Update on Phytochemical and Biological Studies on Rocaglate Derivatives from *Aglaia* Species#

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ABSTRACT

With about 120 species, *Aglaia* is one of the largest genera of the plant family Meliaceae (the mahogany plants). It is native to the tropical rainforests of the Indo-Australian region, ranging from India and Sri Lanka eastward to Polynesia and Micronesia. Various *Aglaia* species have been investigated since the 1960s for their phytochemical constituents and biological properties, with the cyclopenta[*b*]benzofurans (rocaglates or flavaglines) being of particular interest. Phytochemists, medicinal chemists, and biologists have conducted extensive research in establishing these secondary metabolites as potential lead compounds with antineoplastic and antiviral effects, among others. The varied biological properties of rocaglates can be attributed to their unusual structures and their ability to act as inhibitors of the eukaryotic translation initiation factor 4A (eIF4A), affecting protein translation. The present review provides an update on the recently reported phytochemical constituents of *Aglaia* species, focusing on rocaglate derivatives. Furthermore, laboratory work performed on investigating the biological activities of these chemical constituents is also covered.

Introduction

Since the first phytochemical report of the tetracyclic triterpene, aglaiol, from the leaves of the oriental plant *Aglaia odorata* in 1965 [1], many studies have appeared describing the chemical constituents of *Aglaia* species in terms of elucidating the structurally diverse natural products present. Examples of secondary metabolite compound classes isolated from *Aglaia* species include bisamides [2–4], flavonoids [2, 5], lignans [6], and triterpenoids, particularly of the baccharane, cycloartane, and dammarane types [7–9]. Also, more than 100 biogenetically related oxygen-containing heterocyclic secondary metabolites have been isolated to date. They are characteristic of many *Aglaia* species and are known col-

Dedicated to Professor Arnold Vlietinck to recognize his important contributions to natural product research on the occasion of his 80th birthday in 2021.

ABBREVIATIONS

5′-UTRs	5′-untranslated regions
ADC	antibody-drug conjugate
BCSCs	breast cancer stem cells
CDX	cell line-derived xenograft
CPBF	cyclopenta[*b*]benzofuran ring system
DDR	didesmethylrocaglamide
DDX3	DEAD-box RNA helicase 3
EBOV	Ebola virus
eIF4A	eukaryotic initiation factor 4A
EV71	enterovirus 71
FLT3	FMS-like receptor tyrosine kinase 3
HCV	hepatitis C virus
HepG2	human hepatoblastoma cell line
HEV	hepatitis E virus
IRF1	interferon regulatory factor 1
MDR1	multidrug-resistance protein 1
MPNST	malignant peripheral nerve sheath tumor
NSCLC	non-small cell lung carcinoma
p53/Puma	p53 upregulated modulator of apoptosis
PC-3	prostate cancer cell line
PDA	pancreatic ductal adenocarcinoma
PDX	patient-derived xenograft
PHB1/2	prohibitins 1 and 2
PIM1	proto-oncogene serine/threonine-protein kinase
Raf-MEK-ERK	mitogen-activated protein kinases (MAPKs) involved in cell proliferation and survival

lectively as rocaglamide (**1**) derivatives. Such compounds have been divided into 3 sub-classes: (i) CPBFs ("flavaglines" or "rocaglates"); (ii) cyclopenta[b]benzopyrans ("thapsakins" or "aglain" derivatives), and (iii) benzo[*b*]oxepines ("thapoxepines" or "forbaglines"), which are thought to be formed by the cycloaddition of a cinnamic acid amide and a flavonoid unit [10, 11]. Of these 3 groups, rocaglates are the most potently bioactive and well-investigated chemical constituents of *Aglaia* species and thus the main focus of the present article.

