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Chapter 8

Bioactive Coumarins and Xanthones From Calophyllum Genus and Analysis of Their Druglikeness and Toxicological Properties

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Chapter Outline

INTRODUCTION

Several authors have stated that the pharmaceutical industry is facing a productivity crisis, as there is stagnancy in the number of new drug approvals and an increasing expenditure in the research and development processes [1]. Among the several strategies depicted to solve this problem, natural

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products have been proposed as molecules that could effectively be introduced into the pharmaceutical market [2]. Undoubtedly, natural products have been produced as a result of millions of years of evolution of terrestrial and marine organisms in the struggle of adapting to a number of abiotic and biotic stresses; therefore they are encoded to be bioactive [3]. Natural products have been historically used in the pharmaceutical industry for the discovery of bioactive compounds and as precursors for the development of new active molecules, for example: paclitaxel, docetaxel, vincristine, vinblastine, penicillin, and more recently trabectedin and romidepsine [4].

More recently, the use of standarized herbal extracts has been a clear example of the development of new "botanical" drugs, such as Veregen and Fulyzaq, currently approved by the US Federal Drug Administration (FDA) for the treatment of genital warts and HIV-associated diarrhea [5], respectively. In addition, Sativex, a titrated extract containing δ-9-tetrahydrocannabinol, has been approved since 2005 in several countries (Canada, UK, Germany, and New Zealand) for the treatment of spasticity due to multiple sclerosis and central and peripheral neuropathic pain of various origins [3]. In this context, it is clear that advances in rapid genomics sequencing coupled with manipulation of biosynthetic pathways, as well as the recent advances in metagenomics, may provide vast resources for the future discovery of pharmaceutical agents derived from natural products [6].

The *Calophyllum* genus is a large group of tropical tree species belonging to the Calophyllaceae family (180–200 species) that represents an important source of natural products and is mainly distributed in the tropical regions of Asia, Africa, the Americas, Australasia, and the Pacific Islands [7]. These species contain coumarins and xanthones with significant biological activities, such as: cytotoxic, antiviral, chemopreventive, antisecretory, cytoprotective, analgesic, and antimicrobial [8,9]. Most species of this genus are used in folk medicine to treat peptic ulcers, malaria, tumors, infections, venereal diseases, hypertension, pain, and inflammation, among other health problems [10].

BOTANICAL BACKGROUND OF *CALOPHYLLUM* GENUS

The *Calophyllum* species are quite complex and difficult to classify; even most taxonomists who have studied this genus speak of the confusion and frustration experienced when trying to differentiate its species, as it seems challenging to establish definitive boundaries between them [11]. The *Calophyllum* genus belongs to a family of plants known as Calophyllaceae according to the classification system APGIII (Angiosperm Phylogeny Group) [12]; it was formally included in the Clusiaceae or Guttiferae, from which it was segregated. It consists of nearly 187 species; 179 thrive in the Old World and are distributed mainly in the Indo-Malaysian region; the most important one is *C. inophyllum*. On the other hand, only eight species are found in the New World and are distributed from Mexico to Argentina and the Caribbean region; the most important one is *C. brasiliense*. There is a widespread belief that this taxon is the origin of all (or most) other American species of this genus [13]. Most species of this genus

are medium-size trees, although some of them very high while others are shrubs. The wet tropical rainforest of the lowlands is the habitat of most species, but some are found in drier areas or at higher altitudes, and many are in flooded areas. There are several taxonomic characteristics of the genus. The outer bark is red with diamond-shaped fissures, and the oppositely arranged leaves are very distinctive, with narrow parallel veins alternating with resin canals often borne on petioles. The inflorescence is a racime, or a thyrse of flowers grow from the leaf axils or at the ends of branches, with terminal or axillary inflorescences. The sepals and petals are arranged in hermaphrodite flowers. The fruit is a berry (drupe fruit) with thin layers of flesh over a large seed; it produces a white or yellow latex (Figs. 8.1 and 8.2) [7].

CHEMISTRY OF CALOPHYLLUM GENUS

The first chemical analysis of *Calophyllum* was published in 1950 by Polonsky and Ormancey-Potier [14]. These authors elucidated the chemical structures of inophylollide, calophylollide, and calophyllic acid isolated from the leaves and fruits of *C. inophyllum*. Afterward, several species have been widely studied for their phytochemical content [8,10,15]. Generally speaking, the *Calophyllum* genus has a wide variety of triterpenes belonging to different groups: fridelane (frideline, fridelan 3β-diol, and canophyllol), oleanane (canophylic acid and apetalactone), lupane (betulinic acid), taraxerane (taraxerol and taraxerone), and finally, the group of adianane (3β-simiarenol).

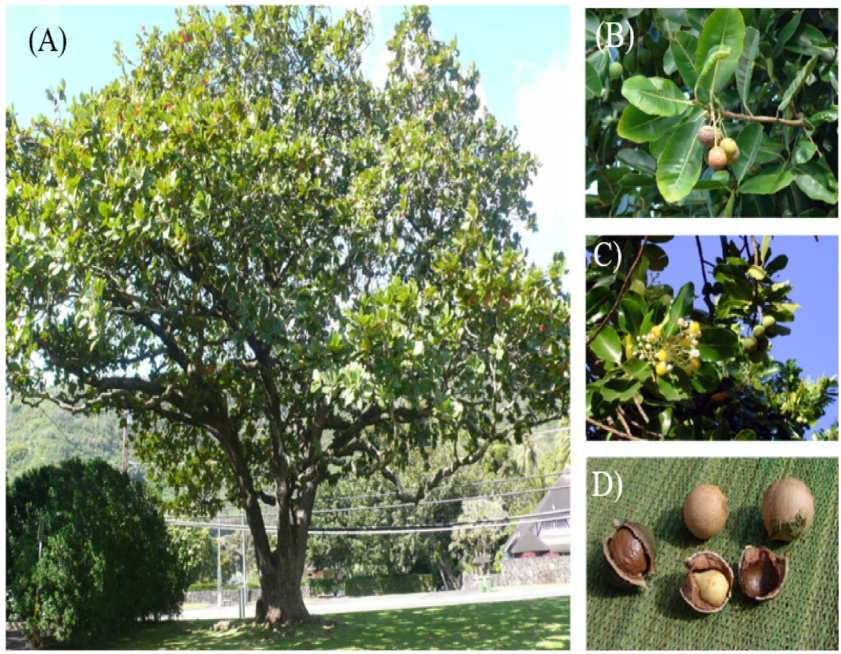

FIG. 8.1 *Calophyllum inophyllum*. (A) Tree; (B) Leaves; (C) Inflorescence; (D) Fruit.

