to predict the adverse liability profile, which may result from the interaction of our compounds with G protein-coupled receptors (GPCRs), ion channel, transporters, kinases, and nuclear receptors, which is fundamental since this category of receptors is considered the main target for 649 highly toxic chemicals and thus linked to adverse effects [16]. This server provides a complementary computational approach and generates results as targets profile bar marked with color-coded system, in which red, yellow, and green colors represent the number of potential interactions. In addition, this server provides also a heatmap of the selected compounds (https://toxpro.bhsai.org/login, accessed on 29 January 2023).

2.3 Molecular docking part

2.3.1 Preparation of both enzymes and ligands

The 3D crystal structure of the protein and ligands is required for performing the molecular docking analysis. The macromolecular target for evaluation of the 3D structure of serotonin receptor protein registered under protein data bank (PDB) code: 7E2Y, was retrieved and downloaded from the Research Council for Structural Biology (RCSB), which can be accessed through www.rcsb.org [17].

RCSB Protein Data Bank (RCSB PDB) empowers new discoveries in scientific research and education by enhancing the accessibility to information related to protein structure, as well as tools for exploring, visualizing, and analyzing the experimentally determined 3D structures of protein archived in PDB database and also provide computed structure models from AlphaFold DB and Model Archive. All such data can be examined in light of external annotations, which can provide a structural perspective on protein biology.

The 3D structure of ligand compounds was obtained from PubChem database [18]. The three-dimensional conformers of compounds 1, 2, and 4 were downloaded in Structure-data file format (.sdf format) and further converted through Open Babel GUI® program [19], to the PDB format. The three-dimensional conformer of compound 3 was not available in the PubChem® database, so its structure was designed and converted to .pdb format through ACD/ChemSketch (version 2012, Advanced Chemistry Development, Inc., Toronto, ON, Canada) [20].

2.3.2 Protein molecular docking and building complexes

In order to perform a coherent prediction regarding the ligand–protein binding process, molecular docking analysis was performed. In this context, the Autodock® Vina module was utilized throughout each and every computation that was carried out. The docking protocol outlined by

Seeliger and de Groot [21] was applied to the molecular docking procedure. The dimensions of the grid box that were set during docking analyses were 92.13 × 83.63 × 80.06 centered on the ligand at *X*, *Y*, and *Z* axis, respectively, with a standard spacing of 1 between each of the internal grid points. BIOVIA Discovery Studio 2020 was utilized in order to create a visualization of the interactions between the protein and ligand complex [22].

3 Results and discussion

3.1 Determination of canonical smiles

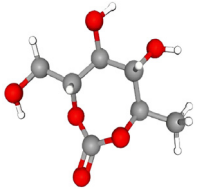
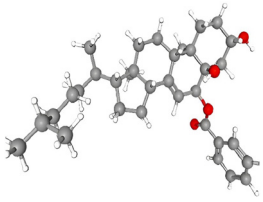
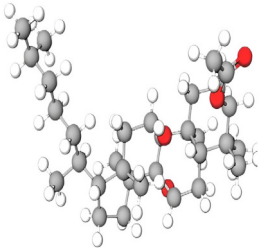
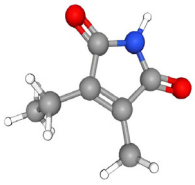
This step is considered primordial before performing computational analysis. Details about the molecular formula, 3D structure, PubChem Identifier, and canonical smiles are presented in Table 1.

3.2 Molecular properties and medicinal chemistry parameters

3.2.1 Molecular property estimation

It is well known that the biological efficacy of a drug is directly linked to the molecular properties of the compounds that enter into its composition. The molecular properties of each compound are graphically represented in Figure 1, while numerical data are reported in Table 2. Based on ADMETlab 2.0 database, the molecular weight (MW) of all tested compounds could be considered optimal,

