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REVIEW

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A unique high-diversity natural product collection as a reservoir of new therapeutic leads

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Plants represent a rich source of structurally diverse secondary metabolites, which can be exploited in the development of new clinically important compounds. Indeed, due to their biodiversity, medicinal plants represent the largest library of compounds that has ever existed. To date less than 1% of this vast biodiversity has been exploited in drug discovery, due to several factors, including the lack of an appropriate multidisciplinary perspective. Here we review the successful application of computer-aided methods in screening a unique and high-diversity *in house* collection library composed of around 1000 individual natural products, isolated mainly from indigenous plants collected in biodiversity-rich countries, especially of the tropics and subtropics, and enlarged with their semi-synthetic and synthetic derivatives, as well as plant material extracts, up to around 2000 components. During the last ten years, the *in house* library has provided several lead compounds that have been developed, and in some cases patented, as anticancer and antimicrobial agents. The main classes of the library are described, including (but not limited to) alkaloids, terpenoids, Diels–Alder-type adducts, isoflavones, chalcones, and cannabinoids. The main focus is on the chemical characteristics and biological activity of these identified compounds, with particular attention being given to those currently under patent or in the preclinical phase. We also assess the use of computer-aided methods in screening this unique and diverse *in house* collection of natural products that, over the last ten years, has provided some lead compounds that have been developed, and in some cases patented, as anticancer and antimicrobial agents. Finally, this review highlights the potential use of plant food extracts as a source of nutraceuticals and functional foods. The multidisciplinary approach described herein may further motivate research groups involved in natural product chemistry to potentially benefit from a limitless source of novel bioactive compounds.

1. Introduction

Since ancient times, nature has been an invaluable source of medicines and traditional remedies, mainly in the form of herbs, animal products, and inorganic materials. The high biodiversity of medicinal plants has made a notable contribution to the development of natural homemade treatments for multiple diseases.[1,2] In traditional methods, plant extracts were tested to evaluate their potential biological activity: further extraction and purification steps were usually guided by biological assays, an approach known as bioassay-guided fractionation.[3] Indeed, plants represent a rich source of structurally diverse secondary metabolites that can be exploited in the development of new clinically important compounds.[4–6] It is no coincidence that a significant proportion of commercial drugs either occur naturally or are derived from natural products by means of total synthesis and/or semi-synthesis transformations, and/or biotransformation studies of advanced synthetic precursors.[7–9] However, towards the end of the 20th century, the development of large synthetic libraries of small molecules led to the scaling back of research on the use of natural products. This was partly a result of the advent of new techniques, such as high-throughput screening (HTS) and combinatorial chemistry, which met the the major pharmaceutical companies' need for acceleration of research and discovery processes,[10–13] and partly due to the slowness and inefficiency of bioassay-guided drug discovery processes. In addition, the isolation of bioactive chemical constituents presented a number of technical challenges, in particular the variability of the source material, the difficulty of isolating the active constituents, and the costs of collection.[1,14] Notably, taxon abundance considerably affected the drug discovery process, and

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most plants of interest were indigenous to biodiversity-rich countries, especially those in the tropics and subtropics.[15] These considerations notwithstanding, combinatorial libraries have proved disappointing in practice, which suggests that diversity within a biologically relevant 'chemical space' is more important than library size.[9] In 2012, Newman and Cragg investigated the main source of new drugs over the period 1981–2010.[16] Their analysis indicated that 66% of new chemical entities are "formally synthetic", since 17% of them correspond to synthetic molecules containing pharmacophores derived directly from natural products, and 14% are actually modeled on the basis of their molecular targets. Accordingly, only 35% of the novel chemical entities can be classified as truly synthetic in origin (*i.e.*, devoid of natural inspiration).[16] Awareness that the number of novel chemical entities in drug discovery was declining led to a renewed interest in rediscovering natural products, including the construction of small focused collections (consisting of 300–3000 compounds) that include many of the structural characteristics of natural products – a process known as diversity-oriented synthesis.[11,17] As several scientists have claimed, we appear to be entering a New Golden Age of natural products drug discovery.[18] Accordingly, natural products exhibit an enormous structural and chemical diversity that cannot be matched by any synthetically based corporate screening library, and they remain the single most productive source of leads in modern drug discovery projects, often providing chemical structures as useful platforms for the development of drugs, or for the understanding of biological processes.[13,19] The advent of new techniques for the separation, purification and characterization of novel compounds has significantly improved the efficiency of these processes. Today, an important challenge is the generation of high-quality libraries of natural products that might enable the rapid identification of lead compounds for drug discovery progression.[10,20–25] In addition, the advent of powerful and user-friendly tools for informatics applications in chemistry and biology has further promoted the revolution of natural products screening in drug discovery.[26–28] Moreover, knowledge of the metabolic profile of a biological matrix is a powerful tool for driving investigations of its properties.[29] This review discusses a unique high-diversity library composed of around 1000 individual natural products, mainly isolated from indigenous plants collected in biodiversity-rich countries, especially in the tropics and subtropics, and enlarged with their semi-synthetic and synthetic derivatives, as well as plant material extracts, including up to 2000 components. In addition, the review highlights the successful application of computer-aided methods for screening this unique and diverse *in house* collection of natural products. Over the last ten years, this has yielded some lead compounds that have been developed, and in some cases patented, as anticancer and antimicrobial agents. Recently, one of these compounds was profiled up to preclinical studies. Moreover, the usefulness of the in house library was the driving force behind COST Action: CM1407 "Challenging organic syntheses inspired by nature – from natural products chemistry to drug discovery", chaired by Bruno Botta. Alongside this COST Action about 54 research groups around Europe put together more than 1000 synthetic and natural compounds to be tested for anticancer, antiproliferative, and antibacterial activity as well as many other biological activities. Finally, this review highlights the potential use of plant food extracts as a source of nutraceuticals and functional foods. The review is intended to provide a guideline on exploiting the maximum potential from both physical and virtual collections of natural compounds within the drug discovery program. We anticipate that it will encourage researchers, having in hand a pool of chemical diversity entities, to consider the approaches described herein both when creating their own screening collection and when applying screening approaches for the identification and optimization of bioactive small molecules.

Francesca Ghirga

Francesca Ghirga was born in Rome, Italy, in 1985. She received her master's degree in pharmaceutical chemistry and technology in 2010 at the Sapienza University of Rome, and obtained her PhD degree in pharmaceutical sciences. Since 2014 she has been a postdoctoral researcher at the Italian Institute of Technology. She is the author of 52 publications and four patents. Her primary research interest is centered on natural products chemistry and supramolecular chemistry, with a particular focus on the development of chemical libraries of novel natural products and both the design and synthesis of small bioactive molecules and their derivatives.

Deborah Quaglio

Deborah Quaglio was born in Caracas, Venezuela, in 1988. She received her master's degree in pharmaceutical chemistry and technology in 2013 from the Sapienza University of Rome. She completed her PhD in pharmaceutical sciences in 2016. During this time, she spent 9 months at the CERM, University of Florence, where she studied cell metabolomics. Since 2019 she has been a Research Fellow at the Department of Chemistry and Technology of Drugs. She is the author of 32 publications and three patents. Her current research interests are focused on the discovery and development of small organic molecules and natural compounds with antitumor activity.

2. Creating a unique collection of natural products

A unique *in house* library of about 1000 bioactive natural compounds, mostly isolated from plants indigenous to biodiversity-rich countries and collected from 1970 onward, is stored at the Organic Chemistry Laboratory of the Department of Chemistry and Technology of Drugs (The Sapienza University of Rome, Italy). In the early 1970s, an interest in plants used in traditional medicine in South America led Professor Franco Delle Monache to systematically investigate their chemical constituents. Several new chemical entities were isolated, and their structures were fully elucidated. On the basis of the results obtained, Professor Delle Monache and his collaborators had the serendipitous idea of creating a unique and diverse *in house* library of natural products. Today that collection consists of natural products belonging to different classes (see table 1, variably substituted flavonoids, benzophenones, xanthones, anthraquinones (Box 1), alkaloids, steroids, terpenoids, *etc*.) of organic compounds that have been fully characterized. It exhibits a wide range of pharmacophores, a high degree of stereochemistry, and a large range of molecular weights: 20% are lead- or drug-like (molecular weights 400–550 Da), 40% are hit-like (molecular weights 300–400 Da), 23% are fragment-like (molecular weights 200–300 Da), 9% have molecular weights of < 200 Da, and 8% are macrocycles (Fig. 1). These properties make a major contribution to the ability of the collection to provide bioactive compounds. The collection was then enlarged by the addition of other natural small molecules from commercially available sources, and of synthetic or semi-synthetic derivatives.

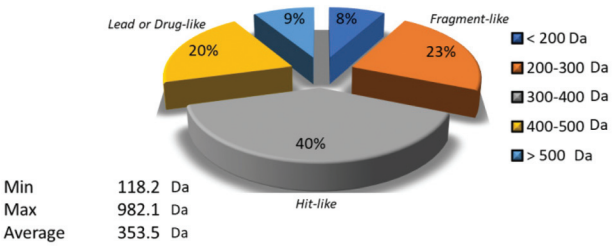

Fig. 1 Main features (MW) of the *in house* library of natural products.

Box 1 Anthranoid biosynthesis

Anthranoids are natural products isolated from different genera of fungi, such as *Aspergillus* and *Penicillium*, and from plants belonging to the families Aloaceae and Hypericaceae family, the latter was formerly included in the Guttiferae (Clusiaceae L.) family, and among the genera, mainly from *Aloe*, *Cratoxylum*, *Hypericum*, *Harungana*, and *Vismia*. Their name derives from their structural similarity to anthracene, independently from their degree of unsaturation.[70] The anthranoids can be divided into two main groups, namely the alizarin type and the emodin type, according to their different biogenetic pathways. Alizarin (**47**) and other related derivatives are biosynthesized in plants belonging to the family Rubiaceae, through a combination of the shikimate (**44**) and mevalonate pathways (Scheme 1a).[71–73]

The emodin-type anthranoids, conversely, are biosynthesized from the acetate pathway by the enzyme octaketide synthase (OKS), a type III polyketide synthase (PSK) that catalyzes the condensation of a unit of acetyl coenzyme A with seven molecules of malonyl coenzyme A. Successively, the octaketide (**48**) undergoes a series of aldol condensation and dehydration steps that lead to the anthrone scaffold (**49**). Oxidation of the emodin-type anthrone (**49**) by molecular oxygen gives emodin (**50**) or other related anthraquinones (see Scheme 1b)[71,72,74,75]

In Scheme 2 the biosynthesis of several anthranoids, pro-

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Silvia Cammarone

Silvia Cammarone was born in Sezze, Italy, in 1993. She received her master's degree cum laude *in pharmacy in 2019 from the Sapienza University of Rome. Currently she is a PhD student on the international PhD program in "Molecular design and characterization for the promotion of health and well-being: from drug to food" at the Sapienza University of Rome, under the supervision of Professor Bruno Botta. Her research is focused on the synthesis of natural compounds and the evaluation of their role in anticancer therapies.*

duced mainly from plants or fungi, is reported. The octaketide (**48**) is the common precursor which can form cyclic intermediates according to different sequences of aldol, dehydration, and enolization steps. The methyl group of chrysophanol anthrone (**51**) can be oxidized to give the aloe emodin anthrone (**52**), which in turn by glucosylation leads to the formation of aloin A and B (**53**), mixtures of diastereoisomers of anthraquinones with laxative properties found in succulent plants belonging to the genus *Aloe*.[75] **51** can also be oxidized to the anthraquinone chrysophanol (**54**), which is the precursor of aloe emodin (**55**) and rhein (**56**) by further oxidation steps of the methyl group to the hydroxymethyl group and carboxylic acid, respectively. The bicyclic intermediate is the precursor of many other anthranoids. By cyclization of the non-aromatic third ring of the latter, followed by decarboxylation, atrochrysone carboxylic acid (**57**) is formed, which in turn by a further decarboxylation step gives the anthrone atrochrysone (**58**). Dehydration of **57** followed by enol rearrangement leads to the endocrocin anthrone (**59**), which in turn gives the anthraquinone endocrocin (**60**) by oxidation. In some plants a simple decarboxylation of **60** can give **50** (not shown). As mentioned earlier, **58** is the precursor of other anthranoids. For example, torosachrysone (**61**) is formed from **58** by simple methylation of the phenol moiety. Dehydration and enol rearrangement of **58** leads to the emodin anthrone (**49**), which in turn can be oxidized to **50**. Methylation of **50** gives physcion (**62**). Radical coupling followed by oxidation of **49** leads to hypericin (**63**), a naphthodianthrone with antidepressant activity isolated from *Hypericum perforatum*.[76] Vismiones are prenylated anthrones which have been mainly isolated from the genus *Vismia*. Their common precursor is deacetylvismione A (**64**), which is derived from **58** by prenylation of the aromatic ring.[70] Finally, the ferruginines, isolated from the Guttiferae, are biosynthesized from **48** by multiple aldol, dehydration, and enolization steps, which form the ketonaphtodiol intermediate (**65**). The latter is converted by a double prenylation into vismin (**66**), the precursor of all ferruginines, which are formed by further modifications such as prenylation, oxidation, or cyclization of prenyl side chains.[70] All of the anthranoids have several biological activities. Among these, vismione has shown antiprotozoal,[77] antimalarian,[78] and anticancer activity, blocking the Hedgehog pathway that is involved in a number of solid tumors.[79]

Currently, all the components of this collection are incorporated into an informatic version of the library, and their chemical and physicochemical features are analysed by means of cheminformatics tools, showing a satisfactory level of chemical diversity.