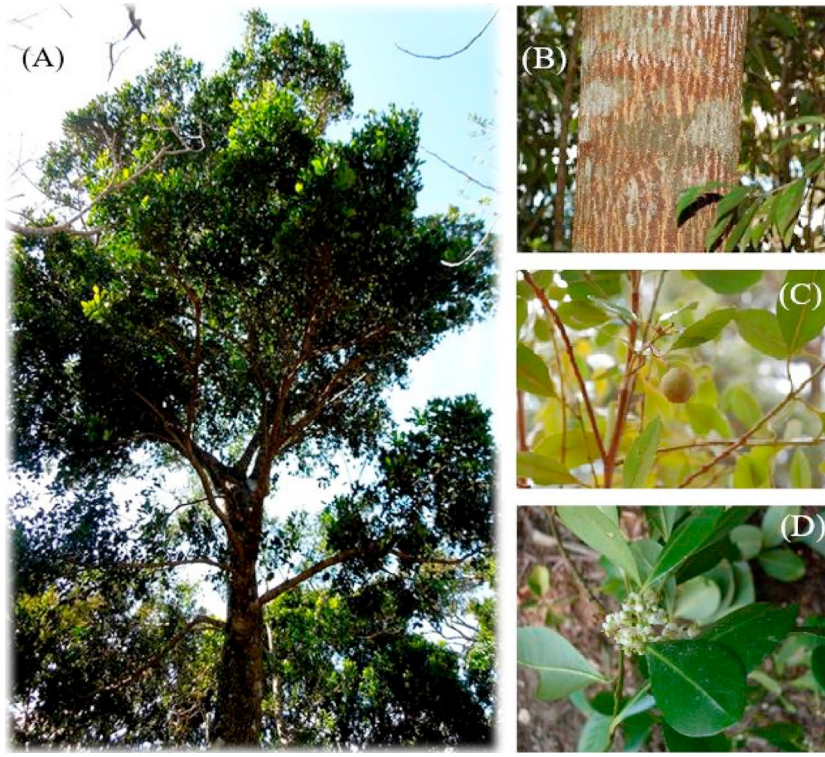

FIG. 8.2 *Calophyllum brasiliense*. (A) Tree; (B) Bark; (C) Fruit; (D) Leaves and Flowers.

Polyphenols such as flavonoids, mainly quercetin and epicatechin, have been isolated from the leaves of several species of this genus. Also, small phenolic compounds such as gallic acid, shikimic acid, or protocatechuic acid, have been isolated from the leaves. Coumarins (Fig. 8.3) and chromanones are common in this genus. Most of the species biosynthesize these compounds in the leaves, some of them can be used as biomarker metabolites and exhibit interesting pharmacological activities. Xanthones (Fig. 8.4) have been isolated from the wood and bark of several species of this genus.

Active Coumarins From *Calophyllum* Species

Several kinds of coumarins have been isolated from the leaves, bark, fruits, and latex of the *Calophyllum* species, among them tricyclic, tetracyclic, and mammea type (Fig. 8.3). Table 8.1 presents a detailed account of the active coumarins isolated from different *Calophyllum* species from 2010 to 2015 on Scopus database. Several dipyrano tetracyclic coumarins have received attention due to their anti-HIV activity, such as calanolides, inophyllums, and cordatolides; these compounds are nonnucleoside-reverse transcriptase inhibitors. Among the most relevant of anti-HIV coumarins are the calanolides. Calanolide A was completely protective against HIV-1 replication,

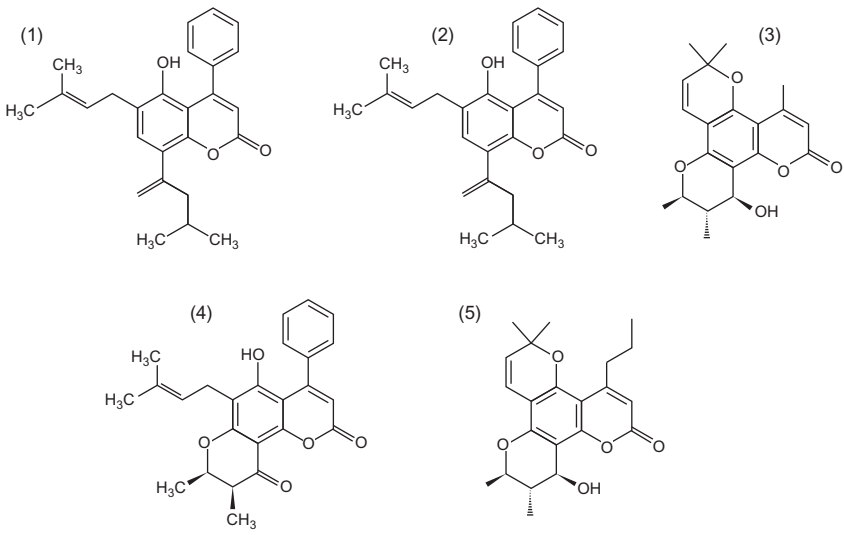

FIG. 8.3 Pharmacologically active coumarins isolated from the species of *Calophyllum*. Mammea A/BA (1), Gut-70 (2), Cordatolide A (3), Calocoumarin A (4), Calanolide A (5).

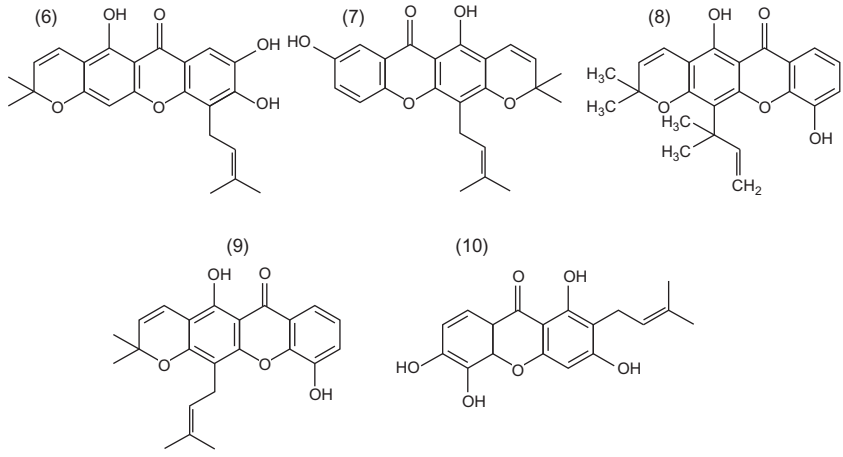

FIG. 8.4 Pharmacologically active xanthones isolated from *Calophyllum* species. (6) Caloxanthone A; (7) Brasixanthone; (8) Blancoxanthone; (9) Trapezifolixanthone; (10) Xanthone V.

showing an $IC_{50} = 5.9 \pm 1.9$ μM [46]. It even reached clinical phase II, where it showed to be apparently safe at therapeutical levels in plasma [47]. Inophyllums isolated mainly from *C. inophyllum* also have shown to be active against HIV; for example, inophyllum B and P have shown $IC_{50} = 38$ and 130 nM, respectively [48]. Cordatolides A and B isolated from *C. cordato-oblongum* have shown $IC_{50} = 12.3$ and 19 μM, respectively, against HIV-1[49].

Other kinds of active coumarins isolated from this genus are mammea-type coumarins, such as mammea A/BA, A/BB, B/BB, B/BA, Gut-70, among others. They have shown to be active against several types of cancer cell lines,

TABLE 8.1 Pharmacologically Active Coumarins and Xanthones Isolated From *Calophyllum* Species (2010–2015)

Species	Coumarines Or Xanthones	Pharmacological Effect	Observations	Ref.
C. inophyllum	Calophyllolide	Osteogenic activity	[+] 30 μM	[16]
		Antimicrobial properties against *Staphylococcus aureus*	[+]16 μg/disk	[17]
	Inophinnin Inophinone Soulatrin, Phylattrin Pyranojacareubin Rheediaxanthone A Macluraxanthone 4-Hydroxyxanthone Caloxanthone C, Brasixanthone B, Trapezifolixanthone	Antiproliferative effect against B-lymphocyte cells (Raji)	[Δ]1.0–9.9 μg/mL	[18]
		Colon carcinoma cells (LS174T)	[Δ]μ6.51 – 3.1 g/mL	
		Human neuroblastomacells (IMR-32)	[Δ]μ5.53 – 72.0 g/mL	
		Skin carcinoma cells (SK-MEL-28).	[Δ]μ6.51 – 75.0 g/mL	
	Inophyllin A	Induces oxidative stress mediated-apoptosis in Jurkat T lymphoblastic leukemia cells	[Δ]50 μM	[19]
	Furanoxanthone Inophinnin Inophyllin A Macluraxanthone Pyranojacareubin 4-Hydroxyxanthone Caloxanthone B	NT		[20]