Table 1: Structural formula and canonical smiles of the selected compounds

Extracts	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

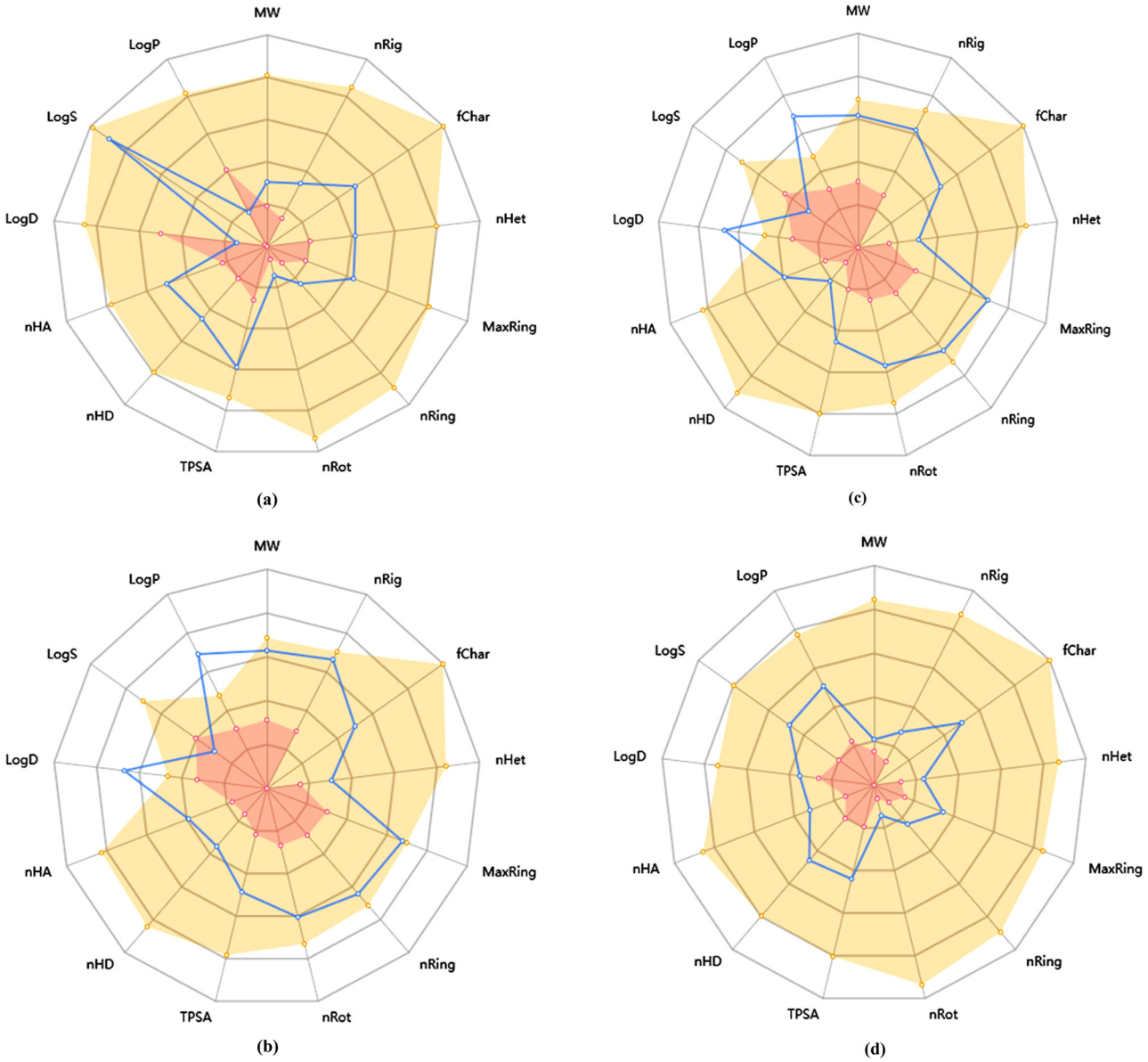


Figure 1: Radar plots describing the molecular characteristics of the selected compounds: (a) data of compound 1, (b) data of compound 2, (c) data of compound 3, and (d) data of compound 4.

Table 2: Molecular propertycalculations of the selected compounds

Extracts	Compound	Molecular properties											
		MW	nHA	nHD	nRot	nRing	Max ring	nHet	fChar	nRig	TPSA	log *P*	log *D*
n-BuOH	1	192.06	6	3	1	1	7	6	0	8	96.22	−1.662	−0.993
	2	522.37	4	2	8	5	17	4	0	27	66.76	6.731	5.967
EA	3	502.40	4	1	7	5	18	4	0	23	59.06	6.698	5.884
	4	139.06	3	3	1	1	5	3	0	6	56.25	1.552	1.374
Optimal values	—	100–600	0–12	0–7	0–11	0–6	0–18	1–15	−4–4	0–30	0–140	0–3	1–3

MW: molecular weight; nHA: number of hydrogen bond acceptors; nHD: number of hydrogen bond donors; nRot: number of rotatable bonds; nRing: number of rings; MaxRing: number of atoms in the biggest ring; nHet: number of heteroatoms; fChar: formal charge; nRig: number of rigid bonds; TPSA: topological polar surface area; log *P*: log of the octanol/water partition coefficient; log *D*: log *P* at physiological pH 7.4.

since it is situated between 100 and 600. It is well known that when MW increases, the drug absorption rate will systematically decrease, and also its diffusion and transport processes, which suggest that compounds 1 and 4 could be absorbed more easily than the other tested compounds [23].

The number of hydrogen bond acceptors (nHA) and hydrogen bond donors (nHD) for all tested compounds is situated in the acceptable range of values as indicated in Table 2, and this information is crucial since these two parameters have a direct impact on the diffusion of molecules across cell membranes [24], which could mean that our compounds may have an important oral bioavailability property.

Interestingly, all tested compounds also have an optimal number of rotatable bonds (nRot) and rings (nRing). Indeed, nRot is a topological parameter that measures molecular flexibility and represents a crucial descriptor of the oral bioavailability of drugs [25], which means that the binding affinity and flexibility of molecules are directly proportional to the number of rotatable bonds that compose it [26]. The rings system also plays an essential role in drug scaffolds, and scientists have agreed to say that an oral drug candidate with a few numbers of aromatic rings is more likely to be developable [27], which suggests that compounds 1 and 4 could be potential candidates.

Remarkably, it is interesting to underline that the four tested compounds have optimal number value of heteroatoms (nHet) but also of formal charge (fChar) and rigid bonds (nRig). These parameters are very important; for example, an important proportion of active pharmaceutical ingredients and excipients contains heteroatoms as a common fragment [28], while rigidity of bounds may clinically stabilize favored conformations of target proteins [29]. On the other hand, formal charge allows to estimate the way electric charge distribution in a molecule, considered nowadays crucial to determine the behavior and fate of inhaled pharmaceutical aerosols [30].

Topological polar surface area (TPSA) is closely related to the hydrogen bonding capacity of a molecule and a suitable predictor of drug transport process, bioavailability, and blood–brain barrier penetration [31]. This parameter determines also the physiochemical aspect of molecule, especially clarifications about its polarity [32].

TPSA of concerned products was found in the range of 56.25–96.22 $Å^2$ and is well below the 140 $Å^2$ limit. This means that the probability for the molecule to cross membranes is significantly reduced [33], which suggests that only compounds 2, 3, and 4 could possibly pass membranes. In addition, the comparative study revealed that our compounds exhibited better results than quercetin and 4-hydroxy isoleucine, which were, respectively, 131.36 and 83.55 $Å^2$, as shown in Table 3. It should be noted that molecules with TPSA values higher than 140 $Å^2$ are expected to exhibit poor intestinal absorption [34].