In addition, the library includes several plant food extracts which were investigated for their potential use as nutraceuticals and functional foods (Box 2). One of the main peculiarities of this collection is that around 30% of the library compounds are classified as unique (*i.e.* they are not available from other commercial or literature sources) (see Table 1). A large number of these unique compounds belong to the chemical class of polyphenols (anthranoids, xanthones, benzophenones, flavonoids, and chalcones). In the 1980s, a chemosystematic investigation of the genus *Vismia* allowed the identification of no less than ten new compounds belonging to this class.[30–38] This genus (which belongs to the family Guttiferae) is mainly confined to the tropical and subtropical regions of South and Central America, where 80% of the known species are found. In the late 1970s a study of *Vismia baccifera* var. *fer-*

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Bruno Botta

Bruno Botta is a Full Professor of chemistry at the Sapienze University of Rome. He is the author of 198 publications, including six patents. He was nominated Deputy Rector for the Internationalization of the University of Rome "La Sapienza" in November 2014, and he was Chair of the Cost Action CM-1407 "Challenging organic syntheses inspired by nature – from natural products chemistry to drug discovery". His main research interests are the structural elucidation and synthesis of biologically active compounds derived from living plants, and both the synthesis and host–guest studies of artificial receptors of the resorcarene family of compounds.

ruginea and *Vismia decipiens* led to the isolation and structural elucidation of two new prenylated anthranoids (a polyphenol subclass that can be chemically described as dihydroxyanthraquinones, dihydroxydianthrones and dihydroxyanthrones) from the chloroform extract of the berries, which were named ferrunanthrone (**1**) and vismione A (**2**).[33,34,37] The occurrence and the distribution of these natural products was of great interest in relation to determining the chemotaxonomy of the genus *Vismia*. Subsequently, during a phytochemical study of *Vismia guaramirangae*, γ-OH-ferruginin A (**3**) and γ,γ'-diOH-ferruginin A (**4**) were isolated and identified for the first time from a chloroform extract from the berries.[32,34] A study conducted on the fruits of *Vismia japurensis* showed, in addition to the γ-OH-anthrone B (**5**), the co-occurrence of acetylvismione B (**6**) in this species.[38] Other species were further investigated, leading to the identification of vismione H (**7**) from the roots of *Vismia guineensis*.[31] New anthranoids were even found in another genus of the tribe Vismiae, when 3-*O*-geranylemodin (**8**) and vismione C (**9**) were isolated from the acetone extract of the berries of *Psorospermum febrifigum* and from the roots of *Psorospermum glaberrimum*.[30,35,39,40] Furthermore, a new xanthone, cadensin C (**11**), was identified during an investigation of the roots of *Vismia guaramirangae*.[36,41]

Box 2 Plant food extracts

Recently foodstuffs have been considered in terms not only of their nutritional value but also of their possible beneficial roles in human health. New terms such as "nutraceuticals" and "functional foods" have been coined, in which two concepts, "food" and "health" formerly always close but separate, are combined together. In fact, foodstuffs may play a pivotal role in human health, and thus a knowledge of the chemico-biological profile of foodstuffs represents an important and challenging area of investigation. It is well established that different variables help to determine the characteristics that arise from the combination of genomic and metabolic responses to environmental conditions (field conditions, weather, and agronomic practices).[182] Thus, metabolomic analysis represents the most suitable approach for investigating the metabolome in biological matrixes (cell, organ, or organism), which contains the downstream products of genomics, transcriptomics, and proteomics as well as the results of genome–environment interactions. Different analytical methodologies can be applied when performing a metabolomic analysis, but a combination of several different techniques greatly reduces the effects of each individual one's limits, and enables a more complete metabolic profile to be obtained. In particular, both untargeted and targeted methodologies have been applied to food extracts present in the library in order both to carry out metabolic profiling and to investigate how environmental and genetic factors may affect the metabolic pathways involving primary and secondary metabolites. The unique library is enriched by a range of extracts (*e.g.* tomato,[183] celery,[184] pepper,[185] olive oil,[186–189] blueberry[190]). The white celery (*Apium graveolens* L.) "sedano bianco di Sperlonga" PGI ecotype was characterized by means of NMR, MS, HPLC-PDA, GC-MS, and spectrophotometric analyses[184] in order to obtain the metabolic profile of the edible parts (blade leaves and petioles), which is also related to quality, freshness, and biological properties. Differences between blade leaf and petiole extracts with regard to the concentrations of sugars, polyalcohols, amino acids, organic acids, phenols, sterols, fatty acids, phthalides, chlorophylls, tannins, and flavonoids were detected. Moreover, several phenolic compounds could represent markers of the investigated ecotype, being responsible for the scavenging properties and antimutagenic activity towards the oxidative DNA damage mediated by *t*BuOOH. Sobolev *et al.*[185] investigated the red sweet pepper (*Capsicum annuum* L.) ecotype "Cornetto di Pontecorvo" in order to identify possible differences between fruits grown in the open field and under glass. Furthermore, a comprehensive pepper metabolite profile for seeds, peel, and pulp was obtained, which suggested that peel and seeds, which are often regarded as waste products due to their low digestibility, could be a good source of bioactive compounds, especially polyphenols, which are important from a nutritional point of view. Preliminary biological tests that were performed in relation to α-amylase suggested a possible functional role for this pepper ecotype in the control of glucidic metabolism.

Olive oil represents an important food matrix for investigation. The study of olive oil relates to a number of issues, including chemical profile characterization, the identification of fraud and/or adulteration, shelf-life, determination of quality parameters, and a combination of chemical analysis and organoleptic evaluation (panel

Andrea Calcaterra

Andrea Calcaterra was born in Rome, Italy, in 1984. He received his master's degree in chemistry in 2009 from the Sapienza University of Rome, and obtained his PhD in pharmaceutical sciences in 2012. From 2013 to 2020 he was a postdoctoral fellow at Sapienza University. In 2020 he was appointed Assistant Professor (Researcher type B) of organic chemistry at Sapienza University. He is the co-author of 31 publications, one book chapter and two patents. His research is focused on the isolation, total synthesis and biological evaluation of natural products from plants, and on the synthesis and supramolecular chemistry of resorc[4]arene macrocycles.

Table 1 List of all unique natural products in the collection

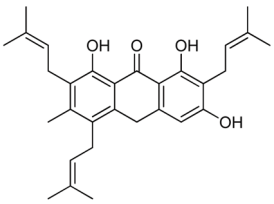
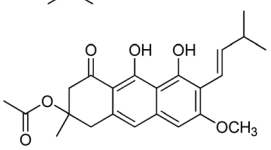
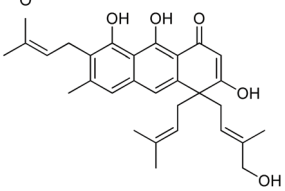
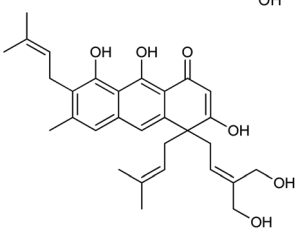
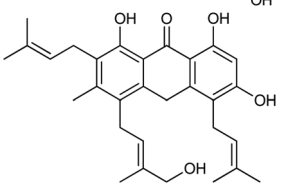
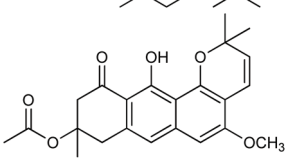
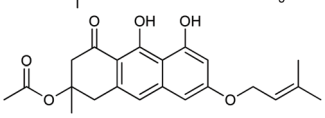
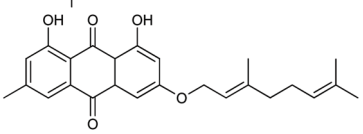
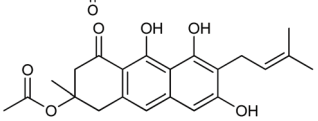
Mol.	Common name	Chemical structure	M.W. (g mol^{-1})	Molecular formula	Source	Ref.
		Phenolic compounds **Anthranoids**				
1	Ferruanthrone		460.6	$C_{30}H_{36}O_4$	*Vismia baccifera* var. *ferruginea* (Hypericaceae family)	34, 37
2	Vismione A		398.5	$C_{23}H_{26}O_6$	*Vismia baccifera* var. *ferruginea* (Hypericaceae family)	33, 37
3	γ-OH-ferruginin A		476.6	$C_{30}H_{36}O_5$	*Vismia guaramirangae* (Hypericaceae family)	32, 34
4	γ,γ'-OH-ferruginin A		492.6	$C_{30}H_{36}O_6$	*Vismia guaramirangae* (Hypericaceae family)	32, 34
5	γ-OH-anthrone-B		476.6	$C_{30}H_{36}O_5$	*Vismia guaramirangae* (Hypericaceae family)	38
6	Acetylvismione B		396.4	$C_{23}H_{24}O_6$	*Vismia japurensis* (Hypericaceae family)	38
7	Vismione H		384.4	$C_{22}H_{24}O_6$	*Vismia guineensis* (Hypericaceae family)	31
8	3-*O*-Geranylemodin		408.5	$C_{25}H_{28}O_5$	*Psorosporum febrifigum* and *Psorosporum glaberrimum* (Hypericaceae family)	30, 35
9	Vismione C		384.4	$C_{22}H_{24}O_6$	*Psorosporum febrifigum* and *Psorosporum glaberrimum* (Hypericaceae family)	30, 35

Table 1 *(Contd.)*

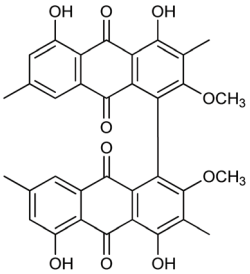
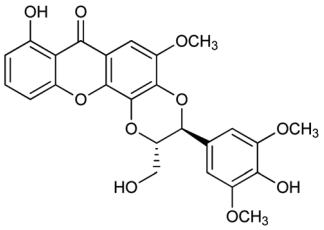
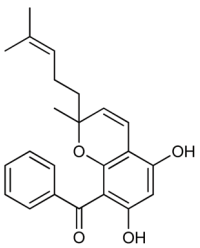
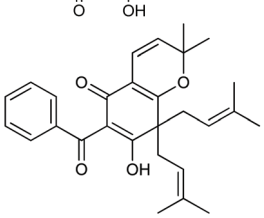
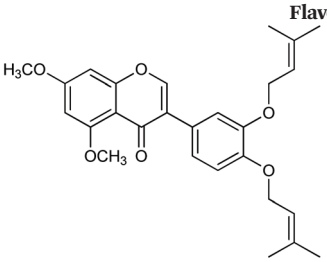
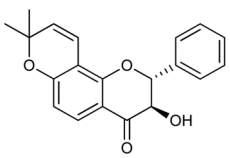
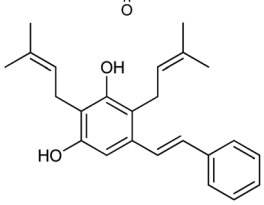
Mol.	Common name	Chemical structure	M.W. (g mol^{-1})	Molecular formula	Source	Ref.
10	Trachyphone		594.6	$C_{34}H_{26}O_{10}$	*Cassia trachypus* (Leguminosae family)	42
		Xanthone				
11	Cadensin C		482.4	$C_{25}H_{22}O_{10}$	*Vismia guaramirangae* (Hypericaceae family)	36
		Benzophenones				
12	Clusiachromene C		364.4	$C_{23}H_{24}O_4$	*Clusia multiflora* (Guttiferae family)	43
13	Machuone		416.6	$C_{28}H_{32}O_4$	*Clusia sadiensis* and *Clusia columaris* (Guttiferae family)	44, 45
		Flavonoids				
14	Glabrescione B		450.5	$C_{27}H_{30}O_6$	*Derris glabrescens* (Leguminosae family)	46
15	3-OH-lonchocarpin		322.4	$C_{20}H_{18}O_4$	*Lonchocarpus eriocaulinasis* (Leguminosae family)	47
16	Longistylin B		348.5	$C_{24}H_{28}O_2$	*Lonchocarpus longistylus* and *L. violaceus* (Leguminosae family)	48