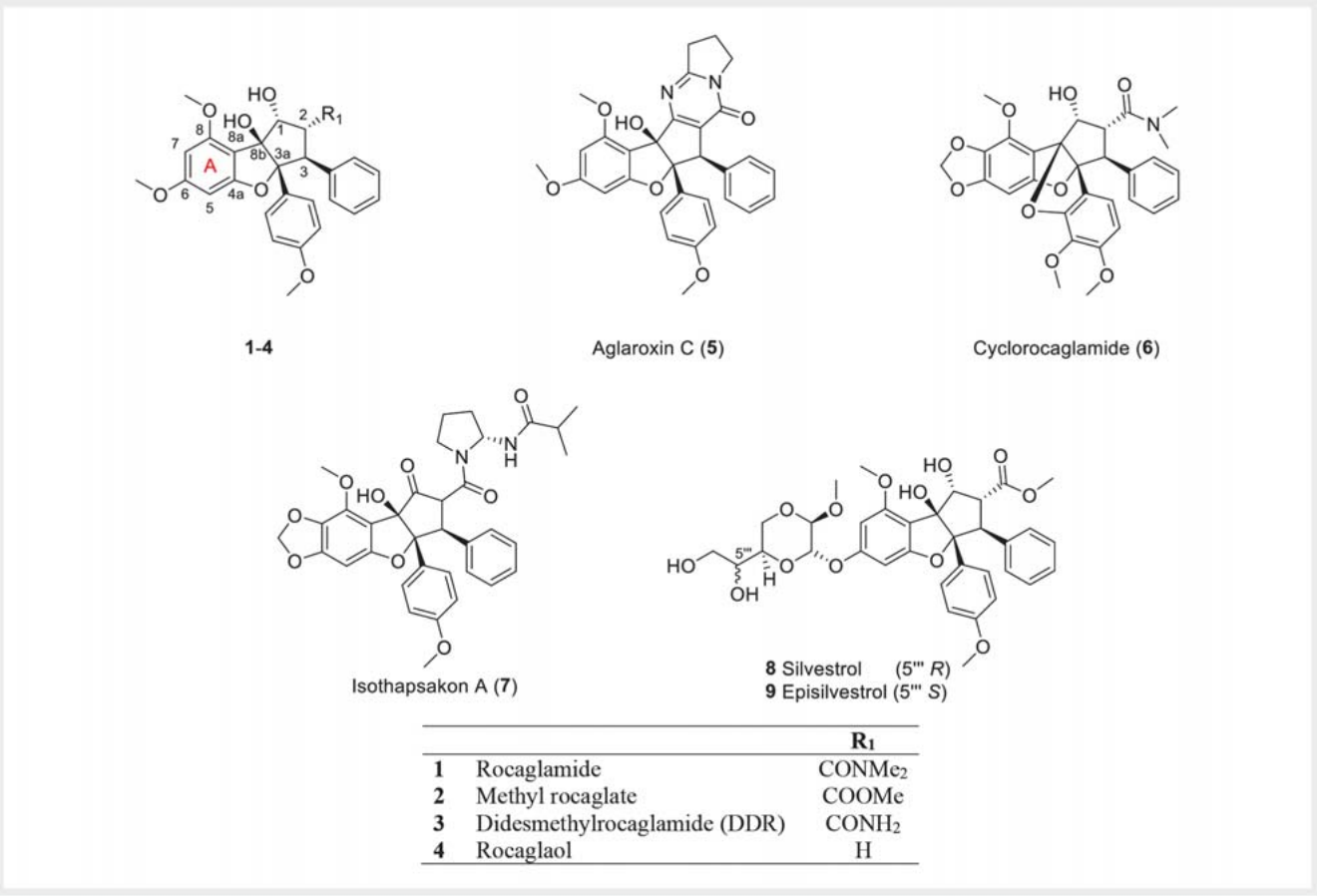
(−)-Rocaglamide (**1**), a CPBF, was the first compound obtained in its class and was isolated in 1982 from the dried roots and stems of *Aglaia elliptifolia* as an *in vivo*-active antileukemic agent [12]. In subsequent years, more than 60 rocaglate derivatives have been isolated and structurally identified from several *Aglaia* species [13, 14]. With most substitutions occurring at the C-1 and C-2 positions of their phenyl rings (▶ **Fig. 1**), some examples of rocaglate derivatives are methyl rocaglate (**2**) [15, 16], DDR (**3**) [13, 15], rocaglaol (**4**) [17], aglaroxin C (**5**) [18], cyclorocaglamide (**6**) [19], and isothapsakon A (**7**) [20]. Another rocaglate derivative that has garnered much scientific attention is silvestrol (**8**), which was isolated and structurally characterized along with its 5‴-epimer, episilvestrol (**9**) [21]. These 2 rocaglate congeners were purified from the stem bark of *Aglaia foveolata* (originally misidentified taxonomically as *Aglaia silvestris*) collected in Indonesia and contain an unprecedented dioxanyl ring connected to the CPBF core at the C-6 position of the phenyl ring A (▶ **Fig. 1**). The presence of a dioxanyl ring was demonstrated to enhance the cytotoxic potency of rocaglates [2, 22, 23] and has led to extensive work on the synthesis and structure-activity requirement exploration of rocaglamide, silvestrol, and related analogs [22–28].