On the other hand, the partition coefficient (log *P*) is principally used in rational drug design to determine the molecular hydrophobicity [35]. Indeed, the hydrophilic/lipophilic nature of drug molecule has a great impact on drug absorption and metabolism, drug–receptor interactions, as well as their toxicity [36]. log *P* and log *D* values of the studied products were found to be in the range of −1.662 to 6.731 and −0.993 to 5.967, respectively. The fact that only compound 4 respected Lipinski's rule of five; this implies that for the other compounds, some structural modifications and formulation strategies are mandatory to improve their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties

Table 3: Comparative study of the drug-likeness features of the selected compounds with other chemicals

Tested compound/ compared chemicals	Drug-likeness parameters								GSK rule	Golden triangle
	MW	TPSA	log *P*	QED	Fsp^3	MCE-18	NPscore	Pfizer rule		
Compound 1	192.06	96.22	−1.662	0.443	0.857	20.923	1.943	Accepted	Accepted	Rejected
Compound 2	522.37	66.76	6.731	0.290	0.735	119.288	2.363	Rejected	Rejected	Rejected
Compound 3	502.40	59.06	6.698	0.309	0.969	138.048	3.078	Rejected	Rejected	Rejected
Compound 4	139.06	56.25	1.552	0.550	0.143	7	0.501	Accepted	Accepted	Rejected
Quercetin	302.04	131.36	2.155	0.434	0	19	1.701	Accepted	Accepted	Accepted
4-Hydroxy isoleucine	147.09	83.55	−2.63	0.49	0.83	4	0.99	Accepted	Accepted	Rejected
12-Hydroxyoctadec-9-enoic acid	298.467	57.53	4.575	0.327	0.833	2	1.614	Rejected	Rejected	Accepted
d-Limonene	136.13	0	4.368	0.485	0.6	15	2.359	Rejected	Rejected	Rejected
Optimal values	100–600	0–140	0–3	>0.67	≥0.42	≥45	−5~5	log *P*<3	MW ≤ 400; log *P* ≤ 4	200 ≤ MW ≤ 50;−2 ≤ log *D* ≤ 5

[37]. It is also interesting to note that log *P* value of compound 4 was considered more acceptable than those of 12-hydroxyoctadec-9-enoic acid (4.575) and d-limonene (4.368) values [35–37].

3.2.2 Medicinal chemistry parameters

Drug likeness may be defined as a complex balance of various molecular properties and structure features, which quantitatively determines whether a particular molecule could be considered a suitable oral drug candidates [38]. In this study, the medicinal chemistry parameters were also predicted, and as reported in Table 4, the quantitative estimate of drug likeness (QED) score, which is referred to as a measure of drug likeness based on the concept of desirability [39], was found to be slightly close to optimal value only for compound 4 (0.550), while those scores of compounds 2 and 3 indicate that they could be too complex to become an oral drug candidates with respect to bioavailability process [40]. It should be noted that the QED values of quercetin, 4-hydroxy isoleucine, 12-hydroxyoctadec-9-enoic acid, and d-limonene were not optimal as well [34–37].

However, the synthetic accessibility score (SAscore) of all tested compounds seems to be optimal. This score is specifically designed to estimate the ease of synthesis of drug-like molecules depending on fragment contributions and molecular complexity [41], considered nowadays an important factor in the early drug discovery phase.

This could also mean that our compounds could be helpful as active pharmaceutical ingredients, especially to synthesize new drug candidates. The Fsp^3 values of almost all tested compounds could be considered optimal, except for compound 4, whose value was below reference. Sp^3, refers to the hybridization that occurs when an atom is surrounded by four groups of electrons. Thus, the total number of sp^3 carbons atoms (Fsp^3) is a crucial parameter since it is considered a newer index representing drug likeness, and several studies revealed that when the Fsp^3 value of a compound increases, this will automatically increase its saturation and solubility properties, while melting points will [42,43]. Concerning the Stands for Medicinal Chemistry Evolution (MCE-18) parameter, only compounds 2 and 3 exhibited optimal values and were considered remarkably better than those of quercetin, 4-hydroxy isoleucine, 12-hydroxyoctadec-9-enoic acid, and d-limonene [34–37]. The main function of this parameter is to score molecules by novelty based on their cumulative sp^3 complexity, which could be helpful to provide a new generation of drug candidates [44]. Natural product-likeness score (NPscore) determines how molecules are similar to the structural space covered by natural products [45], and as indicated in Table 4, the NPscore of all compounds is situated in the optimal value range, but the compound 3 value could be considered the best, since the higher the score is, the higher is the probability for a candidate compound to be natural product-likeness [14].

On the other hand, it seems that compounds 1 and 4 respect the Pfizer and GSK rules but do not satisfy the golden triangle rule. Indeed, based on the Pfizer rule, compounds with a high log *P* (>3) are likely to be toxic [46], while compounds respecting the GSK rule may possess favorable ADMET profile [47]. When comparing to other compounds, it seems that compound 1 and 4 are respecting the same rules as 4-hydroxy isoleucine. However, quercetin is the only chemical satisfying the three rules.

3.3 Cardiotoxicity prediction

Several varieties of phyto-compounds such as quercetin, catechin, and isoflavones have already proven their capacities to reduce the risk of cardiac abnormalities [48], but also to enhance cardioprotective effects through a possible

Table 4: Medicinal chemistry parameter prediction of the selected compounds

Extracts	Tested compound	Medicinal chemistry parameters							
		QED	SAscore	Fsp^3	MCE-18	NPscore	Pfizer rule	GSK rule	Golden triangle
n-BuOH	1	0.443	3.862	0.857	20.923	1.943	Accepted	Accepted	Rejected
	2	0.290	4.595	0.735	119.288	2.363	Rejected	Rejected	Rejected
EA	3	0.309	5.475	0.969	138.048	3.078	Rejected	Rejected	Rejected
	4	0.550	3.519	0.143	7	0.501	Accepted	Accepted	Rejected
Optimal values	/	>0.67	<6	≥0.42	≥45	−5–5	log *P* < 3	MW ≤ 400; log *P* ≤ 4	200 ≤ MW ≤ 50; −2 ≤ log *D* ≤ 5

QED: quantitative estimate of drug-likeness; SAscore: synthetic accessibility score; Fsp^3: the number of sp3 hybridized carbons/total carbon count, correlating with melting point and solubility; MCE-18: stands for medicinal chemistry evolution; NPscore: natural product-likeness score.

increase of antioxidant effects. As reported in Table 5, compounds 1 and 4 could be non-cardiotoxic, but with a level of confidence more pronounced for compound 1 (80%).