	Caloxanthone Q Xanthone B Jacareubin 6-Deoxyjacareubin Caloxanthone O Caloxanthone P	NT		[21,22]
C. brasiliense	GUT-70	Inhibition of HIV-1 entry by the modification of membrane fluidity	$^{\Delta}$10 μM	[23]
		Inhibition of Hsp90 in mantle cell lymphoma	$^{\Delta}$1.5 – 6.3 μM	[24]
	Calanolide B and C	Inhibition of HIV-1 reverse transcriptase	$^{\Delta}$20.2 μg/mL	[9]
	Mammea A/BB Mammea B/BB	Anti-M. tuberculosis H37Rv strain activity	$^{\Delta}$31.2 and 62.5 μg/mL	[25]
	Mammea A/BA Mammea A/BB	Cytotoxicity in K562 leukaemia cells	$^{¥}$43.5 μM	[26]
	Mammea A/BB	Antileishmanial activity *in vitro, in vivo* in mice infected with *Leishmaniaamazonensis*.	*18 mg/kg/day	[27]
		Ultrastructural alterations on mitocondrial membrane of *Leishmania amazonensis*	**7.4 μM	[28]
	Soulamarin	Induces plasma membrane permeabilization of *Trypanosoma cruzi* and mitochondrial dysfunction	$^{¥}$219 μM	[29]
	Xanthone III Xanthone V	Antioxidant properties: Prevention of oxidative damage induced by $FeSO_4$	$^{¥}$2.5 μM	[30]

Continued

TABLE 8.1 Pharmacologically Active Coumarins and Xanthones Isolated From *Calophyllum* Species (2010–2015)—cont'd

Species	Coumarines Or Xanthones	Pharmacological Effect	Observations	Ref.
C. soulattri	Soulattrin Caloxanthone C Macluraxanthone Brasixanthone B Trapezifolixanthone	Cytotoxic activity against: SNU-1, HeLa, Hep G2, NCI-H23, K562, Raji, LS174T, IMR-32 and SK-MEL-28 cells	[¥]0.69 – 92.59 μM	[31,32]
	Phylattrin Soulattrin Caloxanthone C Brasixanthone B Trapezifolixanthone	Antiproliferative effect against B-lymphocyte cells (Raji)	[Δ] 1.0 – 8.3 μg/mL	[18]
		Colon carcinoma cells (LS174T)	[Δ]μ6.51 – 52.1 g/mL	
		Human neuroblastomacells (IMR-32)	[Δ]μ7.31 – 72.0 g/mL	
		Skin carcinoma cells (SK-MEL-28)	[Δ]μ7.31 – 75.0 g/mL	
	Soulamarin Caloxanthone B Caloxanthone C Macluraxanthone Trapezifolixanthone Brasixanthone B	NT		[33]
C. flavoranulum	Flavoranulum coumarin	Antiplasmodial Activity Against *Plasmodium berghei*	[Δ]1.5x10^{-5} mg/mL	[34]
C. apetalum	Dipyranocoumarin α-Hydroxytomentolide A	NT		[35]

C. benjaminum	Benjaminin Fuscaxanthone C ß-Mangostin Thwaitesixanthone, Dombakinaxanthone Caloxanthone A	NT		[36]
C. hosei	Hoseimarin Trapezifolizanthone Osajaxanthone β-Mangostin Caloxanthone A	NT		[37]
C. thorelii	Calothorexanthone 1,4,8-Trihydroxyxanthone δ-Tocotrienol 1,7-Dihydroxyxanthone Globuxanthone.	Antioxidant activity tested using DPPH free radical scavenging assay	[Δ]13.63 – 17.46 µg/mL	[38]
	Thoreliones A and B Oxy-thorelione A	Cytotoxic activity against MCF-7	[¥]7.4 µg/mL	[39]
		HeLa	[¥]9.3 µg/mL	
		NCI-H460	[¥] 10.6 µg/mL	
C. gracilipes	Zeyloxanthanone Gracixanthone Trapezifolixanthone	Cytotoxic activity against MCF-7, HTC-116,PC3, VERO and RAW264.7 cells.	[¥]8.00 – 26.00 µM	[40]

Continued

TABLE 8.1 Pharmacologically Active Coumarins and Xanthones Isolated From *Calophyllum* Species (2010–2015)—cont'd

Species	Coumarines Or Xanthones	Pharmacological Effect	Observations	Ref.
C. symingtonianum	1,3,5-Trihydroxy-2-(3-methylbut-2-enyl) xanthone 6-Desoxyjacareubine	Antifungal activity against *Gloeophyllum trabeum*, and *Pycnoporus sanguineus.*	$^{\Omega}25\mu g - 50\ \mu g$	[41]
C. nodusum	Nodusuxanthone Trapezifolixanthone A	NT		[42]
C. venulosum	Venuloxanthone Tovopyrifolin C Ananixanthone Caloxanthone I	NT		[43]
C. membranaceum	Brasixanthone F Brasilixanthone B Gracilixanthone Rheediachromenoxanthone 2-Hydroxy-1-methoxyxanthone 2-Methoxy-3-hydroxyxanthone	NT		[44]
C. polyanthum	7,4′-Dihydroxy-6,8-dimethoxy-4-phenylcoumarin 7-Hydroxy-6,8,4′-trimethoxy-4-phenylcoumarin	NT		[45]

**Doses that significantly reduced skin lesions in footpads of mice;*
$^{¥}CC_{50}$=Cytotoxic concentration (μM);
$^{\Delta}IC_{50}$=50% Inhibitory concentration of growth or enzyme activity;
$^{\Omega}$MIC=Minimum inhibitory concentrations;
$^{+}EC_{50}$=Concentration that increased the alkaline phosphatase (ALP) early marker molecule of osteoblastic differentiation in MC3T3-E1 cell line ($\mu mol \cdot L^{-1}$),
$^{**}LD_{50}$=lethal doses, NT=non tested.

including PC3, HTC116, U251, and six human leukemic cell lines: BV173, K562, MALM6, HL60, SEM, and a P-glycoprotein over-expressing cell line). These compounds induced caspase-mediated cell death in most of these cells [50,51]. Interestingly, some of these coumarins (mammea A/BA, A/BB, B/BB) also have shown to be active against *Trypanosoma cruzi* and *Leishmania amazonensis* [52,53], inducing disruption of mitochondrial swelling and loss of normal ultrastructure in this parasite [28].

Active Xanthones Isolated From the *Calophyllum* Species

Xanthones have also shown to be active components of this genus with numerous pharmacological activities (Fig. 8.4). Detailed information on pharmacological active xanthones isolated from different *Calophyllum* species from 2010 to 2015, according to the Scopus database. is presented in Table 8.1. Seven antimalarial xanthones were isolated from *C. caledonicum* with $IC_{50} = 0.8–4.4$ μg/mL) [54]. Four xanthones isolated from *C. brasiliense* showed to be inhibitors of sulfotransferases (SULT1A1 and SULT2A1) with values of $IC_{50} = 1.6–7.4$ μM [55]. Xanthones isolated from *C. brasiliense* have also revealed to be active against Chagas disease with $IC_{100} = 153–213$ μM values against tripomastigotes [56]. Xanthones also possess antiviral activities; for instance, five pyranoxanthones isolated from *C. blancoi* showed to be highly active against the coronavirus ($EC_{50} = 3–15$ μg/mL) [57]. In addition, brasixanthones A-D isolated from *C. brasiliense* displayed 100 percent of antiproliferative activity over the Epstein-Barr virus in Raji cell line [58].