A pivotal paper exploring the cellular mechanism of activity of the rocaglates was published in 2008 [29], in which Pelletier and his associates reported silvestrol as an inhibitor of protein translation by modulating the activity of the eIF4A, an RNA helicase subunit of the eIF4F complex. This work has been complemented by further mechanistic reports from the same group [30, 31], with selected rocaglates also being documented to act at the cellular level to modulate the Raf-MEK-ERK pathway via targeting PHBs 1/2 [32], MAPK [33], and FLT3 and the microRNA-155 (*miR-155*) gene [34]. Such biological studies have laid the foundation for developing rocaglamide and silvestrol as potential drug leads against different disease states, including, in particular, cancer, and more recently, certain viruses.

This review describes the work on rocaglates from *Aglaia* species, primarily in terms of their phytochemical isolation, structural characterization, and biological activities, as reported from 2014 to 2020. It is intended as an update of the 2 previous review articles we wrote on this same topic in 2006 [22] and 2014 [23] and includes experimental contributions from the respective laboratories of the 3 current senior authors. Additionally, a summary is included of the collection of several *Aglaia* species from 3 Southeast Asian countries (Indonesia, Laos, and Vietnam) under various formal Memoranda of Agreement (MOA) with the University of Illinois at Chicago, as part of 2 multi-institutional collaborative research projects funded by the U. S. National Cancer Institute (NCI) [35–37]. To assist with the writing of this review, we searched the SciFinder literature database (Chemical Abstracts Service, Columbus, OH, USA) using keywords such as *Aglaia*, rocaglamide, and silvestrol and then categorized and refined for relevant publications and patents from 2014 onward.

Taxonomy and Collection

The genus *Aglaia* is a large group of plants, mostly comprised of trees, belonging to the family Meliaceae. These species have a natural distribution spanning the tropics from Sri Lanka and India, east to the Pacific through Burma, southern mainland China, Taiwan, Vietnam, Malaysia, Indonesia, the Philippines, New Guinea, Northern Australia, and the Western Pacific. In the volume "A Taxonomic Monograph of The Genus *Aglaia* Lour. (Meliaceae)", Dr. Caroline Pannell (University of Oxford, Oxford, U. K.), a leading taxonomic specialist of this genus, included 104 species belonging to *Aglaia* [38]. However, today, approximately 120 *Aglaia* species are recognized [39]. The largest concentration of this genus is found in Indonesia, followed by Malaysia, the Philippines, and the Indochina region (including Thailand and southern mainland China). In the taxonomic system, *Aglaia* belongs to the tribe Aglaieae within the subfamily Melioideae and is made up of 2 taxonomic sections, namely, the section *Aglaia* and the section *Amoora* [38]. In 2005, based on a phylogenetic study, Muellner and coworkers [40] recognized 3 taxonomic sections within the genus, viz., the



		R_1
1	Rocaglamide	$CONMe_2$
2	Methyl rocaglate	COOMe
3	Didesmethylrocaglamide (DDR)	$CONH_2$
4	Rocaglaol	H

▶ **Fig. 1** Examples of selected flavaglines (**1**–**9**) isolated from various *Aglaia* species.

sections *Amoora, Neoaglaia*, and *Aglaia*, which are defined morphologically primarily by their fruit characteristics and by the numbers of flower parts.

All *Aglaia* species are woody, ranging in size from a few meters high to large trees up to 40 m tall. The bark of these trees and their branches tend to exude a sticky white latex when incised. Most species have imparipinnate leaves, although these are occasionally simple or trifoliolate. Characteristically, branchlets and leaves are covered by an indumentum of peltate scales or stellate hairs, which may be used to identify specific species. The flowers of the genus members are small (1–10 mm long) and subglobose or ellipsoid and unisexual, typically set in a terminal and axillary paniculate inflorescence. In classifying the species, the staminal tube in the female flower provides the most taxonomic information. The fruits are either ellipsoid or pear-shaped, dehiscent or indehiscent, and covered by a stellate type of scales or hairs, with the pericarp thick and pliable. The outer layer of the seed coat (referred to as the aril) is usually fleshy [38].