Table 1 *(Contd.)*

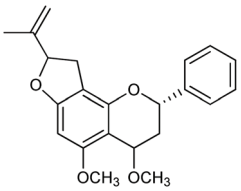
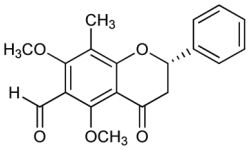
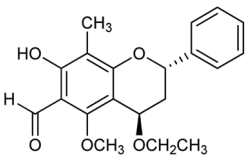
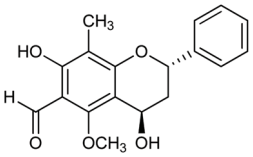
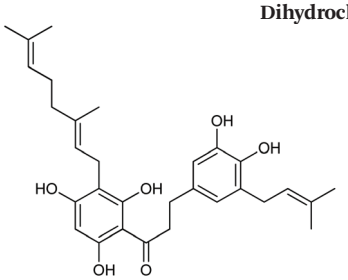
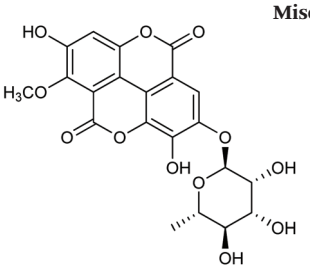
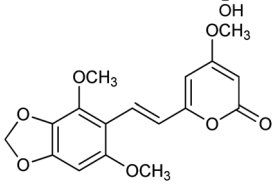
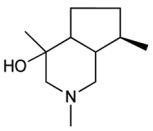
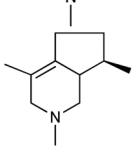
Mol.	Common name	Chemical structure	M.W. (g mol^{-1})	Molecular formula	Source	Ref.
17	Methylhildgardtol A		352.4	$C_{22}H_{24}O_4$	*Tephrosia hildebrandtii* (Leguminosae family)	49
18	6-Carbaldehyde-5,7-diOMe-8-Me-flavanone		326.3	$C_{19}H_{18}O_5$	*Petiveria alliacea* (Petiveriae family)	51
19	6-Carbaldehyde-4-OEt-7-OH-5-OMe-8-Me-flavanone		342.4	$C_{20}H_{22}O_5$	*Petiveria alliacea* (Petiveriae family)	52
20	6-Carbaldehyde-4,7-diOH-5-OMe-8-Me-flavanone		314.3	$C_{18}H_{18}O_5$	*Petiveria alliacea* (Petiveriae family)	52
		Dihydrochalcone				
21	2′,3,4,4′,6′-PentaOH-5-Pr-3′-Gr-dihydrochalcone		494.6	$C_{30}H_{38}O_6$	*Esembeckia leiocarpa* (Rutaceae family)	53
		Miscellaneous				
22	3-OMe-4-*O*-ramnoside ellagic acid		462.4	$C_{21}H_{18}O_{12}$	*Rubeus imperialis* (Rosaceae family)	54
23	4-OMe-6(11,12-methylendioxy-10,14-diOMe)-styryl-2-pyrone		332.3	$C_{17}H_{16}O_7$	*Polygala sabulosa* (Polygalaceae family)	55
		Alkaloids				
24	Oxyskytanthine		183.3	$C_{11}H_{21}NO$	*Skytanthus acutus* (Apocynaceae family)	56
25	Dehydroskytanthine		165.3	$C_{11}H_{19}N$	*Skytanthus acutus* (Apocynaceae family)	56

Table 1 *(Contd.)*

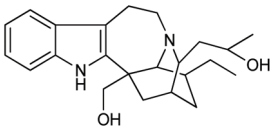
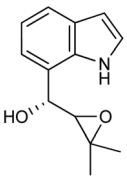
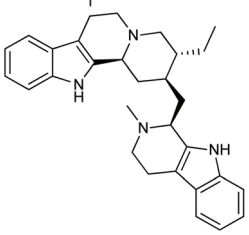
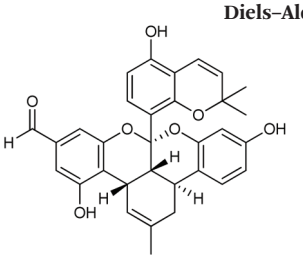
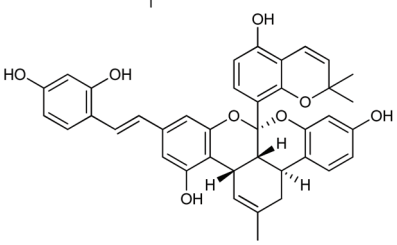
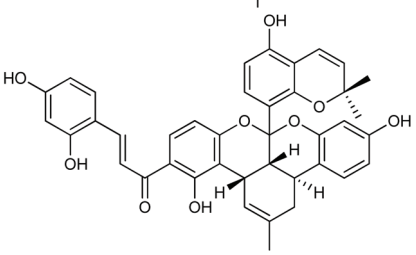
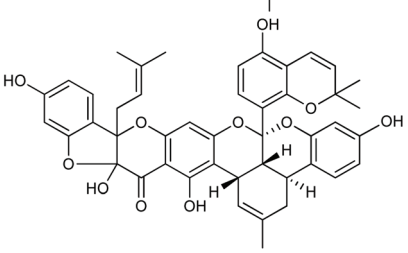
Mol.	Common name	Chemical structure	M.W. (g mol^{-1})	Molecular formula	Source	Ref.
26	19-OH-coronaridina		368.5	$C_{23}H_{32}N_2O_2$	*Ervatamia coronaria* (Apocynaceae family)	57
27	Leiocarpol		217.3	$C_{13}H_{15}NO_2$	*Ervatamia coronaria (Apocynaceae family)*	58
28	Nigritanine		452.6	$C_{30}H_{36}N_4$	*Strychnos nigritana* (Loganiaceae family)	59
		Diels–Alder-type adducts				
29	Soroceal		524.6	$C_{32}H_{28}O_7$	*Sorocea bonplandtii* (Moraceae family)	60
30	Sorocein A		630.7	$C_{39}H_{34}O_8$	*Sorocea bonplandtii* and *Sorocea ilicifolia* (Moraceae family)	60–65
31	Sorocein B		658.7	$C_{40}H_{34}O_9$	*Sorocea bonplandtii* and *Sorocea ilicifolia* (Moraceae family)	60–65
32	Sorocein C		756.8	$C_{45}H_{40}O_{11}$	*Sorocea bonplandtii* and *Sorocea ilicifolia* (Moraceae family)	60–65

Table 1 *(Contd.)*

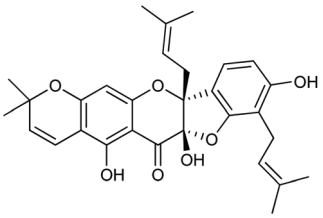
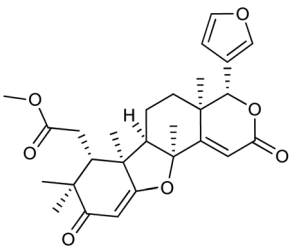
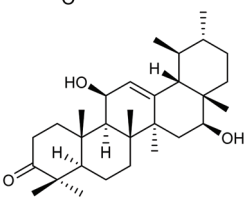
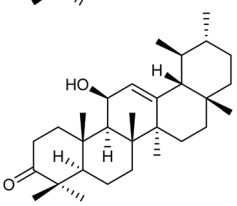
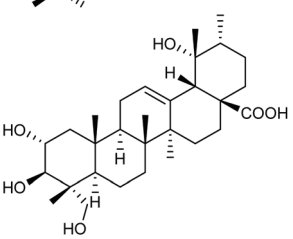
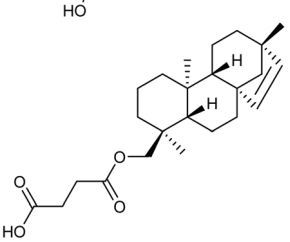
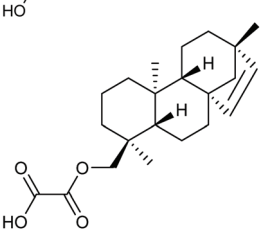
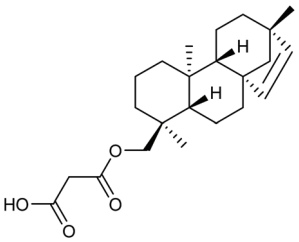
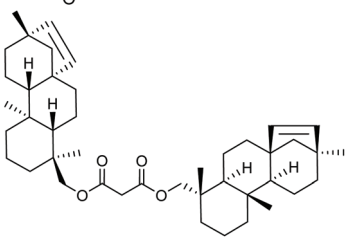
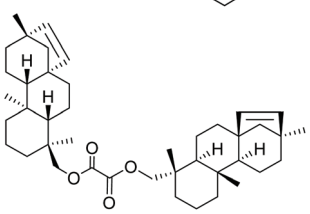
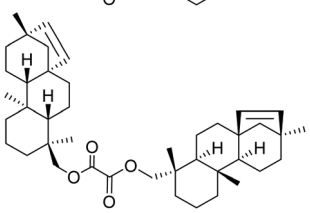
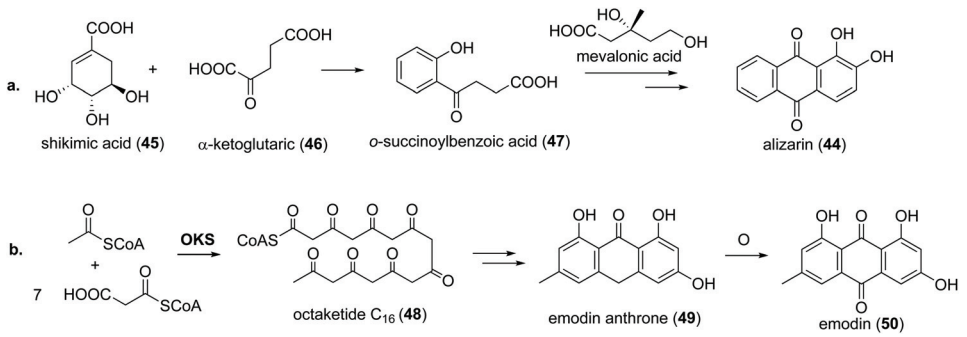
Mol.	Common name	Chemical structure	M.W. (g mol^{-1})	Molecular formula	Source	Ref.
33	Sorocein D		504.6	$C_{30}H_{32}O_7$	*Sorocea bonplandtii* and *Sorocea ilicifolia* (Moraceae family)	60–65
		Terpenoids				
34	Hortiolide A		468.5	$C_{27}H_{32}O_7$	*Hortia columbiana* (Rutaceae family)	66
35	3-Oxo-11β,16β-diOH-urs-12-ene		456.7	$C_{30}H_{48}O_3$	*Protium kleinii* (Burseraceae family)	67
36	3-Oxo-11β-OH-urs-12-ene		440.7	$C_{30}H_{48}O_2$	*Protium kleinii* (Burseraceae family)	67
37	23-OH-tormentic acid		504.7	$C_{30}H_{48}O_6$	*Rubeus imperialis* (Rosaceae family)	54
38	Ent-beyer-15-en-18-*O*-succinate		388.5	$C_{24}H_{36}O_4$	*Fabiana densa* var. *ramulosa* (Solanaceae family)	68, 69
39	Ent-beyer-15-en-18-*O*-oxalate		360.5	$C_{22}H_{32}O_4$	*Fabiana densa* var. *ramulosa* (Solanaceae family)	68, 69

Table 1 *(Contd.)*

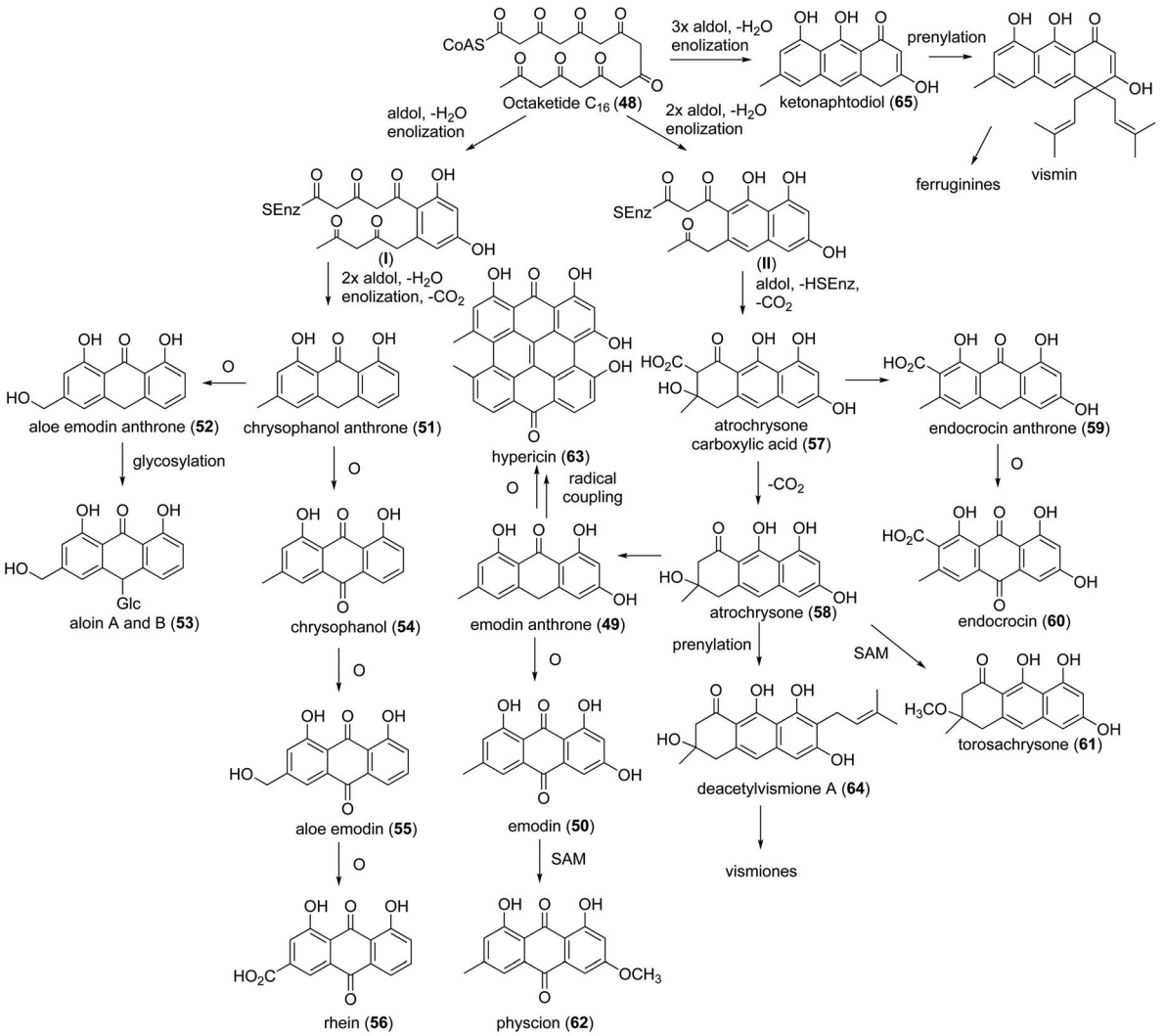
Mol.	Common name	Chemical structure	M.W. (g mol^{-1})	Molecular formula	Source	Ref.
40	Ent-beyer-15-en-18-*O*-malonate		374.5	$C_{23}H_{34}O_4$	*Fabiana densa* var. *ramulosa* (Solanaceae family)	69
41	Ent-beyer-15-en-18-*O*-malonoyl dimer		645.0	$C_{43}H_{64}O_4$	*Fabiana densa* var. *ramulosa* (Solanaceae family)	69
42	Ent-beyer-15-en-18-*O*-succinoyl dimer		659.0	$C_{44}H_{66}O_4$	*Fabiana densa* var. *ramulosa* (Solanaceae family)	69
43	Ent-beyer-15-en-18-*O*-oxaloyl dimer		630.9	$C_{42}H_{62}O_4$	*Fabiana densa* var. *ramulosa* (Solanaceae family)	69