CHEMOINFORMATICS AND TOXICOINFORMATICS IN NATURAL PRODUCTS AND DRUG DISCOVERY

Chemoinformatics and Drug Discovery

Chemoinformatics, also called cheminformatics or chemical information science, has various definitions, for example, "the application of informatic methods to solve chemical problems" or to predict possible toxicological properties derived directly from structural data as compared to other previously known to be experimentally toxic (toxicoinformatics). It is considered as an interface between chemistry and informatics or as a collection of methods used for drug design and discovery [59]. Chemoinformatics comprises a plethora of computational techniques to organize, mine, visualize, and analyze the diversity and coverage of the chemical space of compound collections. The most prominent and widely used example is Lipinski's Rule of Five. The Rule of Five denotes a set of property rules describing orally bioavailable drug space [60]. Briefly during the 1990s, the pharmaceutical industry noticed that too many compounds were terminated in clinical development because of unsatisfactory pharmacokinetics (PK). Lipinski analyzed the attrition problems of the pharmaceutical industry [61] and came to this surprising conclusion: A simple set of physicochemical parameters were associated with 90 percent of orally active

drugs that achieved phase II status. These parameters were later called the "Rule of Five" (RO5) and comprised the following: hydrogen-bond donors <5, molecular mass <500, calculated log P (partition coefficient between water and 1-octanol) <5, the sum of nitrogen, and oxygen atoms (hydrogen-bond acceptors) <10. Extensions to the rule are: polar surface area<140 Å^2, sum of H-bond donors and acceptors <12, and rotatable bonds <10. Compounds that successfully pass through these rules could be called "druggable" or "druglike" [62]. Candidate drugs that conform to these rules tend to have lower attrition rates during clinical trials, and hence have an increased chance of reaching the market [63]. This rule is one of the first examples of chemoinformatics and drug development. As chemoinformatics treats molecules as graphs and its descriptors with associated features (mainly physicochemical properties and biological activity), the ensemble of graphs (set of molecules) and its descriptors form a chemical space in which the relationship between each compound must be defined. This is a basic concept of chemoinformatics [59].

Chemoinformatics and Natural Products

The rules of chemoinformatics are not explicitly taken from rigorous physical models; rather, they are learned inductively from the data [59]. In this context, this tool for the word chemoinformatics is very helpful in order to analyze natural products, as some of them posses highly complex structures. The use of chemoinfomatics approaches in natural products databases could help to reduce hit multiplicity and improve the process of *hit-to-lead* identification. Natural products (NPs) have been selected during evolution to bind to various proteins during their life cycle (e.g., in biosynthesis and degradation) and while exerting their mode of action. As a result, NP structures are good starting points for the discovery and development of protein ligands and ultimately for drug discovery. NPs in general constitute biologically validated starting points for library design, and many of their core structures have been recognized as privileged structures (e.g., benzodiazepine).

Henkel et al. published the first work in which natural products and its pharmacophoric groups were analyzed; they found that some structural units were prone to interact with biological macromolecules [64]. Lately, Lee and Shenider (2001) compared a set of parameters related to Lipinski's Rule of Five for trade drugs and natural products [65]. They discovered that natural product molecules contained an average of 1.4 nitrogen atoms per molecule, one less than the drug set, and the calculated log P values indicate that natural products are more lipophilic than drug molecules (2.9 vs. 2.1). Surprisingly, only 10 percent of all molecules violate the Rule of Five [66].

Feher and Schmidt (2003) found that natural products, on average, contained two more oxygen atoms, were more unsaturated, and possessed fewer sulphur and halogen atoms. Additionally, the number of rotatable bonds was two times less than in commercial drugs, indicating that natural products are more rigid molecules than common drugs [67]. These results were confirmed later by Ertl and Schuffenhauer (2008); they tested a higher database of natural products (130,000 molecules), including marine products, and

confirmed that the majority of natural products do not violate Lipinski´s Rule of Five [68]. Natural products differ from most drugs in their heteroatom distribution, number of rings, and degree of ring fusion; additionally, they are more lipophilic [66]. Natural products must be considered as a very heterogeneous group that varies according to the original organism, the current environment, and their role in nature. Feher and Schmidt also analyzed natural products and seminatural derivatives. It is interesting to note that they found seminatural derivatives to possess a high rate of druglike properties, which indicates that in spite of the complexity of these heterogeneous molecules, natural products may serve as an appropriate inspiration for new drug design.

Toxicoinformatics and Drug Discovery

Among the main problems of drug development and new drug discovery are toxicity and adverse effects. Furthermore, it has been estimated that 95 percent of failures in clinical phases address toxicological issues and adverse effects [69]. Therefore it becomes quite important to assay new active compounds in all toxicological available tests before any clinical testing. In this context, the use of new technologies, such as computational approaches, becomes quite important, as most of in silico approaches are less expensive than in vitro or in vivo experiments and could help make a more accurate decision in drug development. Over the last 30 years, toxicology has improved greatly in the development of new tools for testing. It has become related with other recent disciplines such as chemistry, ecology, genomics, epidemiology, and statistics, and new subspecialities have arisen, such as ecotoxicology, forensic toxicology, molecular clinical toxicology, toxicogenomics, and toxicogenetics, to name just a few examples. The discipline has grown, and so has the amount of new data and toxicity information [70], such as: raw data laboratory, field data statistics, epidemiology reports, hazard monitoring, exposure data, regulatory information, visual images from pathology, genetical expression and proteomic data, physicochemical properties, and QSAR analysis. In this context, toxicoinformatics has emerged as a subdiscipline that can be divided in two terms: first for the collection, management, and dissemination of data, and second for understanding the complex molecular mechanisms of toxicity for obtaining new information from databases that could help to prevent, predict, and treat toxicological effects. Chemical information is inherently a part of toxicology domain, and the development of chemoinformatics supports progress in toxicology [70]. The number of new compounds discovered per year is estimated at 1 million [71], and the information available for these compounds (e.g., pharmacological or toxicological activities, bibliographic text, physicochemical properties, structures, and reactions, among others) is available on line on several public databases. Therefore the use of several chemoinformatic methodologies, such as chemical space, may help predict possible toxicological properties derived directly from the chemical structural data in a chemical space formed by a comparison of different properties with compounds known to be toxic (toxicoinformatics).

Several toxicoinformatic databases have been already published and are available online. Sone et al., published pCEC (Profiles of Chemical Effects on Cells), a

toxicological database with a system of classifying chemicals that have effects on human health; this database stores and handles gene expression profiling information and categories of toxicity data [72]. TOXicology Data NETwork—TOXNET (http://www.toxnet.nlm.nih.gov), is a web-based information system that provides access to information regarding the medical, occupational, pharmacokinetic, toxicological, and environmental effects of biological and chemical substances and is maintained by the National Library of Medicine's Division of Specialized Information Services, a section of the NIH [73]. One of the most popular resources for chemoinformatic and toxicoinformatic approaches, Osiris Property Explorer, allows the user to draw chemical structures and calculate various on-the-fly drug-relevant properties. While drawing a structure, the toxicity risk predictor starts to look for potential toxicity risks, such as mutagenicity, tumorigenicity, irritating effects, and reproductive effects. Toxicity risk alerts are indications that the drawn structure may be harmful concerning the risk category specified. The prediction process relies on a precomputed set of structural fragments that give rise to toxicity alerts in case that they are found in the structure currently drawn. Afterwards, a substructure search process determines the frequency of any fragment (i.e., core and constructed fragments) occurring within all compounds of that toxicity class, since these fragments frequencies belong to the structures of more than 3000 traded drugs. Based on the assumption that traded drugs are largely free of toxic effects, any fragment was considered a risk factor if it occurred often as a substructure of harmful compounds, but never or rarely in traded drugs [74]. Other interesting databases where toxicological information and applications are very useful for toxicological in silico approaches are: Comparative Toxicogenomics Database (http://ctdbase.org/), Toxpredict (https://apps.ideaconsult.net/ToxPredict), and Leadscope Toxicity Database (http://www.leadscope.com). However, a detailed analysis of these are beyond the purpose of this review.