In addition to being non-cardiotoxic, a hypothesis could be the fact that these two compounds may possess a possible cardioprotective effect, which means that they could be potentially effective in increasing and stimulating key heart anti-radical enzymes such as catalase and glutathione peroxidase [49]. This suggests that these compounds could be also effective in increasing the detoxification process and myocardial antioxidant enzyme gene expression, which could be helpful, especially during congestive heart failure following myocardial infarction [50]. It is also interesting to underline that cardiac tissues may also be protected by plant's bioactive compounds via a possible reduction in lipid peroxidation phenomenon [51], and this has also been demonstrated using a rat cardiac H9C2 cell line [52]. It is noted that a high amount of polyphenols have been previously identified in *C. tougourensis* fractions [53,54].

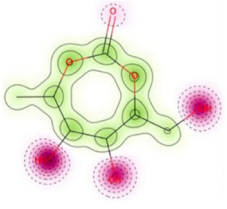
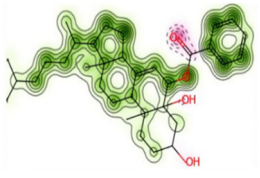
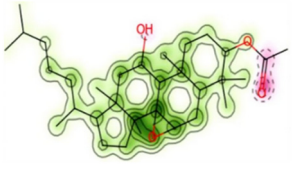
On the other hand, compounds 2 and 3, which have more complex structures, could be potentially cardiotoxic, and even if the cardiotoxic effect of compound 3 seems to be lower than that of compound 2, its potency seems to be more important and even severe. The molecular characteristics of these two compounds may explain their possible cardiotoxicity. Indeed, the chemical groups, molecular size, and number of isomers of a chemical have a direct impact on the formation process of reactive oxygen and nitrogen species, thus affecting cardiac cellular signaling pathways [55]. However, future clinical experiments are mandatory to confirm their possible cardiotoxic effect.

3.4 Toxic liability prediction

In this part, the possible adverse liability profile of the four phyto-compounds with selected receptors and transporters, considered the toxicity targets of 649 known acute and highly toxic chemicals [16] was estimated based on the *Z* score range. This indicates the degree of interaction as follows: >1.96 indicates a high interaction probability and is marked in red, while 1.645–1.96 is marked in yellow and indicates a possible interaction probability, and finally, <1.645 indicates a lack of interaction and is marked in green [16]. As shown in Figure 2, it seems that GPCRs could be considered an important target for compound 1, likewise compound 4. Indeed, the probability for these compounds to interact with adenosine A2A receptor (ADORA2A) was considered high, which is crucial since this receptor is a major target of caffeine and is also crucial for regulating myocardial oxygen consumption [56]. This also suggests that our two compounds may act in synergistic way with methotrexate (MTX), to increase its toxicity, but may also act as a perfect antagonist to block its adverse effect, since in high dose, a severe toxicity may emerge from the interaction between ADORA2A and MTX, causing for the majority of the diagnosed cases, bone erosions, and hepatotoxicity [57]. It is noted that a moderate interaction could also be generated between compound 1 and beta-1 adrenergic receptor (ADRB1), but also between compound 4 and histamine receptor H1 (HRH1).

Concerning ion channel, it seems that compound 4 has a higher probability to establish interaction with three receptors, namely, cholinergic receptor nicotinic alpha 4 subunit (CHRNA4), gamma-aminobutyric acid type a receptor subunit

Table 5: Probable cardiotoxic effect of the four selected compounds

Parameters/ compounds	*n*-BuOH extract		EA extract	
	Compound 1	**Compound 2**	**Compound 3**	**Compound 4**
Prediction	Non-cardiotoxic	Potential cardiotoxic	Potential cardiotoxic	Non-cardiotoxic
Confidence	80%	60%	50%	60%
Potency	NA	Weak or moderate	Strong or extreme	NA
Probability map				

NA: not applicable.