test).[186–188] Although olive oil has been extensively studied all around the world, some metabolites remain unidentified, and the phytochemical composition is a particularly interesting research field. In particular, in our laboratory tetrahydrogeranylgeraniol and dihydrogeranylgeraniol were identified for the first time in both total aliphatic alcohol and waxy fractions of extra virgin olive oil by means of GC and GC-MS methodologies. The presence of these two compounds suggests that they do not originate from hydrolysis of the chlorophyll, as was previously believed, but are present as diterpenic esters.[189]

Although most of the anthranoids, especially the prenylated ones, seemed to occur mainly in the genus *Vismia*, a study conducted in the early 1990s on higher plants from Northeastern Brazil led to the isolation of a new and unique biantraquinone, namely trachyphone (**10**), from the methanolic extract of *Cassia trachypus*.[42] Additional studies of the family Guttiferae allowed the identification of two new benzophenones (compounds structurally related to anthranoids, consisting of aromatic ketones), namely clusiachromene C (**12**) and machuone (**13**), isolated from the fruits of *Clusia multiflora*[43] and from the leaves of *Clusia sadiensis*,[44] respectively. Compound **13** was also isolated from the fruits of *Clusia columnaris*.[45] Among the polyphenols included in the library, the flavonoid subclass, featuring a 15-carbon skeleton with two aromatic rings (A and B) and a heterocyclic ring (C), mainly consists of unique natural products. Particular interest in members of the family Leguminosae, due to the wide variety of flavonoids and rotenoids they contain and their use in traditional medicine, led to the investigation of two closely related genera, namely *Lonchocarpus* and *Derris*. In particular, glabrescione B (**14**), which is one of the most important bioactive compounds in the library (see section 4.4.1) and contains a 5,7-dimethoxyisoflavone core, was isolated for the first time from the seed of *Derris glabrescens* in 1977.[46] In contrast, investigation of the genus *Lonchocarpus* led to the isolation and structural elucidation of 3-OH-lonchocarpin (**15**) from the methanolic extract of the seeds of *L. eriocaulinasis*,[47] as well as the isolation of a prenylated stilbene, longistylin B (**16**), from the methanolic



Scheme 1 Biosynthesis of (a) alizarin-type and (b) emodin-type anthranoids.



Scheme 2 Biosynthesis pathway from octaketide to anthranoids.

extract of the bark and roots of both *L. longistylus* and *L. violaceus.*[48] More detailed investigations revealed that flavonoids could be isolated from several different genera. Consistent with this, a flav-3-ene, namely methyl-hildgardtol A (**17**), was isolated from the roots of *Tephrosia hildebrandtii* (family Leguminosae), collected in tropical East Africa,[49] and three different flavanones were isolated from the genus *Petiveria* (family Petiveriae), a shrub used in traditional medicine in South America for its diuretic, antispasmodic, and anti-inflammatory effects, among others.[50] In particular, 6-car-

baldehyde-5,7-diOMe-8-Me-flavanone (**18**) was isolated from the ethanolic extract of the leaves of *Petiveria alliacea*, and 6-carbaldehyde-4-OEt-7-OH-5-OMe-8-Me-flavanone (**19**) and 6-carbaldehyde-4,7-diOH-5-OMe-8-Me-flavanone (**20**) were isolated from the aerial parts of this plant.[51,52] Among members of the chalcone subclass, which contain a simple 1,3-diphenyl-2-propen-1-one core, the natural occurring compounds 4,2′,4′-triOH-3-Pr-3′-Gr-dihydrochalcone (**21**), which was isolated from the leaves of *Esembeckia leiocarpa*,[53] is available only in this library. Two other interesting natural products belonging to the class of polyphenolic compounds are also unique to this collection, namely 3-OMe-4-*O*-ramnoside ellagic acid (**22**), which was isolated from *Rubeus imperialis*,[54] and 4-OMe-6-(11,12-methylendioxy-14-OMe)-dihydrostyryl-2-pyrone (**23**), which was isolated from all parts of *Polygala sabulosa.*[55]

Several unique natural products belong to one of the largest and most intriguing classes of natural compounds, namely the alkaloids. Indeed, alkaloids are characterized by their great structural diversity, as a result of which there is no uniform classification of these compounds. Oxyskitanthine (**24**) and dehydroskitanthine (**25**), which are piperidine derivatives that possess two *C*-methyl functional groups and one *N*-methyl group, were isolated for the first time from *Skytunthus acutus*, a Chilean member of the family Apocynaceae.[56] 19-OH-coronaridina (**26**) and leiocarpol (**27**), which are both indole alkaloids, were isolated from *Ervatamia coronaria*[57] (family Apocynaceae) and *Esembeckia leiocarpa*[58] (family Rutaceae), respectively. In addition, nigritanine (**28**), which belongs to the carboline subclass of the indole alkaloids, was isolated from the leaves of *Strychnos nigritana* (family Loganiaceae).[59] Recent research identified this alkaloid as a promising candidate for the development of new antimicrobial molecules for the treatment of *Staphylococcus aureus*-induced infections (see section 4.1).

In the early 1990s, a study performed on *Sorocea bonplandii* (a large tree, collected in Santa Catarina, Brazil, and belonging to the family Moraceae) showed that the crude methanolic extract of the roots exhibited an interesting *in vitro* pharmacological profile against several neurotransmitter-induced contractions in guinea pig ileum and rat uterus.[60] In order to isolate and identify the compounds responsible for this biological activity, the crude extract was subjected to a chromatographic separation that allowed the isolation and structural elucidation of five new Diels–Alder-type adducts, named soroceal (**29**), sorocein A (**30**), sorocein B (**31**), sorocein C (**32**), and sorocein D (**33**), together with three previously identified compounds, namely betulinic acid, morusin, and mulberrofuran.[60–62] Compounds **30–33** were also found in *Sorocea ilicifolia*.[63–65]

Terpenoids have been extensively studied, and about the 60% of known natural products belong to this class of compounds. However, several natural products unique to this collection belong to different terpenoid subclasses. A study performed on the ethanolic extract of the wood of *Hortia columbiana* (family Rutaceae) led to the identification of a new limonoid, named hortiolide A (**34**), classified as a tetranortriterpenoid.[66] More recently, during a phytochemical study of the resinous bark of *Protium kleinii* (family Burseraceae), two new pentacyclic triterpenoids, namely 3-oxo-11β,16β-OH-urs-12-ene (**35**) and 3-oxo-11β-OH-urs-12-ene (**36**), were isolated and showed antinociceptive activity.[67] In 2006, some phytochemical studies conducted on *Rubeus imperialis* (collected in Santa Caterina, Brazil, and belonging to the family Rosaceae) indicated the presence of a novel pentacyclic terpenoid, named 23-OH-tormentic acid (**37**).[54] Notably, a study of the air-dried ground leaves of *Fabiana densa* var. *ramulosa* (family Solanaceae), a native shrub of Chile, described the isolation of two diterpenoids, namely the succinoyl and oxaloyl esters of *ent*-beyer-15-en-18-ol (**38–39**) (previously identified in *Baccharis tola* from the resinous exudate of *Fabiana densa* var. *ramulosa*).[68] Recently, a new diterpenoid (**40**) and three dimeric diterpenes (**41–43**), isolated from an extract of the aerial parts of *Fabiana densa* var. *ramulosa*, were reported for the first time.[69] The monomeric diterpenoid exhibited interesting antimicrobial activity (see section 4.5).

3. Computational methods in natural products drug discovery

Computer-aided drug design has revolutionized the approach to the development of therapeutically relevant small molecules over the last three decades.[80] Avoiding a large population of compounds that are unlikely to be successful in relation to drug discovery saves money and time, reducing significantly the number of confirmatory assays and instead focusing experimental efforts and resources on a limited subset of privileged hits/leads. These methods might be broadly classified as structure- and ligand-based approaches, depending on the availability of the three-dimensional structure of the target, or of a large and well-suited set of bioactive ligands, respectively. In both cases, screening a large chemical library *in silico* leads to the selection of a limited number of candidates that are likely to be active against a chosen biological target.[81,82] Pharmacophores are simplified models that describe the most relevant ligand features for bioactivity, which are commonly generated starting from a set of bioactive compounds or from a ligand–receptor complex. In addition, prediction of the absorption, distribution, metabolism, and excretion (ADME)-Tox profile of a given set of compounds generally helps to refine the selection of the most suitable candidates for further testing and development.

It is noteworthy that computational methods were largely exploited to screen the *in house* library of natural products in the search for bioactive ligands for given pharmacological targets. Due to the abundance of protein structures solved by X-ray crystallography, NMR spectroscopy, or electron microscopy, and available in the Protein Data Bank (http://www.rcsb.org/pdb), structure-based approaches were extensively used for this purpose. In contrast, in the few cases where the receptor structure was unavailable or unsuitable for virtual screening, ligand-based or cheminformatics approaches were

pursued. An overview of the application of computational tools in screening the *in house* library is provided below.

Early studies were focused on the identification of small molecule inhibitors of *Mycobacterium tuberculosis* (Mtb) protein tyrosine phosphatase B (PtpB), a virulence factor that is secreted in the cytoplasm of the host cells by Mtb to enhance its survival. Docking-based virtual screening of the *in house* library coupled with biochemical assays highlighted kuwanol E (**67**) as a potent and competitive inhibitor of PtpB (see section 4.2). A subsequent plant-based computational screening highlighted kuwanon G (**68**) and kuwanon H (**69**) as PtpB inhibitors with sub-micromolar potency and efficacy against Mtb survival *in vitro* (see section 4.2 and Chart 1).[83–85] Structure-based approaches have played a crucial role in the study of the Hedgehog (Hh) signalling pathway, both in terms of understanding the structural features of Gli1 binding to DNA, and with regard to identifying and optimizing small molecule inhibitors that act either at upstream levels, *i.e.* targeting the smoothened receptor (SMO), or at downstream levels, *i.e.* targeting the GLI transcription factors, including multitargeting approaches.[86,87] In particular, molecular dynamics (MD) simulations coupled with *in silico* alanine scanning have revealed the hotspot for GLI1 binding to DNA. This theoretical hypothesis was confirmed by experimental studies, thus identifying a putative binding site for small molecules that are able to compete with DNA for binding to the GLI1 zinc finger domain. This site was exploited in the docking-based virtual screening of the library, which highlighted Glabrescione B (GlaB) and the isoflavone scaffold as a privileged pharmacophore for GLI1 inhibition (see section 4.4.1).[86–91] At the same time, the library was screened against available crystallographic structures of the SMO receptor, highlighting multiple natural product chemotypes as profitable SMO antagonists, particularly in the presence of clinically relevant drug-resistant SMO mutations.[92–95]

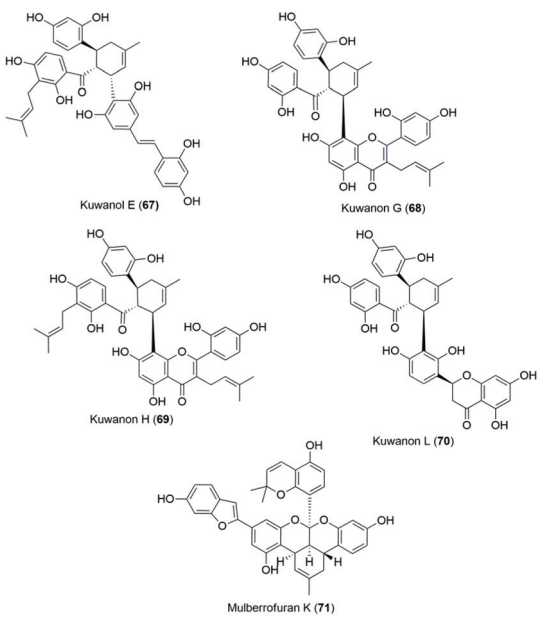


Chart 1 Biologically active D–A-type adducts.