Toxicoinformatics and Natural Products

According to our review and bibliographical analysis, as most of the toxicoinformatics approaches are very novel, there are few studies on toxicoinformatics analysis and natural products databases. In 2014, Saxena tested 80 natural compounds using TEST (Toxicity Estimation Software Tool), which predicts the mutagenicity value, indicating that some natural compounds may be positive in this parameter [75]. In this context, the introduction of more toxicoinformatic studies in natural products represents a novel subject that could help to improve the pharmaceutical development of this type of compounds and could help to develop new libraries of novel structures derived from natural bioactive molecules.

MOLECULAR PROPERTIES OF COUMARINS AND XANTHONES FROM *CALOPHYLLUM* SPECIES

As previously mentioned, it is important to emphasize that many properties of natural products could depend strongly on the data and the chemical space in which they are analyzed. In this context, we obtained some of the main

properties of the principal active coumarins and xanthones isolated from the *Calophyllum* genus in order to analyze their potential as drug leads or as inspiration molecules for the design of new drugs. This was done by setting a chemical space for each group according to their main physicochemical and toxicological properties using the Osiris Molinspiration Property Explorer. The chemoinfomatics analysis consisted of obtaining the main theoretical physicochemical (ChemMine Tools-JoelLib descriptors) and toxicological properties (Osiris-DataWarrior) to determine whether such compounds could be considered for the development of new drugs, as leads, or as hits (drugability). The list contains 70 coumarins and 70 xanthones along with the results of their physicochemical properties (Table A.1, Appendix). The physicochemical properties of coumarins and xanthones were calculated using ChemMineTools based on JoelLib descriptors; a summary of main properties is presented in Table 8.2. The following properties, were evaluated: MW, Log P, number of HBA, number of HBD, acidic groups, aliphatic OH, basic groups, FRB, heavy bonds, heterocycles, hydrophobic groups, MR, number of atoms, number of halogen atoms, B, Br, Cl, I, F, N, O, P, S, and number of bonds.

Table 8.2 shows a summary and comparison of the main properties for coumarins and xanthones. There are molecular differences in both types of compounds, such as molecular weight, number of bonds, log *P*, druglikeness, molecular refractivity, and PSA. This is not surprising, as most of the coumarins possess a higher molecular weight and a more complex structure, which explains the main differences between most of these properties. It is of interest to note that xanthones possess higher values for druglikeness. Most of the coumarins have a similar pattern of physicochemical properties; however, there is heterogeneity in molecular weight (MW), molecular refractivity (MR), number of atoms, number of bonds, and polar surface area (PSA). Inocalophyllin A, B and inocalophyllin A methyl ester are a particular cluster, as they possess higher scores for MW, MR, structural complexity, and higher number of double bonds (i.e., higher value for polarizability of a mole of subtance) that grant them each of the mentioned properties (Figs. 8.5 and 8.6). On the other hand, most of xanthones isolated from *Calophyllum* spp. (Table A.1) possess the same pattern of physicochemical properties; this is because unlike coumarins, these compounds have greater structural homogeneity.

Chemoinformatic analysis indicated that 57/70 coumarins (Fig. 8.6) and 59/70 xanthones (Fig. 8.7) are in compliance with Lipinski's Rule of Five [61]. Among both groups, two compounds, 2-hydroxyxanthone and caledonixanthone-B, possess potential of leadlikeness (Fig. 8.8). These are also members of compound libraries that follow Lipinski's Rule of Five as well as the rule of three [76]; that is, log *P* is not greater than 3; molecular mass is less than 300 daltons; there are no more than 3 hydrogen bond donors and 3 hydrogen bond acceptors; and there are no more than 3 rotatable bonds, so there will be an easier and fast time in delivering optimized drug development candidates. Compounds 2-hydroxyxanthone and caledonixanthone-B have been isolated from *C. caledonium,* and none of them have been tested for any pharmacological properties; therefore both molecules were tested on

TABLE 8.2 Main Physicochemical Properties of Active Coumarins and Xanthones Isolated From *Calophyllum* Genus

Properties	Coumarins		Xanthones		% Difference
	Range of Values	*Average (SD)*	*Range of Values*	*Average (SD)*	
MW	340.46–569.80	405.00 (43.2)	460.53–212.20	319.36 (63)	85.64
Log *P*	9.96–6.65	8.66 (0.86)	13.57–1.34	5.25 (2.14)	3.41
HBA	7.00–3.00	5.00 (1.00)	8.00–3.00	5.00 (1.00)	0.00
HBD	4.00–0.00	1.00 (1.00)	5.00–0.00	2.00 (1.00)	1.00
# acidic groups	1.00–0.00	0.06 (0.23)	1.00–0.00	0.03 (0.17)	0.03
Aliphatic OH	2.00–0.00	0.68 (0.74)	2.00–0.00	0.16 (0.44)	0.52
Log *S*	−3.35–(−6.64)	−5.04 (0.81)	−3.67–(−7.79)	−5.11 (1.01)	0.07
Druglikeness	0.96–(−27.79)	−5.98 (5.51)	0.61–(−12.27)	−1.63 (1.85)	4.35
Hydrophobic groups	0.00	0.00	0.00	0.00	0.00
Molecular Refractivity	225.27–120.09	147.69 (17.7)	70.81–181.70	108.11 (27.00)	39.58
Number of bonds	94.00–46.00	61.97 (8.5)	71.0–26.0	42.23 (11.34)	19.74
PSA	128.20–46.53	77.17 (15.76)	131.36–35.53	81.42 (19.64)	4.25

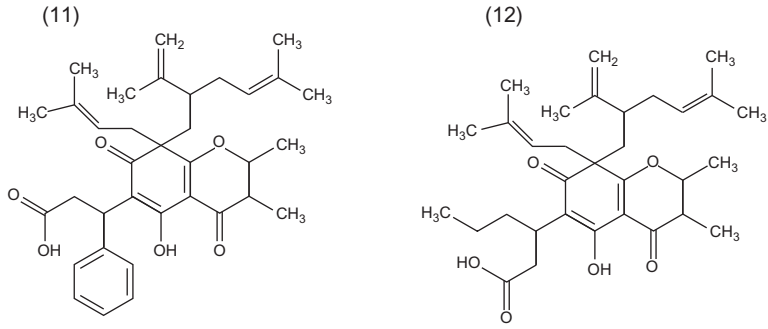

FIG. 8.5 Structures of Inocalophyllin A (11) and Inocalophyllin B (12).