Members of the genus *Aglaia* are found growing in both evergreen and monsoon primary and secondary forests, from sea level to an altitude of 1,500 m, or, rarely, higher, and occur mostly in evergreen forests and less commonly in monsoon forests. *Aglaia* species populations are normally scattered, not in a dense and dominant cluster, and many species have become rare due to forest clearance and may be threatened. Examples include *A. foveolata, A. spectabilis,* and *A. perviridis* [41]. Most species yield good hardwood timber used in construction for buildings, bridges, houses, and furniture. Several species have found traditional medicinal uses, especially the leaves, to treat bodily afflictions, such as wounds, fever, headache, asthma, and jaundice, and as a tonic, e.g., after childbirth [42, 43]. One species, *A. odorata*, often found in cultivation, has a history of wide use in traditional medicine to treat various diseases [44, 45].

During the time of operation of the 2 NCI-supported multi-institutional research projects [35–37], a total of 17 identified species of *Aglaia* were collected for investigation for potential anticancer activity (▶ **Table 1**). The greatest number of collections came from Indonesia (11 species), while a small number each came from Laos (3 species), Vietnam (2 species), and Thailand (one species). Voucher specimens of these collections are in deposit at the Herbarium of the Field Museum of Natural History, Chicago, IL, USA, and at herbarium institutions in the county where each collection was made.

Phytochemical Reports of Rocaglate (Cyclopenta[*b*]benzofurans) Constituents of *Aglaia* Species (2014–2020)

In 2014, our group at The Ohio State University (OSU) published a comprehensive review focusing on rocaglamide (**1**), silvestrol (**8**), and their structurally related bioactive compounds [23], inclusive of isolation of several new rocaglates from 4 *Aglaia* species (*Aglaia cucullata, A. edulis, A. odorata*, and *A. perviridis*). Recent reports published mainly during the period 2014–2020 have described the isolation of new rocaglate derivatives from only a relatively few *Aglaia* spp., namely, *A. odorata, A. oligophylla, A. perviridis*, and *A. stellatopilosa*. Many of the research reports on the *Aglaia* CPBFs published over the last few years have focused on their syntheses, analog development, and biological activity evaluation, with the last-mentioned topic, in particular, to be covered in subsequent sections of this review.