Similar to Hh, the Notch signalling pathway is also implicated in embryonic development and cell differentiation, and is involved in multiple cancer types, such as T-cell acute lymphoblastic leukemia (T-ALL). At the time of our study, only small portions of the large Notch receptor were available by X-ray crystallography, although they were unsuitable for drug design purposes due to the lack of co-crystallized ligands, or of information on the possible ligand binding site. Unfortunately, no direct Notch inhibitors were available either, since Notch inhibition was mostly achieved through the modulation of gamma secretases, a family of proteins that activate Notch. Consequently, structure- or ligand-based strategies could not be engaged in the design of Notch inhibitors. This task was achieved through the application of a cheminformatics tools, *i.e.* a combination of fingerprint comparison and the maximum common substructure (MCS) search, with the aim of grouping natural products from the *in house* library based on their main scaffold or substructure. With this tool, a representative compound in each cluster was also identified,[96] which was included in the chemically diverse test set for evaluating Notch inhibition. Notably, a chalcone derivative emerged as an effective Notch inhibitor, giving rise to the development of further generations of improved Notch inhibitors with anticancer effects (see section 4.4.2).[97–99]

Recently, molecular docking was used as a reliable tool for selecting potential inhibitors of the zinc-dependent culling deneddylating enzyme CSN5,[100] highlighting pharmacophores that are suited both to coordination of the metal ion, and to the identification of small molecule inhibitors of ArnT-mediated colistin resistance. In the latter approach in particular, computational screening was useful for identifying a diterpene lead, which was subsequently optimized up to the development of more accessible semi-synthetic *ent*-beyerane diterpenes as valuable and cost-effective colistin resistance inhibitors with a potential translational impact (see section 4.5).[69,101,102] Overall, computational approaches facilitated the placement of multiple chemotypes of natural products as hits/leads for pharmacological application. Notwithstanding the relatively limited dimensions of the *in house* library, no overlap between the scaffolds of bioactive compounds was observed using the approaches outlined in this review, which suggests that enhancing the chemical diversity of a compounds library might have a more powerful effect than increasing its size.

4. *In house* natural product collection as a reservoir of privileged scaffolds for drug discovery

Over the years, the *in house* library offered a unique opportunity to identify unexpected new scaffolds for the development of therapeutically relevant molecules. Furthermore, it is still successfully screened *in silico* and *in vitro* for the identification

of hit and lead compounds from previous early-stage drug discovery projects. In this section the main classes of the library are described, including (but not limited to) alkaloids, terpenoids, Diels–Alder-type adducts, isoflavones, chalcones, and cannabinoids. The focus is on the identified chemical characteristics and biological activity of these compounds, with particular attention given to those currently under patent or in the preclinical phase. Also described are the synthetic and semisynthetic derivatives obtained by the process of optimization of active hits up to lead compounds or lead candidates by improving their potency, stability, physicochemical features (*e.g.* water solubility, log *P*, polar surface area PSA), chemical properties, and metabolic and pharmacokinetic parameters.

4.1 Alkaloids

Alkaloids are an important group of secondary metabolites produced by a wide variety of organisms, including bacteria, fungi, plants, and animals. Chemically, alkaloids are characterized by great structural diversity, sharing only the feature of being nitrogen-containing compounds (having one or more nitrogen atoms within a heterocyclic ring).[103] Alkaloids can occur as monomers, dimers (bisalkaloids), trimers, or tetramers. They are classified according to their chemical structure into two broad groups. The heterocyclic alkaloids (also known as typical alkaloids) contain nitrogen in the heterocyclic ring and originate from amino acids, whereas the nonheterocyclic alkaloids (also known as atypical or proto-alkaloids) contain a nitrogen atom derived from an amino acid which is not part of the heterocyclic ring.[104] Heterocyclic alkaloids are further divided into 14 subclasses, which include indoles, isoquinolines, pyrrolizidines, pyrrolidines, quinolizidines, tropanes, purines, piperidines, and imidazoles.[105] Since alkaloids contain in their chemical structure a nitrogen atom capable of accepting protons, and one or more amine-donating hydrogen atoms, they are able to bind enzymes, receptors, and proteins through hydrogen bonds, and this confers their high biological activity. Therefore these compounds have been extensively studied because of their biological activity and their pharmacological properties (*e.g.* anticancer, antibacterial, antiviral, and central nervous depressant activity).[106,107]

Alkaloids may also contain a large number of chiral centers, which has made their enantioselective separation from plant sources more difficult. Recently, increasing interest in plant alkaloids and their use for therapeutic purposes has led to the development of different extraction methods. However, the isolation yields are generally very low, and the methodology is expensive and time-consuming. With the aim of overcoming these issues, numerous attempts at synthesis have been made in order to easily access enantiomerically pure alkaloids.[108] In nature, the biosynthesis route to benzylisoquinoline alkaloids (BIAs), namely morphine, codeine, berberine, and papaverine, is a Pictet Spengler (P–S) cyclization, the precursor of which is a chiral tetrahydroisoquinoline (THIQ), namely *S*-norcoclaurine (NC).[109] Indeed, the enzymatic pathways of BIAs share a common route, in which the first committed step consists of a formal P–S condensation of 4-hydroxyphenylacetaldehyde (4-HPAA) with dopamine, stereospecifically catalyzed by the enzyme norcoclaurine synthase (NCS).[109] Accordingly, the synthesis of alkaloids has become possible via a P–S reaction, which has been widely studied for its application in the preparation of tetrahydro-β-carbolines (THBCs) and THIQ.[110–113]

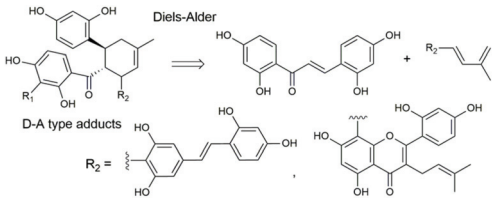
In the last few decades, a metathesis-based process has also been kept under consideration as a potential tool for accessing the pyrrolizidine and quinolizidine cores, typical of alkaloids.[114,115]

Within the *in house* library outlined in this review, alkaloids represent around 20% of all natural compounds, including the different subclasses mentioned above, and are found in more than 10 plant families, including the Apocynaceae, Loganiaceae, Berberidaceae, Papaveraceae, and Rubiaceae. The largest subclass of alkaloids in the library is represented by β-carbolines, which feature a common tricyclic pyrido[3,4-*b*] indole ring. Based on the saturation of the N-containing six-membered ring, β-carbolines are categorized as fully aromatic (BCs), dihydro- (DHBCs), and tetrahydro- (THBCs) β-carbolines.[116]

In a recent study, 39 alkaloids from the *in house* library were screened towards Gram-positive (*Staphylococcus aureus* ATCC 25923) and Gram-negative (*Escherichia coli* ATCC 25922) reference bacterial strains in order to identify potential new antibacterial agents. Interestingly, greater selectivity towards the human pathogen *S. aureus* was noted, especially for the BC alkaloids.[117] In fact, the rare heterodimer alkaloid nigritanine (**38**), which was isolated from different African species of the genus *Strichnos* (see section 2),[59] as well as some of its analogs (*i.e.*, speciociliatine, mytragine, and paynantheine) showed very high activity against the reference strain of *S. aureus* ATCC 25923. Furthermore, the antibacterial activity of nigritanine and its monomeric analogs was evaluated against three multidrug-resistant clinical isolates of *S. aureus*. It was found that only nigritanine had a low cytotoxicity and a very high activity with an MIC value of 128 μM, a feature not observed for the other BC analogues tested. From a chemical standpoint, **38** is a heterodimer alkaloid formed by the union of a corynane and a tryptamine unit. The analysis of the antibacterial activity related to the corynane scaffold indicated that the heterodimer showed a substantially higher activity than the monomeric analogs, highlighting the essential role of the tryptamine unit. These results confirmed the trend observed for the BC homodimer, for which dimerization increases the antibacterial activity, possibly because the larger molecule is less susceptible to bacterial efflux. Thus the heterodimer alkaloid nigritanine has emerged as a promising scaffold for the development of potent and selective antibacterial compounds with low cytotoxicity.[117]

4.2 Diels–Alder-type adducts

Diels–Alder (D–A)-type adducts are a class of complex polyphenolic natural compounds that are mostly isolated from moraceous plants. The main natural source of such compounds is the root bark of mulberry tree (*Morus alba* L., *Morus nigra* L., *etc.*),



Scheme 3 Retrosynthesis approach to D–A-type adducts.

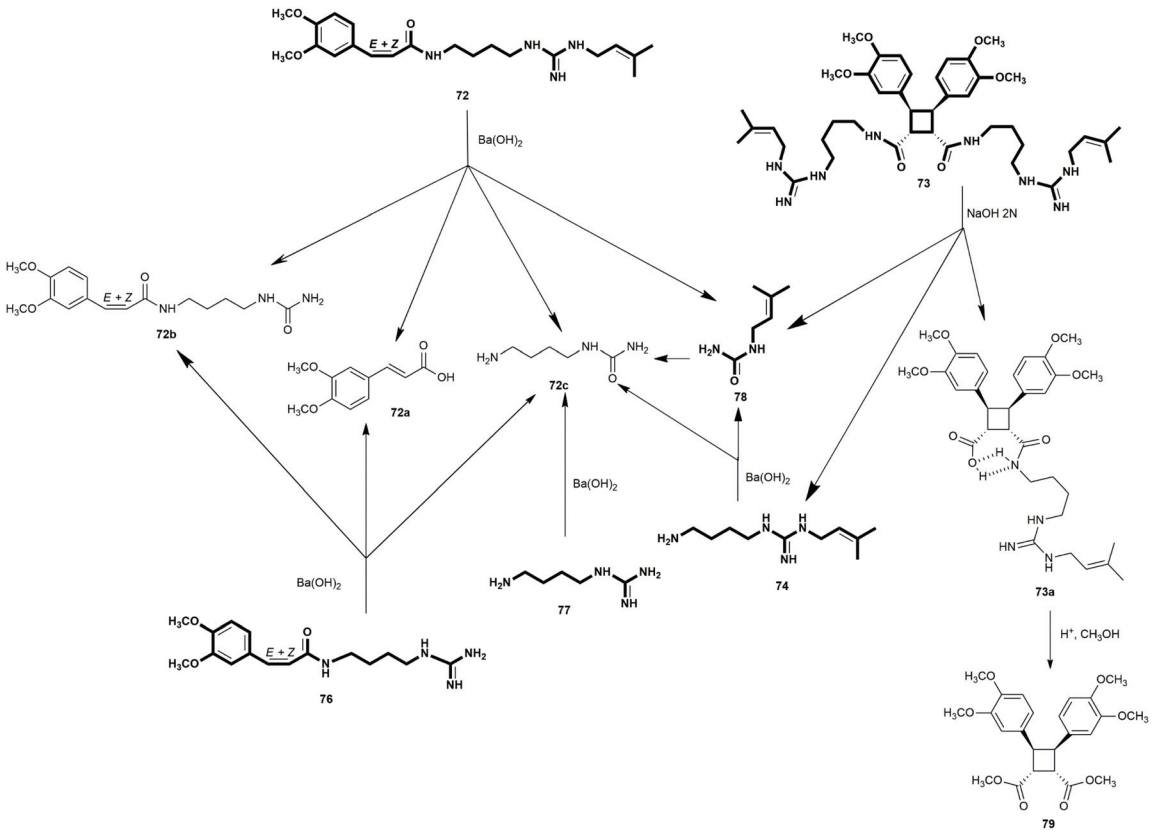
which is well known in traditional Chinese medicine as "Sang-Bai-Pi". About 40 kinds of optically active D–A-type adducts have been isolated from Japanese *Morus* root bark and Chinese crude drug "Sang-Bai-Pi", and the chemical structure of some of these compounds is shown in Chart 1. Their complex chemical structure, including a central cyclohexene ring endowed with different stereocenters, suggests that their biosynthesis proceeds through an enzymatic Diels–Alder [4 + 2] cycloaddition between two partners among flavonoids, chalcones, stilbenes, and 2-arylbenzofurans as precursors (Scheme 3).[118]

Such compounds showed several kinds of biological activity, including antioxidant,[119] anticancer,[119] antibacterial,[120] antifungal,[120] hypoglycemic,[121] and antitubercular activities,[84] among others. In 2013, six natural compounds were discovered as new inhibitors at low concentration of the protein tyrosine phosphatase B (PtpB), a virulence factor that is secreted by *Mycobacterium tuberculosis* and is essential for its survival.[84] An *in silico* screening of the library, followed by enzymatic and kinetic studies and MS assays, led to the identification of several natural compounds, belonging to the classes of isoflavanones, anthrones, anthraquinone dimers, and D–A type adducts. Kuwanol E (**67**), the only representative of the last class, was identified as the most potent inhibitor of PtpB from *M. tuberculosis*, with a K_i value of 1.6 ± 0.1 μM.[84] Compound **67** was therefore a promising antitubercular agent, but the scarce availability of the natural occurring sample in the *in house* library has limited biological studies. In order to confirm its chemical structure and set up a synthesis strategy capable of providing more material for further assays, in 2016 the first total synthesis of kuwanol E was reported, achieved through a convergent synthesis strategy, including a key biomimetic intermolecular Diels–Alder [4 + 2] cycloaddition.[122] In 2018, additional D–A-type adducts and other natural products isolated from *M. nigra* roots bark were screened.[85] The two most potent compounds that were found to inhibit PtpB at sub-micromolar concentrations were again two D–A-type adducts, namely kuwanon G (**68**) and kuwanon H (**69**). These results highlighted the relevance of the D–A-type scaffold in the development of promising candidates for the treatment of tuberculosis.[85] Another structurally very similar D–A-type adduct, namely kuwanon L (**70**), was also extracted and isolated from the root bark of *M. nigra* L. This compound was evaluated for anti-HIV activity and was shown to be an allosteric inhibitor of HIV-1 integrase *in vitro*.