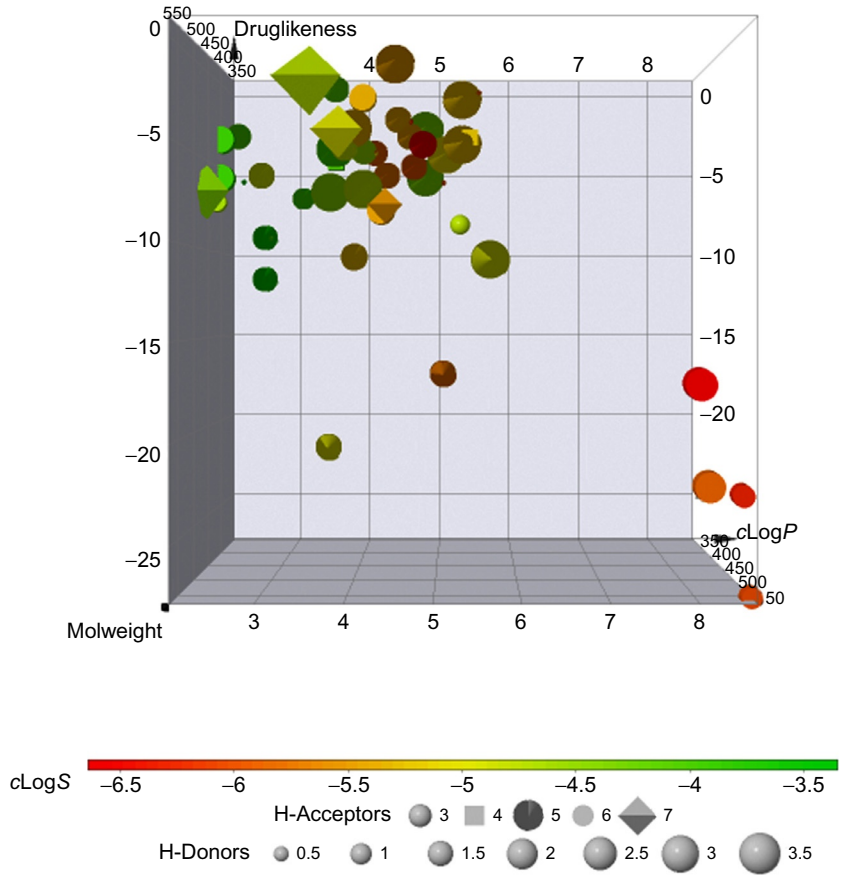

FIG. 8.6 Druglikeness properties of coumarins. Druglikeness score (*Y*-axis) vs. *c* log *P* (*X*-axis) vs. MW (*Z*-axis). Aqueous solubility (*c* log *S*) is indicated by color depending on value. Number of H-acceptors is indicated by type of the figure. Number of H-donors is represented by size of the figure. Properties were calculated using DataWarrior.

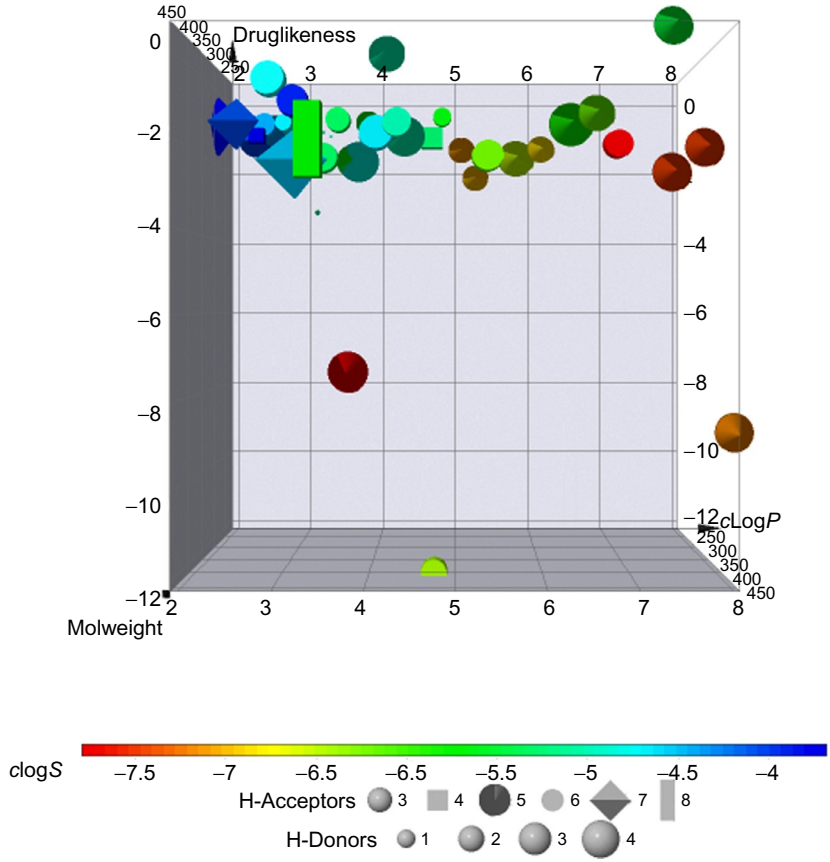

FIG. 8.7 Druglikeness properties of xanthones. Druglikeness score (*Y*-axis) vs. *c* log *P* (*X*-axis) vs. MW (*Z*-axis). Aqueous solubility (c log *S*) is indicated by color depending on value. Number of H-acceptors is indicated by type of the figure. Number of H-donors is represented by size of the figure. Properties were calculated using DataWarrior.

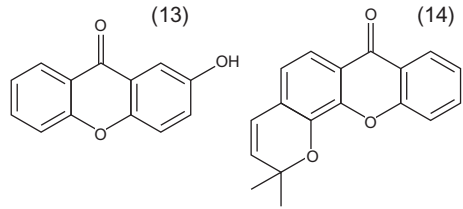

FIG. 8.8 Lead compounds from *Calophyllum* genus. (11) 2-Hydroxyxanthone; (12) Caledonixanthone B.

a SwissTarget prediction tool available online [77], which predicts bioactive molecule targets based on their similarity with known ligands of already known drugs. Interestingly, 2-hydroxyxanthone has a 100 percent probability of interacting with MAOA and MAOB, which speak of its potential as

antidepressant drugs and correlates with literature, as there are several studies of xanthones and MAO inhibition [78]. On the other hand, caledonixanthone B has a 58 percent probability of interacting with dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), which is overexpressed in a variety of diseases, including a number of human malignancies, such as haematological and brain cancers. It is interesting to note that this compound has proved to be active against different tumor cell lines, such as HL-60 [79]; however, these data should be experimentally tested in order to validate such results. Most of the coumarins and xanthones have log *S* values of less than −3.5 and are in compliance with most drugs, as more than 80 percent of the trademarked drugs have an estimated log *S* greater than −4. This is very interesting because the aqueous solubility of a compound significantly affects its absorption and distribution characteristics.

On the other side, most of the coumarins (Fig. 8.3) and xanthones (Fig. 8.4) isolated from *Calophyllum* spp. have negative values for druglikeness. However, it is important to highlight that positive value states that a molecule contains mainly fragments that are frequently present in commercial drugs, although it doesn't necessarily mean that these fragments are well balanced concerning other properties. For instance, a molecule may have a druglike moiety due to its lipophilic fragments. This molecule will have a high druglikeness score, although it wouldn't really qualify for being a drug because of its high lipophilicity. These results indicate that coumarins (e.g., inocalophyllin A,B and their methylesthers, mammeas A/AA dehydrocyclo F, A/BA cyclo F, C/OB, C/OA, C/AB, A/BA cyclo F, and Gut-70) and xanthones (e.g., 2″-isoprenyl-3″-hydroxy-dihydrofurano-demethyl-calabaxanthone, 1,5-didydroxy-6-(4-hydroxy-3-methyl butyl) xanthone, and apetalinone A) should be structurally modified in order to be easily formulated and introduced in clinics, as they have very low values of druglikeness.