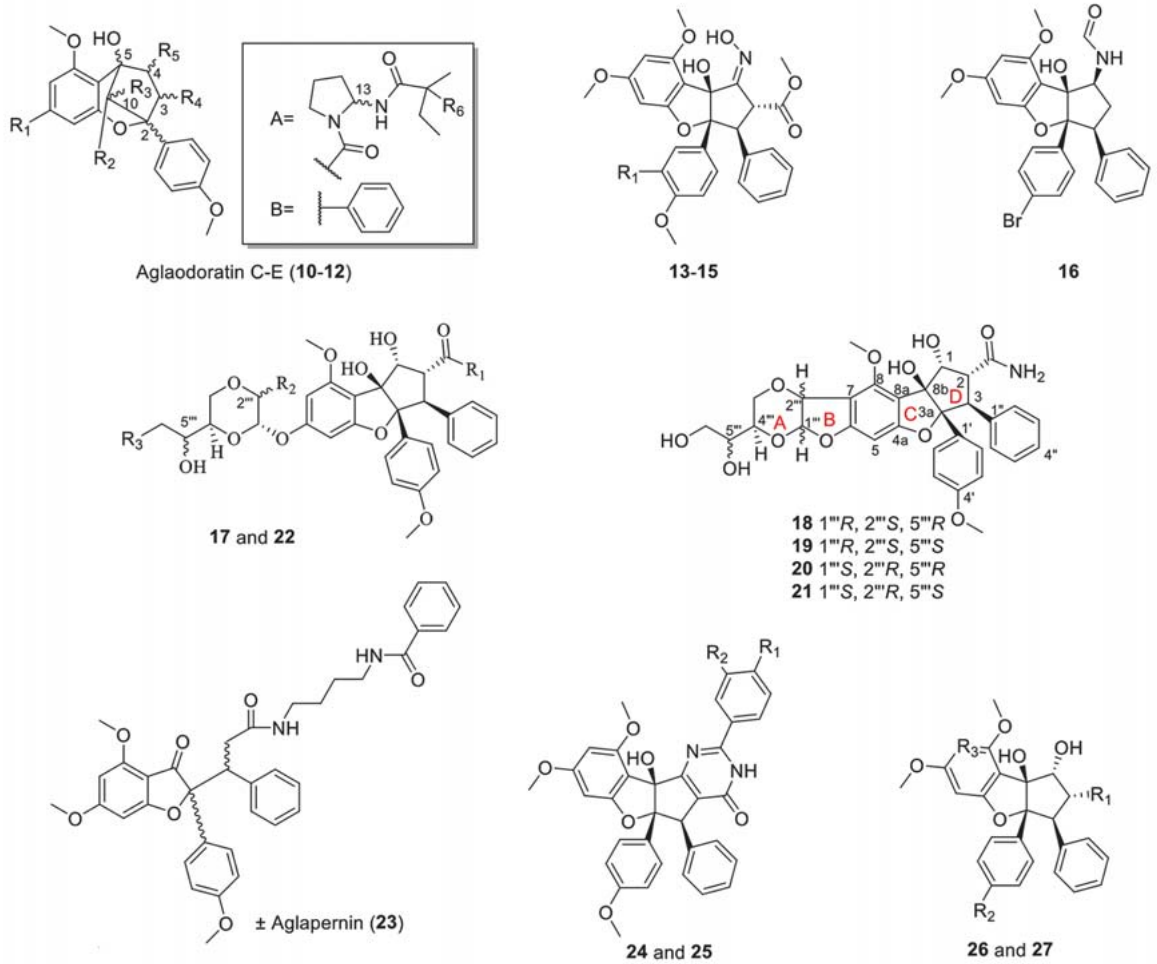
In 2015, An et al. reported the structures of 8 new benzo[*b*]oxepine derivatives (aglaodoratins A–H) [46], and 8 new cyclopenta[*b*]benzopyrans, biosynthetic precursors to rocaglates (aglapervirisins B–J) [47], from the leaves of *A. odorata* and *A. perviridis*, respectively. Of these 16 secondary metabolites, aglaodoratins C (**10**), D (**11**), and E (**12**) (▶ **Fig. 2**) were observed to exhibit moderate cytotoxicity, with their IC_{50} values in the range from 0.097 to 6.25 µM, against 3 human cancer cell lines (HT-29 colon cancer, SMMC-7721 hepatocellular cancer, and MG-63 osteosarcoma). Additionally, aglaodoratin C (**10**) inhibited cellular proliferation by arresting cells at the G2/M cell-cycle phase and thereby inducing apoptosis in HepG2 liver cancer cells *in vitro* [46]. In contrast, aglapervirisins B–J were either only weakly active or noncytotoxic for a panel of 4 human cancer cell lines [47]. Interestingly, Kong and colleagues recently published a further phytochemical investigation on the leaves of *A. perviridis*, where they reported 4 new aglain glycosides (aglapervirisins J–M) with weak anti-inflammatory activity, as determined by an *in vitro* nitric oxide inhibition assay using the RAW264.7 mouse macrophage tumor cell line [48].

Two separate groups reported the similar compounds rocaglamide AY (**13**) [49] and aglaodoratin I (**14**) [46], isolated from the leaves of *A. oligophylla* and *A. odorata*, respectively. Both these rocaglates have an oxime group at the C-1 position, but aglaodoratin I (**14**) possesses a hydroxy group at the C-3′ position instead of a hydrogen atom, as seen in rocaglamide AY (**13**) (▶ **Fig. 2**). The limited quantity of **14** obtained prevented it from being evaluated for cytotoxicity [46]. Although no biological test results were reported for rocaglamide AY (**13**) either, it was mentioned that many rocaglamide congeners possess insecticidal properties against the agricultural insect pest, *Spodoptera littoralis* [49]. Another paper published in 1999 reported a similar compound **15**, also from the twigs and leaves of *A. odorata*, which exhibited moderate insecticidal activity (LC_{50} of 1.3 ppm) toward *S. littoralis* larvae [10]. Additionally, a rocaglaol derivative **16**, with a formamide group at the C-1 (*S*) position and a bromine at C-4′ instead of a methoxy group, was found to have cytotoxic activity ranging from 0.5–2.3 nM against an array of human cancer cell lines [50], suggesting an amide at C-1 may lead to a more potent cytotoxic effect when compared to rocaglate derivatives with a more typical hydroxy group found at this position.

▶ **Table 1** *Aglaia* species collected in Southeast Asia under 2 NCI-funded research projects.