Moreover, compound **70** is also capable of inhibiting HIV-1 replication in cell cultures.[123,124] In further studies, kuwanon L was found to inhibit both the integrase and the reverse transcriptase from HIV-1, making this natural product an attractive lead for the development of multi-target anti-HIV drugs. Soroceal (**29**), sorocein A (**30**) and B (**31**), and mulberrofuran K (**71**) also belong to the Diels–Alder-type adducts class, and have been isolated from *Sorocea ilicifolia* and *Sorocea bonplandii*,[60] both of which are plants used in Brazilian traditional medicine (see section 2). These compounds have a complex tricyclic acetal moiety in the center of their chemical structure. Such architecture derives from intramolecular acetalization of the carbonyl group of D–A-type adducts with two phenolic side groups. Such a reaction can also occur as a result of treatment of common D–A-type adducts with sulfuric acid.

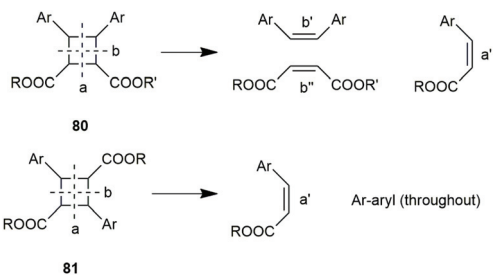
4.3 Guanidine-containing natural products

Plants represent an important source of guanidine-containing natural products with intriguing structures and promising biological activity. Several guanidine derivatives were isolated from various members of the Leguminosae, Tephrosieae, Urticaceae, Compositae, Fumariaceae and Fabaceae. The interest in guanidine-containing natural compounds is mainly due to their impressive biological activity, but is also related to their roles as bases, catalysts, membrane transporters, nucleotide mimetics, peptide mimetics, and molecular probes.[125–129] The major challenges are related to the isolation and structural identification of guanidine natural products, which occur in very complex and polar mixtures that are difficult to separate. As a consequence of this, a number of efforts have been focused on methods of synthesizing natural guanidine and its derivatives. Fascinatingly, the guanidine group has played a pivotal role in medicinal chemistry, becoming a key motif in the design and synthesis of new drugs. Synthetic guanidine derivatives, such as pinacidil, had attracted great interest as potential antihypertensive drugs that could block adrenergic nerve activity through central and/or peripheral mechanisms.[130–132] Several studies investigated the structural relationship between natural or synthetic guanidine derivatives and adrenergic blocking activity, indicating that small structural changes markedly decrease the activity.[133–135] Interestingly, Delle Monache *et al.* conducted an extensive investigation of the Venezuelan plant *Verbesina caracasana* (family Compositae). The authors reported that a crude methanol extract of this plant, when intravenously administered to mice, induced biological effects such as erection of hair, and initial stimulation and subsequent blockade of breathing.[136,137] A biologically controlled purification, followed by silica gel chromatography, yielded a series of active guanidine derivatives (**72–78**) which were extensively studied and patented as hypotensive drugs (Scheme 4).[138] The structural elucidation of the active compounds was performed by ^{1}H and ^{13}C NMR, MS and a set of reactions. Caracasanamide (**72**), isolated from the less polar fractions, was the first compound to be identified. Alkaline hydrolysis was chosen as a powerful tool for structural elucidation. Accordingly, the treatment of **72** with barium hydroxide yielded dimethoxycinnnamic acid (**72a**), a mixture of (*E*)- and (*Z*)-forms of the 4-[(3,4-dimethoxy-



Scheme 4 Basic and acid hydrolysis for structural elucidation of guanidine derivative from *Verbesina caracasana*.

cinnamoyl)arnino]butylurea (**72b**), 3-methyl-but-2-enylurea (**78**), and 4-amino-1-ureidobutane (**72c**) (Scheme 4). NMR analysis indicated that compound **72** subjected to hydrolysis featured a guanidine group (RNHC(NH)NHR′), and it was isolated as a mixture of the (*E*)- and (*Z*)-forms. Taking all of the data together, it was assigned the structure 1-[(3,4-dimethoxycinnamoyl)amino]-4-[(3-methyl-2-butenyl)guanidino]-butane. Due to its higher solubility in water, the (*Z*)-form was easily purified by crystallization. The structure of **72** was also confirmed by synthesis of the (*E*)-form.[136,137,139] From a biogenetic point of view, **72** seems to be formed from guanidine by a three-step process involving addition of the base, prenylation, and acylation. Using a biomimetic approach, the (*E*)-form of **72** was synthesized. The most promising procedure involves the alkylation of *N,N′*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea with 4-bromo-2-methyl-2-butene, the displacement of the methylthio group with 1,4-diamminobutane, and acylation with (*E*)-3,4-dimethoxycinnamic acid activated as a mixed anhydride with diethyl chlorophosphite.[106,112] A second compound, namely caracasandiamide (**73**), was further isolated and was assigned the dimeric structure bis(3′,4′-dimethoxy)-β-truxin-bis [[*N*-(3-methylbut-2-enyl)guanidobutyl]amide].[140] MS analysis suggested that compound **73** is a dimer with a cyclobutane skeleton featuring two phenyl rings and two carboxylic groups. This structural characterization matched with both truxinic (**80**) and truxillic acids (**81**), which can be easily distinguished



Scheme 5 Mass fragmentation of truxinic (**80**) and truxillic (**81**) acids.

by their mass fragmentation, as the cleavage of truxillic acid gives one type of fragment, whereas in the other case two fragments are observed (Scheme 5).

Therefore the analysis of mass fragmentation of **73** indicated the presence of truxinic acid, and along with NMR characterization assigned the structure of a dimer of **72** to **73**.[108] The analytical structural elucidation of **73** was supported by two different treatments. A first alkaline hydrolysis (NaOH 2 N) yielded prenylagmatine (**74**), 3-methyl-but-2-enyl urea (**78**), and a crystalline product (**73a**) (Scheme 4). ^{1}H NMR spectrum analysis of **73a** showed that the signals of the truxinic moiety were integrated in a 2 : 1 ratio with respect to the signals of the alkyl diaminobutane moiety. In addition, ^{13}C

NMR revealed the presence of two distinct carbonyl resonances and double signals for the aromatic and cyclobutane carbons, thus suggesting the hydrolysis of a prenylguanidobutanamine unit. Due to the presence of a hydrogen bond between the acidic and amido groups, the hydrolysis of the second prenylguanidobutanamine was achieved using strongly acidic conditions (H_2SO_4 2 N in MeOH). This led to the formation, in addition to the expected prenylurea (**78**) and prenylagmatine (**74**), of the methyl ester **79** (Scheme 4).[140,141] Extensive chromatographic purification of the more polar fraction of the extract allowed the isolation and structural elucidation of other compounds that featured the guanidine group.[142] The elution order was as follows: 1-amino-4-(3-methylbut-2-enyl)guanidinobutane (**74**); 1-(3,4-dimethoxycinnamoyl)amino-4-guanidinobutane (**76**); 1-amino-4-guanidinobutane (agmatine) (**77**); *N*-(3-methylbut-2-enyl)guanidine (galegine) (**78**). Two of the compounds, **74** and **78**, were previously identified as products of hydrolysis of **72** and **73**. Compound **78**, in turn, is the product of alkaline hydrolysis ($Ba(OH)_2$) of **74** along with **72c**. The latter product is also obtained by hydrolysis of **77** with $Ba(OH)_2$. The NMR spectrum data for **76** were almost coincident with those for **72**, except for the signals of the 3-methyl-2-butenyl chain.

4.4 Polyphenolic compounds

4.4.1 Isoflavones. Isoflavones are colorless polyphenols belonging to a widespread group of natural products called flavonoids. They are secondary metabolites produced by a branch of the general phenylpropanoid pathway by which flavonoid compounds are synthesized in higher plants. Despite the distribution of isoflavones within the plant kingdom being almost entirely restricted to species of the family Leguminosae, of which soybean is the richest source, they show a surprising degree of structural diversity.[143] Whereas most flavonoids have ring B attached at position C-2 of ring C, isoflavones characteristically have a 3-phenyl-chromen-4-one skeleton (Fig. 2).

Although they are not steroids, isoflavones have structural similarities to estrogens, and particularly to estradiol. This peculiarity confers on them pseudohormonal properties, such as the ability to bind estrogen receptors. Therefore they are classified as "phytoestrogens" or "plant estrogens".[144] Isoflavones are considered to be chemoprotective agents, and can be used as an alternative or additional therapy for a wide range of hormonal disorders, including several types of cancer (*e.g.* breast cancer), osteoporosis, and menopausal symptoms.[145–148] Within the *in house* library, isoflavones represent around 30% of the flavonoid class and are characterized by significant chemical diversity generated through the varied distribution of hydroxyl, methoxyl, *C*- and *O*-prenyl groups on their core as well as the different oxidation levels and the presence of extra heterocyclic rings (*i.e.* isoflavan, isoflavanquinone, and pterocarpan derivatives).[46,149,150] Moreover, synthetic isoflavones inspired by natural compounds contribute to enrichment of the library collection, making it a unique reservoir of new therapeutic leads (from **82** to **104**). In 2015, a joint investigation by a research group headed by Professor Bruno Botta and experts in the molecular medicine of brain tumors enabled identification of isoflavone glabrescione B (**14**) as a good preclinical candidate for the treatment of Hedgehog (Hh)-dependent tumors (Fig. 2).[89]

The Hh signaling pathway plays an important role in mediating normal development, oncogenesis, tumor progression, stem cell biology, and tumor–stroma interactions.[88,95] Its aberrant activation, caused by several germline or somatic mutations in components of the Hh pathway, has been observed in many tumors, including medulloblastoma (MB) and basal cell carcinoma (BCC).[151] Consequently, pharmacological Hh inhibition at the level of the seven-pass transmem-

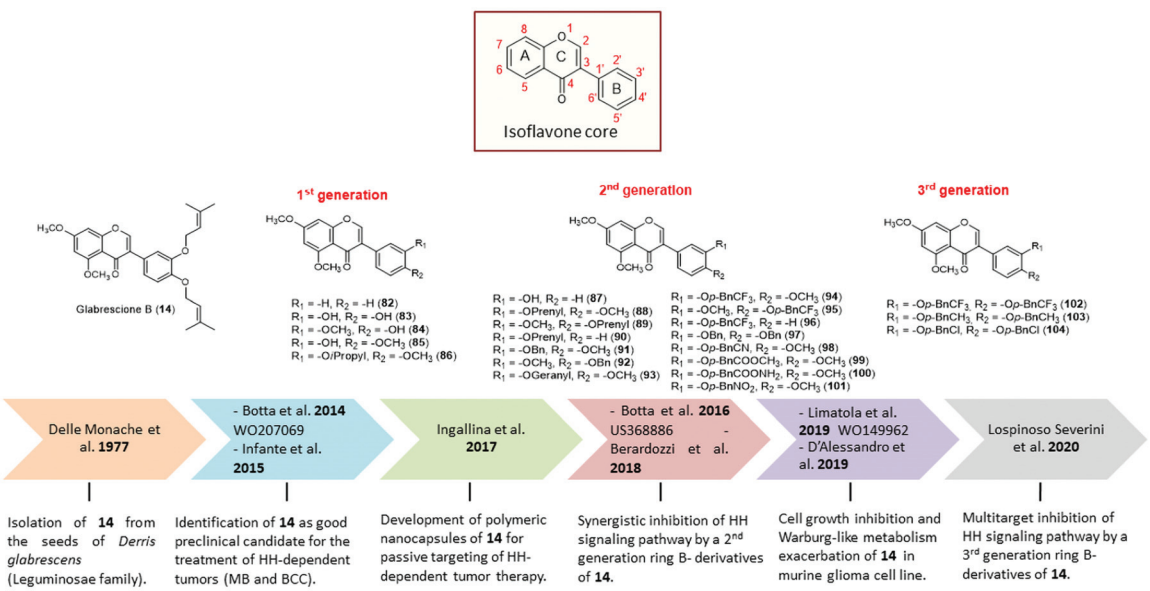


Fig. 2 Timeline for the development of the isoflavone scaffold from hit to lead optimization.