Toxicity is one of the most handicapped issues for molecules used in clinical tests and development as approved drugs. Regarding toxicity, there are four coumarins (mammea A/AB dioxycalanocyclo-F, inophyllum G-1, G-2, and calocoumarin C) that theoretically possess high reproductive, tumorigenic, and irritant effects; on the other hand, there are 37 coumarins that possess reproductive and irritant effects and 18 coumarins with no tumorigenic, reproductive, mutagenic, or irritant effects, such as inocalophyllin A, B and its methyl ethers, disparfuran B, Gut 70, mucigerine, mammea A/AA, A/AA dehydrocyclo F, C/OA, A/BA, B/BA, B/BA cyclo F, A/BD, A/BA cyclo F, B/BA, C/OB, and A/BD. These results indicate that most of the coumarins should be studied in order to investigate their possible reproductive effects as well as for its irritant properties so as to identify if their structures could be improved to get better therapeutic results. This last property probably was detected by our study because Osiris DataWarrior involves an algorithm that relates toxicological properties with structure, as there are several examples of commercial coumarins that possess irritant properties, such as osthole, 7-hydroxycoumarin, and byakangelicin, used in cosmetics. These properties could be improved with changes in the type of formulation, as most of these compounds are irritating only to the skin [80]. Nonetheless, the interaction on the reproductive effect must be deeply studied before using this type of

compounds in clinics, as there are few examples of the interaction of coumarins in reproductive effects and teratogenicity. Xanthones, on the other hand, present very different toxicological profiles as compared with coumarins (Figs. 8.9–8.10). There are 48 that are nonirritant and do not possess

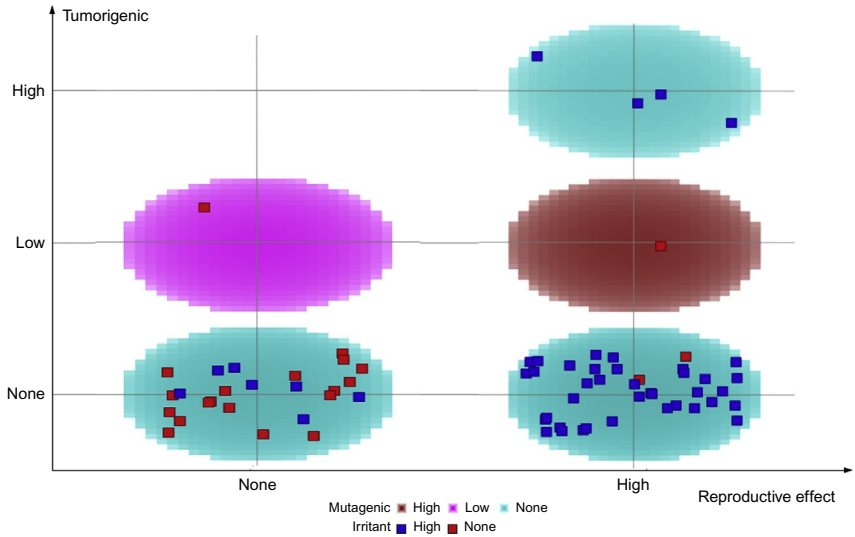

FIG. 8.9 Toxicological properties of coumarins. Reproductive effect (X) vs. Tumorigenic effect (Y). Each square represents a coumarin. Irritant effect is shown as *red* (none) or *blue* (high). Mutagenic effect is showed in background: *red* (high), *purple* (low), *pale blue* (none). All properties were estimated and calculated using DataWarrior.

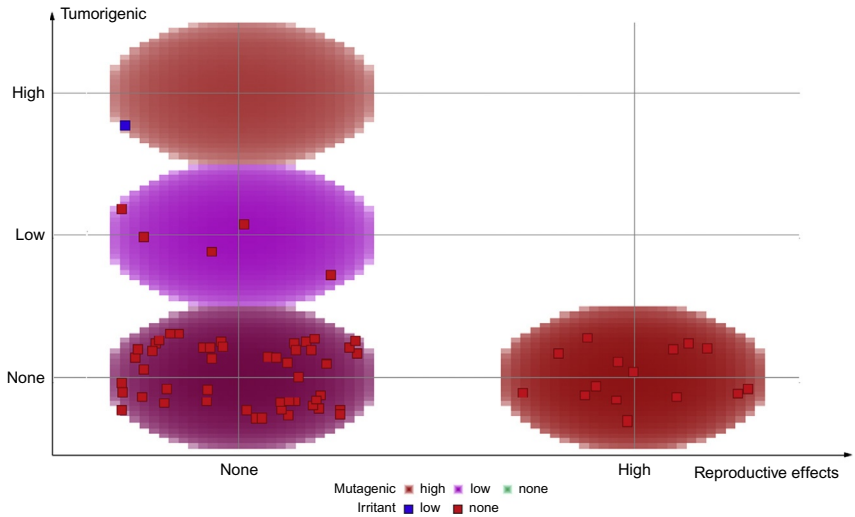

FIG. 8.10 Toxicological properties of xanthones. Reproductive effect vs. tumorigenic effect. Irritant effect is shown as *red* or *blue* (high or none). Mutagenic effect is showed in background with *purple* (high), *red* (low) and *green* (none). Each square represents a xanthone. All properties where estimated and calculated using DataWarrior.

reproductive or tumorigenic effects; nevertheless, 63 could posses highly mutagenic effects. These results indicate that most of the xanthones should be experimentally investigated in order to delve more into their mutagenic properties, as most of them could be considered for clinics.

CONCLUSIONS

Pharmacological and biological research on *Calophyllum* spp. has increased over the last few years due to its potential as an important source of pharmacologically active compounds against HIV-1, intracellular parasites, and cancer cell lines, among others. Further studies should be focused on experimental toxicological research (reproductive and mutagenic effects) as well as structural improvements in order to validate whether xanthones and coumarins have real opportunities for the development of new active drugs. The vast majority of xanthones and coumarins isolated from this genus are in compliance with Lipinski's Rule of Five, but even so there are two xanthones (2-hydroxyxanthone and caledonixanthone-B) with leadlikeness potential that apparently could interact with MAO and DYRKA1, respectively.

Methods

Chemotoxicoinformatic Analysis: Properties calculations and data visualization was determined using the freely available software DataWarrior-*Cheminformatics Program for Data Visualization and Analysis* [81]. For chemical clustering and heatmap calculations and visualization, we used ChemMine tools, which is an online service for analyzing and clustering small molecules. Numerical data clustering was performed accordingly to Euclidian distance between column *z*-scores for calculated properties, based on a single linkage method and numerical data (properties based on JoeLib descriptors).

Target search of compounds: A chemoinformatic analysis of possible targets was performed on SwissTarget prediction online server [82], (Swiss Institute of Bioinformatics; http://www.swisstargetprediction.ch/ accessed on 01.03.15).

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APPENDIX

TABLE A.1 Active Coumarins and Xanthones Isolated From *Calophyllum* Species Used for the Analysis

Coumarins	*c* Log *P*	*c* Log *S*	Xanthones	*c* Log *P*	*c* Log *S*
Mammea A/AB cycle E	4.4	−5.5	Caloxanthone E	1.9	−3.8
Calaustralin	3.6	−4.2	3,8-Dihydroxy-1,2,4-trimethoxyxanthone	2.2	−4.1
Mammein	4.8	−4.4	1,3,6-Trihydroxy-5,7-dimethoxyxanthone	2.2	−4.1
Cordatolide B	3.0	−4.0	1,3,5-Trihydroxy-2-methoxyxanthone	2.3	−4.1
Isodisparfuran A	4.1	−5.8	1,3,8-Trihydroxy-7-methoxyxanthone	2.3	−4.1
Calanolide A	3.9	−4.5	1,5,6-Trihydroxyxanthone	2.3	−4.0
Mammea A/AC	5.1	−5.1	1,3,5-Trihydroxyxanthone	2.3	−4.0
Costatolide	3.9	−4.5	Dehydrocycloguanidine	2.5	−3.7
Cordatolide A	3.0	−4.0	1,7-Dihydroxy-3,6-dimethoxyxanthone	2.5	−4.4
Calanolide B	3.9	−4.5	3,6-Dihydroxy-1,5-dimethoxyxanthone	2.5	−4.4
Pseudocordatolide C	3.0	−4.0	1,3-Dihydroxy-2,5-dimethoxyxanthone	2.5	−4.4
Calophylolide	3.6	−4.1	7-Hydroxy-1,2,8-trimethoxyxanthone	2.5	−4.4
Isomammeisin	5.3	−5.3	1,3-Dihydroxy-7,8-methoxyxanthone	2.5	−4.4
Isomammein	4.8	−4.4	1,7-Dihydroxy-3-methoxyxanthone	2.6	−4.4
Oblogulide	2.3	−3.3	1,6-Dihydroxy-5-methoxyxanthone	2.6	−4.4