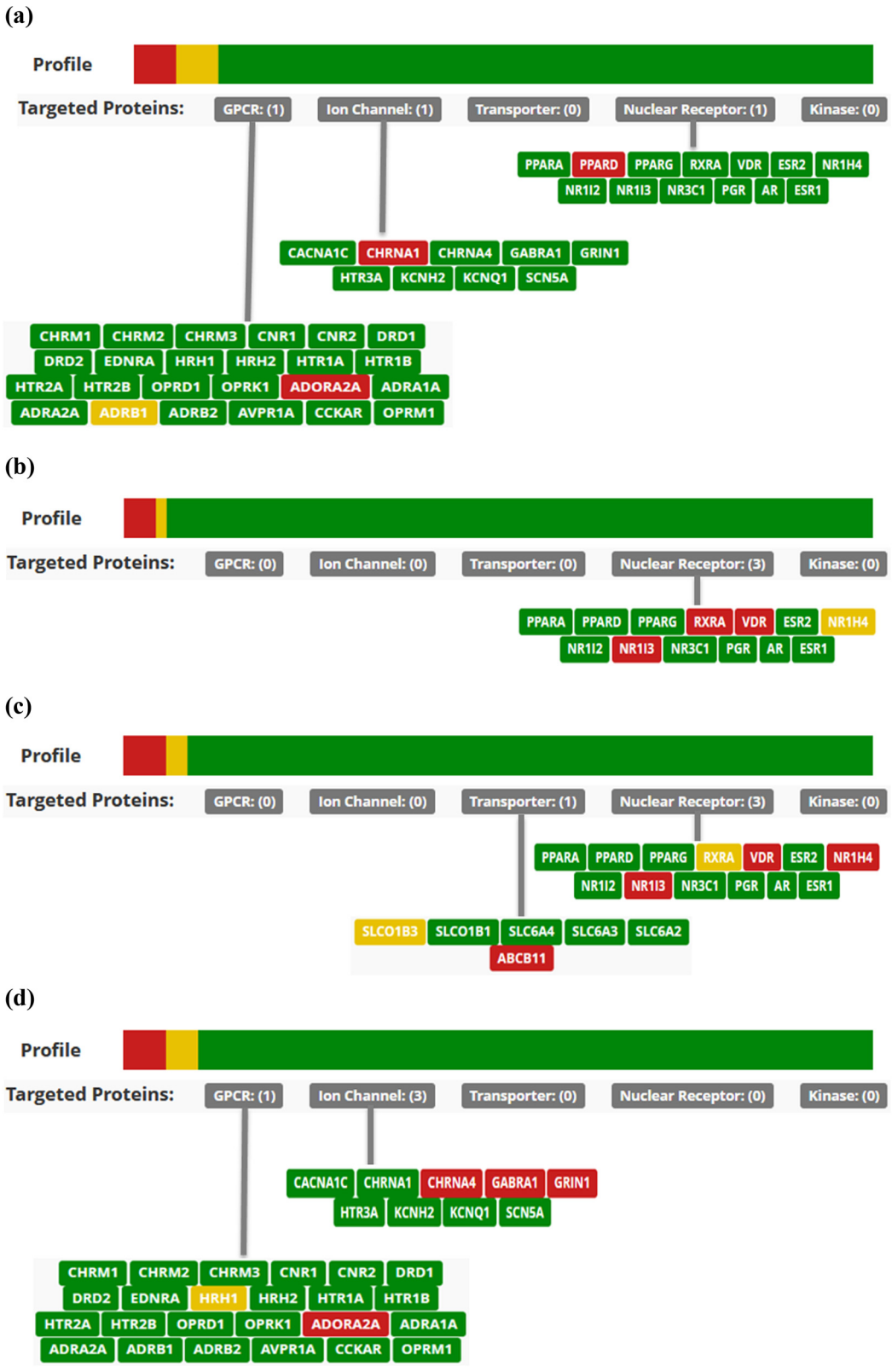


Figure 2: Toxic liability profile of the selected compounds: (a) data of compound 1, (b) data of compound 2, (c) data of compound 3, and (d) data of compound 4.

alpha 1 (GABRA1), and glutamate ionotropic receptor NMDA type subunit 1 (GRIN1), while compound 1 may only interact with cholinergic receptor nicotinic alpha 1 subunit (CHRNA1). It has been reported that nicotinic ligand-gated ion channels are the target for many toxic chemicals, including marine toxins such as lophotoxins and neosurugatoxin, principally