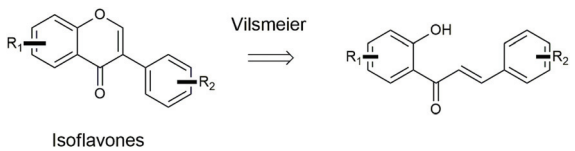
brane receptor SMO has emerged as an attractive anticancer strategy as highlighted by the development of Vismodegib, Sonidegib, and Glasdegib, three FDA-approved drugs for the treatment of metastatic and locally advanced BCC, and newly diagnosed acute myeloid leukemia.[87,152,153] However, drug-resistant mutations occurring at the SMO receptor as a consequence of pharmacological overstimulation, coupled with Hh activation downstream or independently of SMO, have indicated the need to identify a novel strategy for inhibition of Hh signaling. Particular interest has been shown in the identification of molecules that are able to target glioma-associated oncogene (GLI) transcription factors, the final effectors of the Hh pathway, whose druggability has been assessed by means of isoflavone **14**, and the synthetic inhibitor GANT-61, although the latter suffers from chemical instability.[88,154] Based on knowledge of the crystallographic structure of the zinc finger domain of GLI1 (GLI1ZF) in a complex with DNA, together with NMR studies as well as computational and experimental mutagenesis, the structural requirements of GLI1/DNA interaction were identified.[89] By a combination of docking-based virtual screening of the *in house* library towards the DNA-binding site of GLI1 and *in vitro* transcriptional assays, **14** was identified as a novel small molecule that was able to bind GLI1ZF and interfere with DNA interaction. The naturally occurring isoflavone GlaB showed significant anticancer efficacy against Hh/GLI-dependent MB and BCC as well as cancer stem cells (CSCs) both *in vitro* and *in vivo*, and therefore it was patented as a useful treatment for Hh-dependent tumors.[89] GlaB was isolated and characterized for the first time by Delle Monache *et al.* in 1977, making the *in house* library its unique source (see section 2).

Extraction methods allow the recovery of only very limited amounts of pure compound **14**, so in order to support its initial stage of development the total synthesis of **14** was achieved in just three steps, through the deoxybenzoin intermediate, with an overall yield of 15% (Scheme 6).[86,90]

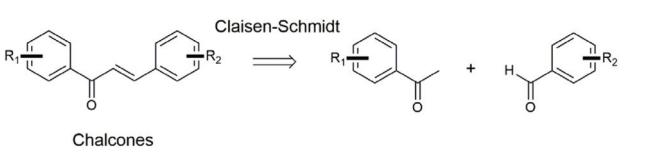
To gain insight into the molecular mechanism underlying Hh signaling modulation, the NMR technique was used and the pivotal role of ring B of **14** in its direct interaction with GLI1ZF was highlighted. Moreover, the contribution of *O*-prenyl chains to its biological activity was demonstrated by the synthesis of a first generation of ring B derivatives of **14**, which proved to be inactive. Since isoflavone emerged as a privilege scaffold in Hh inhibition, a second generation of ring B derivatives of **14** with the ability to interact selectively with the SMO receptor or GLI transcription factors was synthesized and tested *in vitro*.[86] The experimental results, supported by molecular modeling predictions, demonstrated that the insertion of a bulky substituent at the *meta* or *para* position of isoflavone ring B (*i.e.* compounds **88**, **95**, and **97**) enhanced the specific affinity of these compounds for GLI or SMO, respectively, providing HH-dependent tumor growth inhibition in primary MB cells at sub-micromolar concentrations (Fig. 2). Interestingly, their simultaneous administration in primary MB cells showed the synergistic inhibition of Hh pathway at both upstream and downstream levels by using doses that were around 20-fold lower than individual compound doses. Based on these findings, further attempts were made to design a single Hh multitarget inhibitor.[91] Accordingly, a third generation of **14**-inspired isoflavones featuring two *para*-substituted benzyloxy moieties in place of the prenyl chains at ring B were synthesized. Among them, compound **103** revealed simultaneous targeting of both SMO and GLI and a strong inhibition of Hh-dependent tumor growth in human and murine MB cells at sub-micromolar concentrations (Fig. 2). Moreover, intratumoral administration of **104** reduced Hh-driven tumor growth in an *in vivo* allograft model of MB by suppressing cell proliferation and promoting apoptosis.

4.4.2 Chalcones. The generic name "chalcone" is derived from the Greek word "chalcos", meaning "bronze", which refers to the color of most natural chalcones. These compounds are one of the most important subclasses of polyphenols, with a widespread distribution in the plant kingdom.[155] Chalcone consists of a simple chemical scaffold featuring two aromatic rings joined by a three-carbon α,β-unsaturated carbonyl system, and among them the E isomer is the predominant configuration. Chalcones have a simple chemistry that allows a multiplicity of substitutions to be made by means of convenient and cost-effective synthesis procedures (Scheme 7).[156]

As a result, these natural products consist of a privileged scaffold that is widely used as an effective template in drug discovery.[157] Chalcones and their synthetic derivatives have shown several interesting biological activities with clinical potential against various diseases.[158–160] In the *in house* library, chalcones represent 20% of the polyphenols class and consist of natural chalcones and dehydrochalcones as well as their synthetic derivatives. As previously described, the SMO receptor is one of the main upstream transducers of the Hh signaling pathway, a key driver in tumorigenesis, and several SMO antagonists are currently being investigated in various stages of clinical trials.[87] However, in addition to common adverse effects, the main drawback of SMO antagonists is their transient effect due to the outgrowth of several resistant mutations in SMO residues located in proximity to the antagonist's site (*i.e.* D473, W281, V321, I408, C469, and Q477), or located distally (*i.e.* W535, L412, T241, A459, and



Scheme 6 Vilsmeier cyclization to form an isoflavone core.



Scheme 7 Retrosynthetic approach to building chalcones.

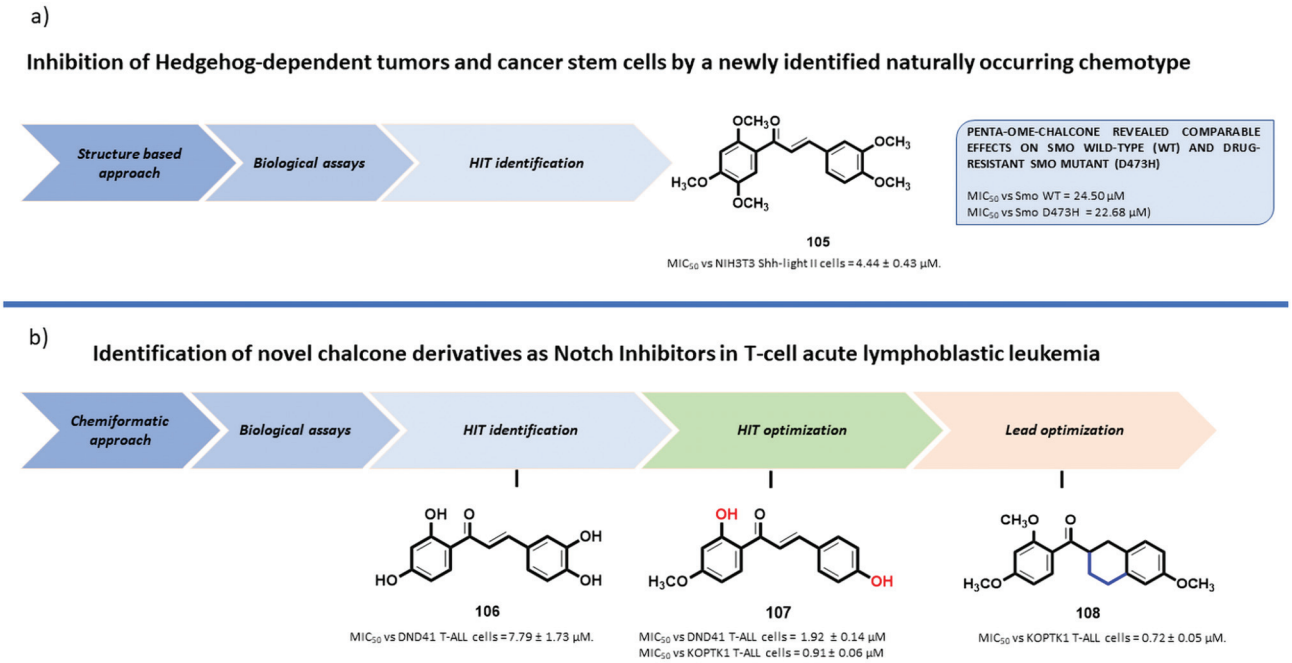


Fig. 3 Discovery of naturally occurring chalcones as anticancer agents.

S533).[152,161,162] Due to the occurrence of drug-resistant SMO mutations, one current challenge for addressing clinical failure is the identification of new SMO antagonists with novel and diverse chemotypes, also exploiting high-resolution structural elucidation and computational modeling techniques.[163] In an effort to identify novel chemotypes of SMO antagonists, the docking-based virtual screening of the *in house* library of natural compounds and their derivatives identified 2′,3,4,4′,5′-pentaOMe-chalcone (**105**) as the most promising compound (Fig. 3a). Chalcone **106** proved to be the most effective Hh inhibitor, with an IC_{50} of 4.44 μM, and also showed efficacy against the drug-resistant form of SMO as well as anticancer effects in MB models *in vitro* and *in vivo*. The straightforward chemistry of this compound made it a versatile building block for developing potential therapeutic agents for Vismodegib- or Sonidegib-resistant tumors.[164] Notch signaling, another important pathway that is hyperactivated in CSCs, is considered to be a rational target in the therapy of several cancers, including T-cell acute lymphoblastic leukemia (T-ALL).[165] Several small molecules, including gamma secretase inhibitors (GSIs) such as MK-0752, RO4929097, and PF-03084014, and Notch1-specific antibodies such as OMP-52M51, were moved to clinical trials for the therapy of Notch-driven tumors.[98,165] Unfortunately, the potential clinical applications of GSIs are limited by primary resistance and/or by severe side effects reducing their widespread therapeutic use.[166] In addition to the approaches described above, several attempts have been made to develop small molecule Notch-blocking agents that are able to target the pathway without interfering with GS enzyme, although their mechanism of action is still being investigated. Since the three-dimensional structure of the Notch receptor was unknown at the time of our study, new chemotypes of Notch-modulating agents from the *in house* library were identified through the cheminformatics clustering approach based on a combination of fingerprint and maximum common substructure (MCS) searches.[97] The representative molecules of the most abundant clusters, selected on the basis of physical availability at the time of the experiments, hit-like features, and chemical diversity criteria, were screened *in vitro* for their ability to reduce Notch-signaling activity and cell growth in Notch-dependent human T-ALL cell lines (DND41, KOPTK1, and TALL-1 cells). Among them, the natural occurring butein (**106**, 2′,3,4,4′-tetrahydroxychalcone, isolated from *Butea*, *Dahlia*, *Coreopsis*, and *Searsia*, was found to be the most powerful Notch inhibitor, with an IC_{50} value of 7.79 ± 1.73 μM in DND41 T-cells (Fig. 3b). Therefore, **106** emerged as a valuable hit compound, and a design-synthesis-bioassay workflow was employed for hit optimization and to overcome undesirable chemical features. Several butein derivatives were synthesized and tested, leading to the identification of a novel potent Notch inhibitor, chalcone **107**, the most effective molecule of the series in impairing DND41 T-cell growth, with an IC_{50} value of 1.92 μM ± 0.14 μM. A dipper investigation demonstrated that **107** suppressed cell growth of several human T-ALL cell lines by promoting apoptosis and G1 cell cycle arrest. Based on the biological evaluations, a structure–activity relationship (SAR) study suggested that there was synergistic activity of 2′- and 4-hydroxy groups (Fig. 3b).[97] Recently, a first generation of chalcone **107** derivatives was designed and synthesized based on hit-likeness and chemical diversity, and their antiproliferative activity and inhibition of Notch1 expression in KOPTK1 cells were evaluated. The best biological

results in this series were obtained for compound **108**, featuring a tetrahydronaphthalene-based scaffold, which was identified as a promising new Notch-blocking agent (Fig. 3b). SAR analysis of the chalcone series confirmed the key roles played by the 2′- and 4-OH groups in both antiproliferative and Notch1 inhibitory activity. In addition, the introduction of a bicyclic ring to the chalcone structure, which reduced the conformational freedom of chalcone mimetic analogs by removing the double bond conjugated to the enone, maintained the ability to exert inhibition of the Notch pathway, although with some differences compared with chalcones.[99]