Mammea A/AA	3.5	−5.0	1,7-Dihydroxyxanthone	2.7	−4.3
Inophyllum A	4.5	−5.5	1,5-Dihydroxyxanthone	2.7	−4.3
Calanolide F	3.9	−4.5	Caloxanthone H	2.7	−4.7
Inophyllum D	4.5	−5.5	Caledonixanthone B	2.8	−4.0
Soulatrolide	2.7	−4.8	6-Hydroxy-1,3,5-trimethoxyxanthone	2.8	−4.7
Gut 70	5.3	−4.7	6-Hydroxy-1,2,5-trimethoxyxanthone	2.8	−4.7
DisparacetylfuranA	2.1	−4.6	1-Hydroxy-6,7-dimethoxyxanthone	2.9	−4.7
Disparfuran B	3.2	−4.7	6-Hydroxy-1,5-dimethoxyxanthone	2.9	−4.7
Calanolide C	2.2	−4.1	3-Hydroxy-2,4-dimethoxyxanthone	2.9	−4.7
Mammea B/BB cyclo F	2.0	−3.9	7-Hydroxy-5,6-dimethoxyxanthone	2.9	−4.7
Mammmea A/BA cyclo F	3.9	−5.3	1-Hydroxy-7-methoxyxanthone	3.0	−4.6
Calanolide D	4.1	−5.3	6-Hydroxy-5-methoxyxanthone	3.0	−4.6
Mammea B/BA cyclo F	2.0	−3.9	7-Hydroxy-8-methoxyxanthone	3.0	−4.6
Mammea C/AB	5.7	−4.9	1,3,5,6-Tetrahydroxy-2-(3-hydroxy-3-methylbutyl) xanthone	3.0	−4.5
Soulatrolone	4.6	−6.2	4-Hydroxyxanthone	3.0	−4.6
Mammea A/BC cyclo F	2.1	−4.4	2-Hydroxyxanthone	3.0	−4.6
Inophyllum C	4.6	−6.2	3-Hydroxyblancoxanthone	3.1	−3.9
Calophyllic acid	3.9	−5.4	Caloxanthone C	3.1	−3.9
Mammea A/BB	5.3	−5.3	1,3.5,7-Tetramethoxyxanthone	3.1	−5.0
Mucigerine	2.7	−4.0	1,2.8-Trimethoxyxanthone	3.2	−5.0
Mammea B/BB	4.8	−4.4	6-Methoxy-2-(methoxycarbonyl)xanthone	3.2	−5.1

Continued

TABLE A.1 Active Coumarins and Xanthones Isolated From *Calophyllum* Species Used for the Analysis—cont'd

Coumarins	*c* Log *P*	*c* Log *S*	Xanthones	*c* Log *P*	*c* Log *S*
Mammea C/OA	2.5	−3.6	7,8-Dimethoxyxanthone	3.2	−5.0
Inophyllum E	4.6	−6.2	2-Methoxyxanthone	3.3	−4.9
Mammea C/OB	2.5	−3.6	Caloxanthone D	3.3	−5.8
Recedesolide	4.8	−4.6	Jacareubin	3.4	−5.3
Calanone	4.8	−6.6	Scriblitifolic acid	3.6	−5.3
Brasimarin C	5.3	−5.6	1,5-Dihydroxy-6-(4-hydroxy-3-methylbutyl)xanthone	3.6	−5.0
Mammea A/AA methoxycyclo F	3.7	−5.0	Osajaxanthone	3.7	−5.6
Mammea A/BA	4.0	−4.7	6-Dehydroxyjacareubin	3.7	−5.6
Mammea B/BA	3.6	−4.0	1,5-Dihydroxy-6-(4-hydroxy-3-methylbutyl-2-enyl) xanthone	3.8	−4.8
Apetalolide	5.1	−6.0	Teysmannic acid	3.9	−5.6
Inophyllum B	4.6	−6.2	2-(3,3-dimethylallyl)-1,3,5,6-tetrahydroxyxanthone	4.0	−4.7
Mammea A/AA cyclo F	5.1	−5.7	Acetylblancoxanthone	4.2	−5.0
Teysmanone B	5.6	−5.9	Caledonixanthone	4.3	−5.0
Calocoumarina A	3.8	−4.8	1,3,5-Trihydroxy-2-isoprenylxanthone	4.4	−5.0
Calopolyanolide C	5.4	−5.2	2-(3,3-Dimethylallyl)-1,3,5-trihydroxy-xanthone	4.4	−5.0
Mammea A/BD	3.5	−4.5	8-(3′,3′-Dimethylallyl)-1,5-dydroxyxanthone	4.7	−5.3
Teysmanone A	4.8	−6.6	Calophyllin B	4.7	−5.3

Brasimarin A	4.3	−5.7	2″-Isopropenyl-3″-hydroxydihydrofuranodemethylcalabaxanone	4.7	−6.4
Calopolyanolide B	3.8	−4.8	1-Hydroxy-3,5,6-trimethoxy-2-(3-methylbut-2-enyl) xanthone	4.8	−5.6
Calopolyanolide A	3.8	−4.8	Thwaitesixanthone	5.1	−7.1
Mammea A/AA	4.0	−4.7	11,12-Dihydrothwaitesixanthone	5.2	−7.0
Dispardiol	3.3	−4.7	Caloxanthone A	5.4	−6.2
Inocalophyllin A	8.0	−6.2	Trapezifolixanthone	5.7	−6.5
Inocalophylliun B methyl esther	8.6	−6.1	Calothwaitesixanthone	5.7	−6.5
Brasimarin B	4.0	−5.4	Methylcalabaxanthone	5.7	−6.5
Inophyllum G-2	4.6	−5.5	Calabaxanthone	6.0	−6.8
Mammea A/AB dioxycalanocyclo F	4.3	−5.5	6-deoxy-x-mangostin	6.4	−5.9
Isomammeigin	4.7	−5.9	Calocalabaxanthone	6.7	−6.2
Inocalophyllin A methyl esther	8.5	−6.3	Pyranojacareubin	6.8	−7.8
Mammea A/BC cycle F	4.2	−5.5	Apetalinone B	7.4	−7.4
Calocoumarin C	4.8	−6.2	Apetalinone C	7.4	−5.4
Inocalophyllin B	8.2	−5.9	Dombakinaxanthone	7.7	−7.5
Mammea A/AC cyclo F	4.2	−5.5	Apetalinone A	8.1	−7.2

ABBREVIATIONS

RT	reverse transcriptase
HIV-1	human immunodeficiency virus type 1
FRB	fraction of rotatable bonds
PSA	polar surface area
MW	molecular weight
MR	molecular refractivity
Log *P*	partition coefficient
Log *S*	solubility
HBA	hydrogen bond acceptors
HBD	hydrogen bond donors
NT	nontested
A (MAOA)	amine oxidase [flavin-containing]
B (MAOB)	amine oxidase [flavin-containing]
1A (DYRK1A)	tyrosine-phosphorylation-regulated kinase
CC	cytotoxic concentration
μM	micromolar
IC_{50}	inhibitory concentration 50 percent
MIC	minimum inhibitory concentrations
EC_{50}	concentration that increased the alkaline phosphatase
LD_{50}	lethal doses 50 percent

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