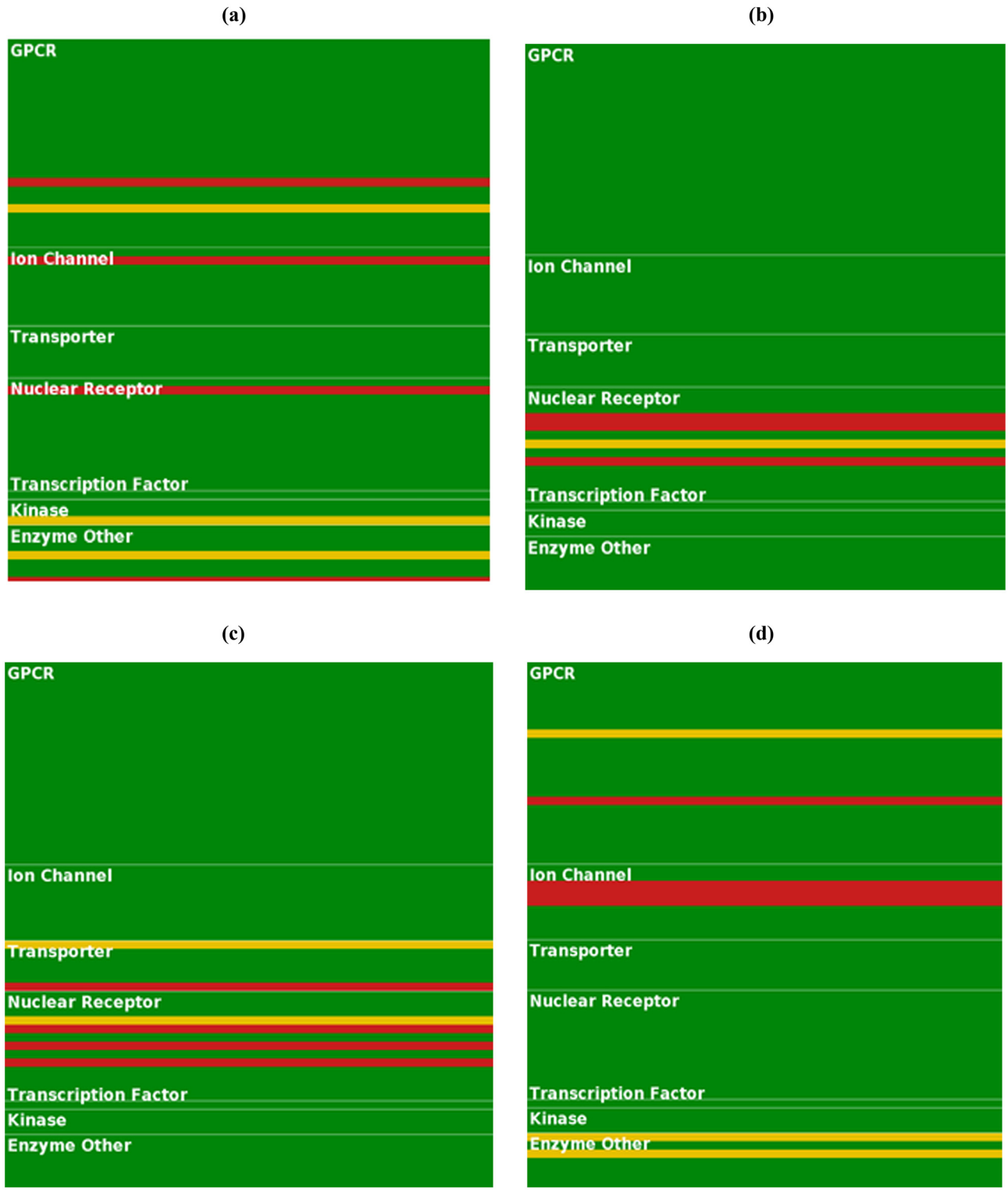


Figure 3: Heatmap prediction of the selected compounds: (a) data of compound 1, (b) data of compound 2, (c) data of compound 3, and (d) data of compound 4.

obtained from gorgonian corals and Japanese ivory shell, respectively [58]. Another study also indicated that this type of receptor is the principal target of neuroactive insecticides [59], and it has been reported that this group of organophosphates may increase the incidence of Parkinson's and Alzheimer's diseases [60], but also responsible for secondary toxic effects in mammals, including the disruption of the cannabinoid system [61]. The possible interaction of compound 4 with GABRA1 suggests that this compound may act in a synergistic way with other toxins such as microcystin, since these categories of toxins may induce a severe damage to cerebrum ultrastructure of zebrafish by defecting the expression of GABRA1 and other subunits [62,63], since GABRA1 is primordial to regulate zebrafish neural circuits, their motility, and larval motility [63,64]. On the other hand, imidacloprid, a well-known pesticide, has been associated with several deteriorations in rat's cognitive functions, especially learning and memory processes by acting on inotropic glutamate receptor (GRIN1) [65,66].

Concerning transporters, data revealed that only compound 3 could be considered a prospective candidate to interact with two transporters, the first one with a high probability and named ATP binding cassette subfamily b member 11 (ABCB11), while in a moderate way with the second one called solute carrier organic anion transporter family member 1B3 (SLCO1B3). Indeed, a novel physiological role of ABCB11 has been revealed by Henkel et al. [65], in which this primary transporter may effectively promote bile acid conservation within the enterohepatic circulation, which could be helpful to remove the excessive amount of cholesterol, while ensuring proper intestinal lipid absorption [67]. However, it has been reported that the accumulation of some drugs in liver tissues such as bosentan, glibenclamide, and metformin may be associated with hepatotoxicity and some forms of intrahepatic cholestasis of pregnancy by directly affecting the function of ABCB11 [68,69]. It is noted that compound 3 could be also effective as a transporter agonist for SLCO1B3, since this transporter is giving a new hope in the treatment of breast and prostate cancers by modulating the transport of estrogen and testosterone [70,71], which could be helpful to develop new strategies to overcome resistance to chemotherapy.

Data also revealed that almost all tested compounds may establish an interaction with five nuclear receptors, except compound 4. Indeed, a high interaction probability could possibly emerge between compound 1 and peroxisome proliferator-activated receptor delta (PPARD), which is very important since the activation of this receptor can considerably attenuate colon carcinogenesis [72] and let us not forget that a long-time exposition to toxic chemicals, including acrylamide, benzene, and organochlorine compounds, may considerably increase the chance to develop this type of cancer [73–75]. At the present time, there is any evidence of the possible interaction of these toxics with PPARD, but several studies have already underlined the possible binding process of these chemicals with PPARα and PPARγ [76–78], which is crucial since these two types of isoforms may have a direct impact on the proliferation or suppression of colon cancer cells [79–81].

However, compounds 2 and 3 seem to be the most active on nuclear receptors and could possibly generate and interact with retinoid X receptor alpha (RXRA), vitamin D receptor (VDR), nuclear receptor subfamily 1, group H, member 4 (NR1H4), and nuclear receptor subfamily 1 group I member 3 (NR1I3). Remarkably, Chen et al. [80] demonstrated that a proper function of RXRA could possibly suppress rat's polymicrobial sepsis at an early stage, but also treat some malignant hematopoiesis diseases like acute promyelocytic leukemia [82]. It is also important to underline that VDR plays a critical role since the expression of 900 genes, which are involved in several physiological functions directly linked to this receptor [83].

Several studies also underlined that VDR may slow down the pathological evolution and complication of oral cancer [84] and cholestatic liver disease [85] via a possible control of the proliferation process, involving key intracellular kinase pathways such as P13 and ROS-dependent ERK/p38MAPK pathways [85–87]. However, it has been reported that VDR dysfunction due to chemotoxins may considerably increase calcium toxicity. This will alter in long-term period motor–cognitive functions, and this has been proved using drug/chemotoxin-induced parkinsonism model in mice [88]. A preclinical study made by Yang et al. [89] demonstrated that regulating the activity of NR1H4 may considerably decrease the probability to develop colon cancer, while NR1I3 may give a promising result in the regulation of drug metabolism and energy homeostasis processes [90]. Finally, it has been reported that the co-administration of drugs may considerably alter NR1I3 and thus the clearance property of the second drug and generate severe toxicity due to a possible imbalance between agonist or antagonist concentrations [91].

Table 6: Total free energy (kcal/mol) calculation of docked compounds

Ligand	Binding affinity (kcal/mol)
Compound 2	−9
Compound 3	−8.3
Compound 1	−6.1
Compound 4	−5.3

Binding affinity is presented from the highest to lowest value.