4.5 Terpenoids

Terpenoids, also known as isoprenoids, represent the largest class of secondary metabolites, and do not usually contain nitrogen or sulfur in their structures. The generic name "terpene" was originally derived from the hydrocarbons found in turpentine, whereas the suffix "ene" indicates the presence of olefinic bonds.[167] Isoprene (2-methylbuta-1,3-diene, C_5H_8) is the basic structural unit of terpenoids. The so-called "isoprene rule" states that all terpenoids are derived from the ordered head-to-tail joining of isoprene units. A head-to-tail fusion is the most common type; however, non-head-to-tail condensation of isoprene units also occurs.[167,168] Accordingly, terpenoids are classified based on the number of isoprenoid units present in their structure, as follows: two isoprenoid units (monoterpenes), three isoprenoid units (sesquiterpenes), four isoprenoid units (diterpenes), five isoprenoid units (sesterterpenes), six isoprenoid units (triterpenes), and eight isoprenoid units (tetraterpenes). In the *in house* library, this class covers around 30% of the natural products and includes monoterpenes, sequiterpenes, diterpenes, and triterpenes. Diterpenes and triterpenes are the largest subclasses of the collection, and the diterpenes also include several semisynthetic derivatives. As was described in section 2, several unique natural products belong to the diterpenoid group. Most of them were isolated from *Fabiana densa* var. *ramulosa* (family Solanaceae), and they consist of diterpenoids **38–40**, namely succinoyl, oxaloyl, and malonoyl esters of *ent*-beyer-15-en-18-ol, and the corresponding dimers **41–43** (Fig. 4a).[68,69] A thorough characterization of the antimicrobial activity of these non-common diterpenes was evaluated against a panel of Gram-negative and Gram-positive bacterial reference strains. Diterpenoid **40** displayed selective activity against Gram-positive bacterial strains with negligible cytotoxicity towards human keratinocytes, whereas the dimeric diterpenes showed no efficacy towards any of the tested microorganisms (Fig. 4a). These results paved the way for the optimization of new diterpene-based drugs for the development of new anti-infective agents. In a recent study aimed at identifying potential inhibitors of Ara4N-dependent colistin resistance, the last-resort treatment for many multidrug-resistant Gram-negative bacteria, a docking-based virtual screening of the *in house* chemical library within the catalytic site of ArnT (the enzyme responsible for colistin resistance mediated by lipid A aminoarabinosylation in *Pseudomonas aeruginosa*) was carried out, leading to the final selection of 18 candidate hits that were then subjected to biological investigations.[169] *In vitro* screening identified diterpene **39** as a promising colistin adjuvant activity, which was able to potentiate colistin activity against colistin-

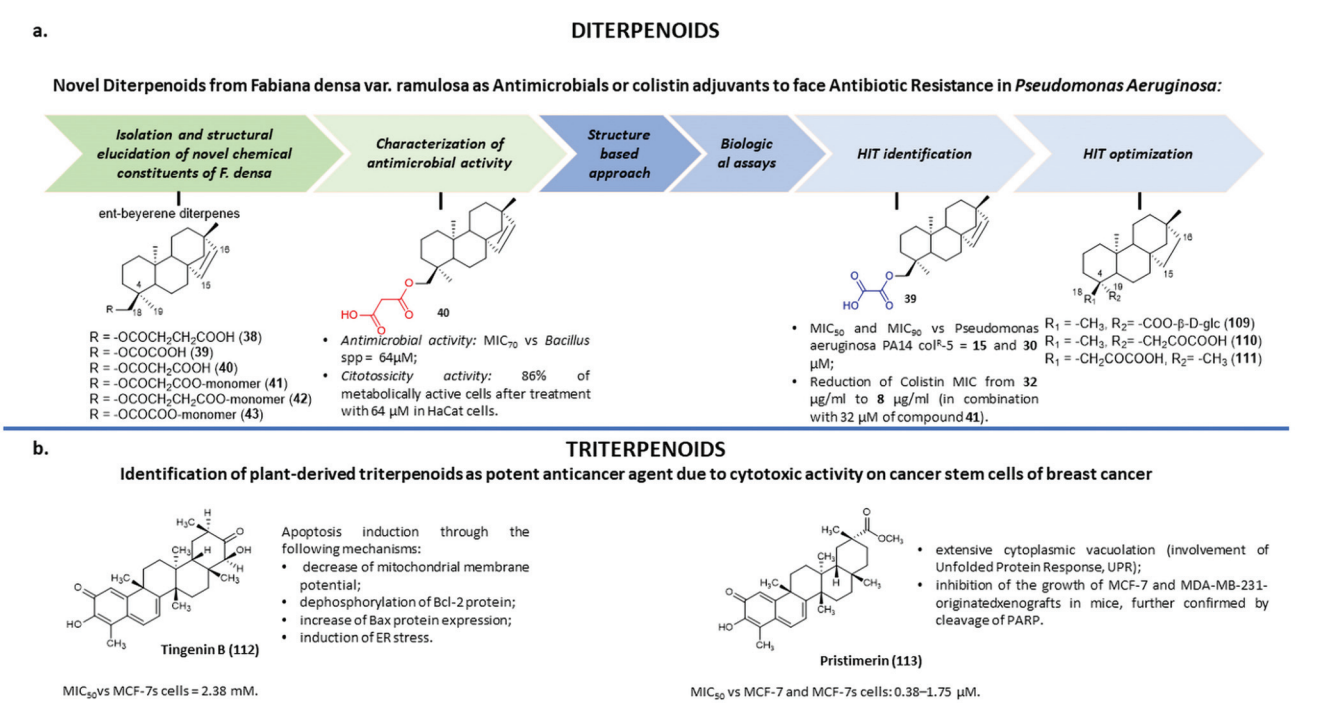


Fig. 4 Discovery of naturally occurring terpenes as antimicrobial agents, colistin adjuvants, or anticancer agents.

resistant *P. aeruginosa* isolates without affecting growth *per se*, and with no activity against colistin-sensitive strains (Fig. 4a). This compound was also effective against colistin-resistant clinical isolates of *Klebsiella pneumoniae*. The binding mode of **39** within the catalytic site of ArnT was investigated by molecular docking simulations, which led to identify two main binding poses. In both poses, the oxalyl group nicely overlapped with the crystallographic phosphate moiety, and established an H-bond interaction with the Lys85 residue, which was shown to be important for ArnT activity. In order to validate the efficacy of the diterpene scaffold as a key platform for further development of ArnT-mediated colistin resistance inhibitors with increased activity, a wide variety of chemical analogs were designed for SAR studies. In particular, different derivatives of diterpene **39** were synthesized with the aim of investigating the role of (i) the length and flexibility of the alkyl chain of the functional group at C-18, (ii) the chirality of C-4, (iii) the presence of a sugar unit to mimic L-Ara4N, and (iv) the unsaturation between C-15 and C-16, with regard to the biological properties of the original diterpene scaffold. Microbiological assays coupled with molecular modelling indicated that for a more efficient colistin adjuvant activity, probably resulting from inhibition of ArnT activity by the selected compounds, and therefore from their interaction with the catalytic site of ArnT, an *ent*-beyerane scaffold is required along with a sugar residue at C-19 to resemble L-Ara4N (**109**), or an oxalate-like group at C-18/C-19 (**110** and **111**). The *ent*-beyerane skeleton, which differs from the parental *ent*-beyerene scaffold in that it lacks unsaturation between C-15 and C-16 and has an absolute configuration at C-4 (R rather than S), was identified for the first time as a privileged scaffold for further development and optimization of valuable colistin resistance inhibitors.[102,170] Triterpenes have relatively complex cyclic structures, most of them being alcohols, aldehydes, or carboxylic acids, and represent the largest class of terpenes in the *in house* library, including friedlane, oleane, ursane, lupan, and steroid types. Triterpenes exhibit a spectrum of important pharmacological activities, and quinone-methide triterpenes in particular, which have been extensively studied over the last few decades, were found to inhibit CSCs that are resistant to standard chemotherapies.[171] Among them, a friedlane triterpene, namely tingenone, proved to be cytotoxic against several cancer types, including breast cancer.[172] Based on this evidence and the availability in the *in house* library of a structurally related quinone-methide triterpenoid, namely tingenin B (**112**), isolated from *Maytenus* species, the *in vitro* cytotoxicity and cell death mechanism of tingenin B in breast CSCs were investigated.[173,174] The results demonstrated that tingenin B (**112**) has a promising cytotoxic activity and an apoptosis induction and/or ER stress-inducing effect towards breast CSCs, although *in vivo* experiments are required in order to demonstrate its potential for the better management of breast cancer (Fig. 4b).[173]

A recent study investigated the mechanism of action of another quinone-methide triterpenoid that occurs in the *in house* library, namely pristimerin (**113**), which exhibited growth inhibitory activity in breast cancer cells.[175,176] The results showed that **113** induced cytoplasmic vacuolation-mediated cell death involving endoplasmic reticulum (ER) stress and protein ubiquitination (Fig. 4b).[175] This study provided a thorough characterization of the pharmacological mechanisms underlying the action of **113** in the treatment of breast cancer.

4.6 Cannabinoids

Phytocannabinoids are a class of natural compounds that occur mainly in plants belonging to the family Cannabinaceae, but are also found in some other higher plants (*Helichrysum umbraculigerum* Less., *Amorpha*, *Glycyrrhiza*, and *Rhododendron*), liverworts (*Radula* species), and fungi. From a chemical point of view, phytocannabinoids can be defined as isoprenylated resorcinyl polyketides that are biogenetically synthesized from geranyldiphosphate and olivetolic acid in the trichomes (epidermal protuberances that cover the leaves). From a biological point of view, this class of natural compounds is best known for the psychotropic compound Δ^9-THC, despite the slow abandonment of the *Cannabis sativa* (or industrial hemp) crop during the period 1950–2000. However, over the last 15 years there has been a renewed focus on phytocannabinoids, on account of the interesting biological properties of some of these compounds.[177] In particular, cannabidiol (CBD) is receiving increasing attention due to its role in the treatment of several neurological disorders,[178] and today represents an effective new option for the treatment of two forms of drug-resistant epilepsy (Lennox–Gastaut syndrome and Dravet syndrome). In 2000 the European Union published a regulation concerning the reintroduction of some industrial hemp cultivars characterized by low Δ^9-THC content which need to be certified and included in the list (ref.). Since then there has been an exponential increase in industrial hemp cultivation all around the world, and particularly in European counties, from 8000 ha in 2011 to 46 700 ha in 2017.[179] However, during these years the loss of cultivation has contributed to the expression of phenotypic features, despite the genomic properties, and therefore a thorough investigation of the effects of different agronomic practices, the seasons, and pedoclimatic conditions on the pattern of primary and secondary metabolite production is needed. Furthermore, renewed interest in this crop is now involving new economic areas ranging from pharmaceuticals to food chemistry, and from the cosmetics industry to bioengineering. Since 2016 the unique library has contained several methanolic extracts from industrial hemp inflorescences, which are characterized by low Δ^9-THC content (below 0.6%). In an attempt to determine the influence of seasonal effects, genomic features, and pedoclimatic and agronomic conditions, different cultivars belonging to both monoecious and dioecious varieties have been investigated. The first published study investigated the chemico-biological characterization of four monoecious cultivars, namely Uso 31, Felina 32, Ferimon, and Fedora, during the flowering period by developing and applying a multi-methodological analytical protocol.[180] The

combination of untargeted (NMR) and targeted (UHPLC, GC-MS, HPLC, and spectrophotometric analyses) methodologies allowed the identification, quantification, and monitoring of selected classes of natural compounds, namely amino acids, sugars, organic acids, cannabinoids, terpenoids, phenols, flavonoids, tannins, and biogenic amines, throughout the flowering period. Some compounds could represent markers of a specific cultivar, namely neophytadiene, nerolidol, and chlorogenic acid identified in Felina 32, whereas alloaromadendrene and *trans*-cinnamic acid were detected only in Uso-31. Although monoecious hemp cultivars are selected for seed production and thus mainly for food purposes, cannabinoid content was found to increase over the flowering period (from June to September). CBD was the most concentrated cannabinoid among the six that were quantified, and higher levels were found in Fedora inflorescences. This study allowed assessment of the activity of the cannabinoid pathway, which increased over the flowering season in the four cultivars examined, reaching a maximum near the end of that period. Moreover, on the basis of metabolic profiling, biological evaluation is ongoing. A further study involving the chemical and biological exploration of both essential oil and aromatic water of *Cannabis sativa* L. inflorescences by the application of a multi-methodological approach was carried out.[181] The chemical composition was investigated in terms of cannabinoid content, volatile components, phenolic and flavonoid patterns, and colour characteristics by means of GC/MS, HPLC, and spectrophotometric methods. In addition, using a wide multidisciplinary approach the potential applications of the essential oil were investigated for different biological activities, including antioxidant and antiradical activity, enzyme inhibition, antimicrobial activity against bacteria and fungi, and comparative cytotoxic activity against tumor and non-tumor cell lines. The results validate the use of this natural product as a rich source of important biologically active molecules, and highlight the role played by naringenin, one of the most important secondary metabolites.

In order to investigate how different agronomic practices affect the phytochemical profile of a single cultivar, the composition of Ferimon monoecious inflorescences was also monitored throughout the season under different conditions, namely irrigation, three levels of nitrogen fertilization, and three levels of phosphate fertilization (in preparation).

5. Conclusions

Historically, a considerable number of drug discovery efforts have been based on natural products. However, many resources still need to be explored in modern natural product research. To date, about 200 000 plant secondary metabolites are included in the Dictionary of Natural Products, of which 170 000 are unique structures.[191] Among all molecules in clinical trials, 15% are plant-related compounds, and 60% of these were isolated from only 10 taxonomic families. It is noteworthy that despite natural products being a unique source of molecular diversity in drug discovery campaigns, less than 1% of this vast biodiversity has been investigated as a potential source of drug candidates. Even traditional medicine from different countries and cultures, which is based mainly on plant remedies, requires further investigation in this context. Recent advances in isolation, purification, and characterization technology, as well as the development of powerful and user-friendly informatic tools, allow the isolation, identification, and screening of novel promising natural scaffolds endowed with biological activity. Thus improvements in natural product investigation, together with the systematic construction of collections and the employment of computer-based screening analyses, will lead to the development of more rational and successful approaches to drug discovery. The present review provides a guideline for researchers on achieving the maximum potential from collections of natural compounds, in terms both of creating a collection of synthetic and natural compounds collection, and of successfully applying screening campaigns for the identification and optimization of bioactive small molecules within the drug discovery program.

Conflicts of interest

There are no conflicts to declare.

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