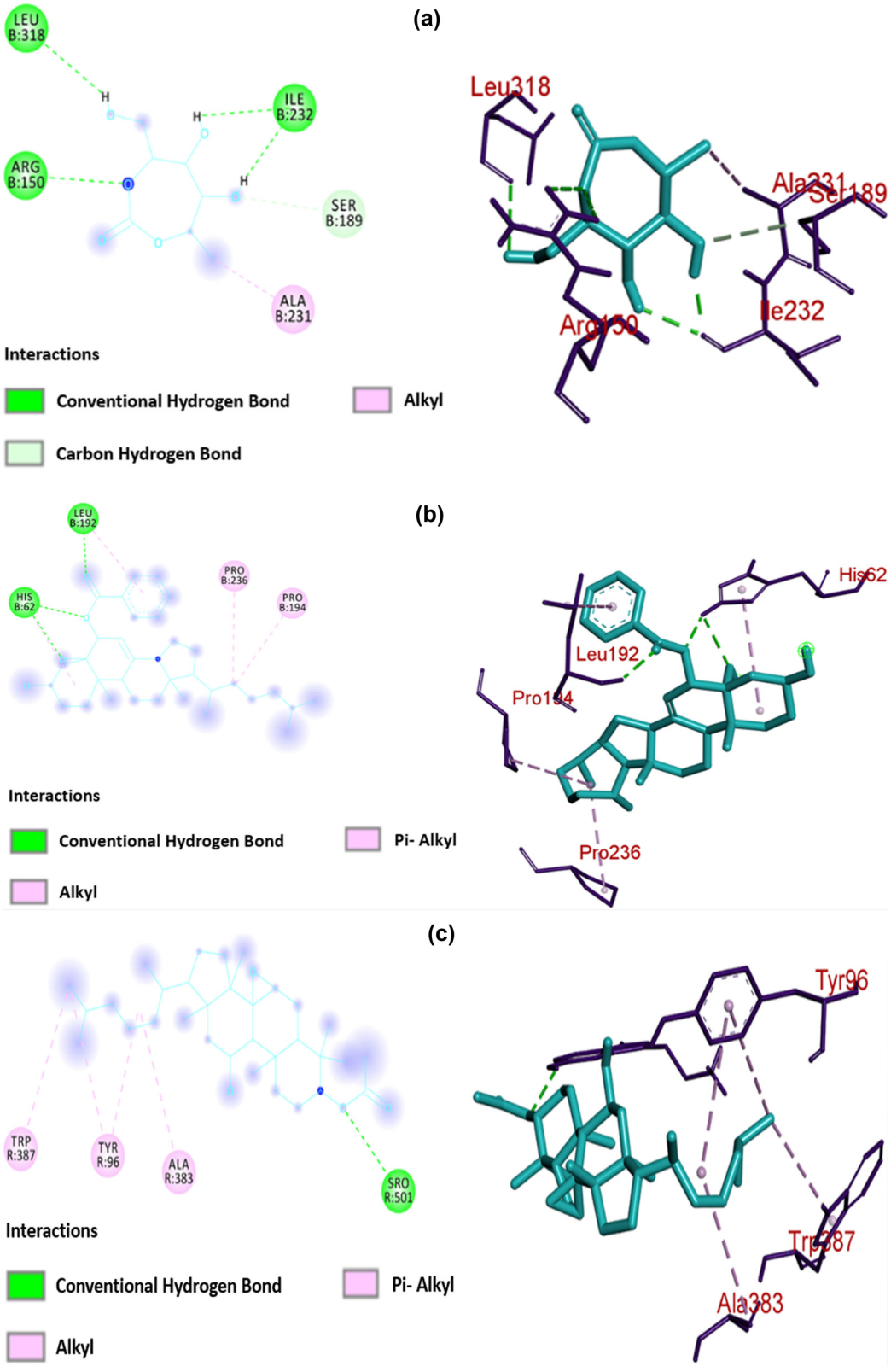


Figure 4: 2D and 3D intermolecular representation of the binding process between compounds and the active site (amino acid residues) of serotonin receptor: (a) compound 1 vs serotonin receptor, (b) compound 2 vs serotonin receptor, (c) compound 3 vs serotonin receptor, and (d) compound 4 vs serotonin receptor.

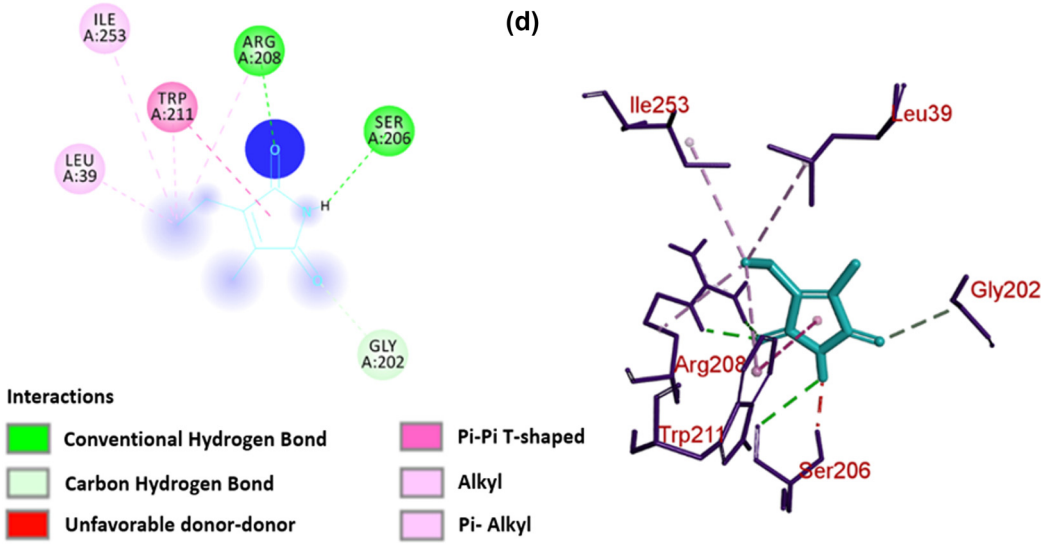


Figure 4: (*Continued*)

It is noted that any interaction has been observed between the four tested phyto-compounds and kinase.

The heatmap of each compound is summarized in Figure 3, and it seems that the heat map is less pronounced for compound 2. This approach could be helpful for future research, especially to compare the results between computational and external experimental safety profile of a drug candidate [92], but also to elaborate a more sophisticated mechanism-based testing strategies, especially those involving animal models [93].

3.5 Molecular docking approach

As a neurotransmitter, serotonin is crucial for the activation process of the largest subtype family of GPCRs. In the current study, an antagonistic effect compound 1–4 on 5-HT2A receptors was studied. The binding affinity values suggested that all compounds are capable to stabilize the protein with energies from −9 to −5.3 kcal/mol (Table 6). Least the docking energy, higher will be the intermolecular interactions of a compound with the target protein, which suggests that compound 2 has the best interaction among all tested candidates.

The 2D and 3D structures depicted in Figure 4b reflect that the compound 2 possess the best score (−9 kcal/mol) that probably stabilizes the protein–ligand complex, by forming conventional hydrogen bond with HIS 62 and LEU 192. It also forms Pi–Alkyl and Alkyl interaction with the residues PRO 194 and PRO 236, respectively. It is also interesting to underline that compounds 2 and 4 shared the same categories of bounds, but compound 4 established two more interactions; carbon hydrogen bond with GLY 202 residue and Pi–Pi T-shaped interaction with TRP 211 residue (Figure 4d). In terms of conventional hydrogen bond and alkyl interaction, compounds 1 and 2 shared the same bounds, but not shared the same residues since compound 1 targeted ARG 150, ILE 232, LEU 318, and ALA 231 residues (Figure 4a). In case of compound 3, it only formed Pi–Alkyl interaction and alkyl interaction with the protein molecule residues as depicted in Figure 4c. It

Table 7: Interaction analyses of amino acids with serotonin receptor (7E2Y)

Compounds/bonds category	Conventional hydrogen bond	Carbon hydrogen bond	Pi–Alkyl interaction	Pi–Pi T-shaped interaction	Alkyl interaction
Compound 1	ARG 150, ILE 232, LEU 318	SER 189	—	—	ALA 231
Compound 2	HIS 62, LEU 192	—	PRO 194, PRO 236	—	PRO 194, PRO 236
Compound 3	—	—	TRP 96, ALA 383, TYR 387	—	TYR 96, ALA 383, TRP 387
Compound 4	SER 206, ARG 208	GLY 202	LEU 39, ILE 253	TRP 211	LEU 39, ILE 253

is noted that Pi–alkyl interaction and alkyl interaction were established by the tested compounds, except for compound 1 as mentioned in Table 7. Hydrogen bonding plays a significant part in the stabilization of the protein–ligand complex. Van der Waals forces and hydrophobic forces also contribute to the stabilization of non-polar ligands [94]. It is also interesting to underline that ligand efficacy is directly related to alkyl chain. Indeed, several hypotheses suggested that ligand's alkyl chain could be involved in receptor activation and increase the receptor/ligand binding affinity by acting as an anchor or by allowing a quick adaptation of a particular receptor in order to accommodate the structural specificities of agonist ligands [95,96]. It is noted that several ligand's alkyl chains have been involved in the activation process of GPCRs [96]. It should be noted that the lowest possible binding energy is indicative of ligands having a significant affinity for the protein molecule; this value can be determined with the help of molecular docking tools and software, which greatly determines and simplifies the nature of binding affinity [97], thus providing crucial information about the possible protein–protein and protein–ligand interactions processes, which play a key role in the organization and homeostasis of biological systems [98].

Let us not forget that an overexpression of serotonin was dramatically associated with several neurodegenerative and neuropsychiatric disorders [99]. Hopefully, at the pharmacological level, recent studies clinically confirmed that the use of serotonin antagonist is giving new hope in effectively treating serious pathological conditions such as hepatic steatosis and dementia, which was proved using serotonin 2A and 5-HT6 receptors, respectively [100,101]. An excessive activity of serotonin may be responsible for some conditions such as migraine, cognitive deficits, and hypertension. That is why, the antagonistic effect of our compounds could be helpful, since they may be used and formulated as blockers to limit the binding process of serotonin with 5-HT2A receptor and thus to limit the activity of serotonin.

4 Conclusion

In terms of novelty, this work introduced recent medicinal chemistry parameters such as Pfizer rule, GSK rule, golden triangle, and MCE-18, but also 12 molecular parameters to reveal the drug-likeness capacities of four phyto-compounds. However, this study's findings revealed that compounds 2 and 3 could be cardiotoxic, making them not suitable candidates to enter in the composition of heart disease medications, since they may produce morphologic lesions in the heart muscle. Concerning liability profile, it seems that our compounds, could preferentially interact with GPCR, ion channels, transporters, and nuclear receptors, which suggests that our compound could be helpful to limit the adverse effects of toxic chemicals, since this category of receptors is considered the main target for 649 highly toxic chemicals. Moreover, the molecular docking method also allowed the visualization of the possible interaction with serotonin 5-HT2A receptor, revealing an antagonistic effect, but with a binding affinity considered more stable for compound 4 (5.3 kcal/mol) and establishing six various types of interaction with active sites of the receptor. This could be helpful in the near future, especially to understand the molecular mechanisms of blockers but also the radioligand binding process and experiments in isolated nervous tissues. However, due to the limitations of this study, it was not possible to experimentally validate the accuracy of the various results related to these theoretical predictions. That is why, wet laboratory experiments should be performed to validate the findings of this preliminary work, but also to properly investigate their features with appropriate preclinical and clinical/observational approaches.

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data generated or analyzed during this study are included in this published article.

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