Country	Species	Voucher specimen
Thailand	*Aglaia elliptica* Bl.	Nantasan s. n.
Laos	*Aglaia* cf. *macrocarpa* King	Soejarto et al. 15399
Laos	*Aglaia* cf. *oligophylla*	Soejarto et al. 15396
Laos	*Aglaia spectabilis* (Miq.) Jain & Bennet	Soejarto et al.15410
Vietnam	*Aglaia* cf. *aquatica* (Pierre) Harms	Soejarto et al. 15176
Vietnam	*Aglaia perviridis* Hiern	Soejarto et al. 14863
Indonesia	*Aglaia* cf. *argentea* Bl.	Riswan ML-039
Indonesia	*Aglaia edulis* (Roxb.) Wall.	Riswan SR-022
Indonesia	*Aglaia elliptica* Bl.	Riswan ML-033
Indonesia	*Aglaia foveolata* Pannell	Riswan KP-034
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS02
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS17
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS18
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS23
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS24
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS24
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS25
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS26
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS26
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS27
Indonesia	*Aglaia foveolata* Pannell	Riswan SR-IS28
Indonesia	*Aglaia foveolata* Pannell	Riswan Z-34
Indonesia	*Aglaia korthalsii* (Miq.) Pellegr.	Kardono SC87
Indonesia	*Aglaia leptantha* Miq.	Riswan SR-IS01
Indonesia	*Aglaia odorata* Lour.	Riswan A-12
Indonesia	*Aglaia odoratissima* Bl.	Riswan SR-072
Indonesia	*Aglaia rubiginosa* (Hiern) Pannell	Riswan Z-55
Indonesia	*Aglaia silvestris* Merr.	Riswan SR-J17
Indonesia	*Aglaia silvestris* Merr.	Riswan SR-068
Indonesia	*Aglaia silvestris* Merr.	Riswan SR-CJR068
Indonesia	*Aglaia teysmanniana* (Miq.) Miq.	Riswan SR-J20
Indonesia	*Aglaia tomentosa* Teijsm. & Binn.	Riswan B-037

In 2016, Othman and colleagues published the isolation of silvestrol (**8**) and its epimer 5‴-episilvestrol (**9**), in addition to several new 2,3-seco-dammarane triterpenoids, from the stems of *A. stellatopilosa*, collected in Sarawak, Malaysia [51]. Earlier, silvestrol (**8**) was reported in a patent application as an antineoplastic constituent of the Malaysian plant *Aglaia leptantha*, which was lat-



		R_1	R_2	R_3	R_4	R_5	R_6
10	Aglaodoratin C (2*S*, 3*R*, 4*S*, 5*S*, 13*R*)	OMe	$R_2 = R_3 = O$		unit B	unit A	OH
11	Aglaodoratin D (2*S*, 3*S*, 4*R*, 5*S*, 10*R*, 13*S*)	OH	H	OH	unit A	unit B	H
12	Aglaodoratin E (2*R*, 3*R*, 4*S*, 5*R*, 10*S*, 13*R*)	OMe	H	OH	unit A	unit B	OH
13	Rocaglamide AY	H	-	-	-	-	-
14	Aglaodoratin I	OH	-	-	-	-	-
15		OMe	-	-	-	-	-
17	Aglapervirisin A (5‴ *R*, 2‴ *S*)	OMe	OMe	Ac	-	-	-
22	2‴ (*R*/*S*) (5‴ *S*)	NH_2	OH	OH	-	-	-
24		Me	H	-	-	-	-
25		H	OMe	-	-	-	-
26	(–)-CR-31-B	CONHOMe	OMe	C	-	-	-
27	eFT-226	CH_2NMe_2	CN	N	-	-	-

▶ **Fig. 2** Structures of cytotoxic rocaglates isolated from various *Aglaia* species and several related synthetic congeners (**10**–**27**).

er re-identified as *A. stellatopilosa* [52]. However, the structures for the isolated compound described in this patent did not show full configurational details and did not distinguish between compounds **8** and **9**. Silvestrol (**8**) and episilvestrol (**9**) were also purified from the leaves of *A. perviridis* in 2016, collected in Yunnan Province of the People's Republic of China [47]. Moreover, our group confirmed the presence of compounds **8** and **9** in a root sample of *A. perviridis* collected in Vietnam [53]. Therefore, to date, cyclopenta[b]benzopyrans containing a dioxanyl ring as in silvestrol (**8**) have been found in only 3 of the approximately 120 *Aglaia* species (i.e., *A. foveolata*, *A. perviridis*, and *A. stellatopilosa*), making them rare constituents of this genus.

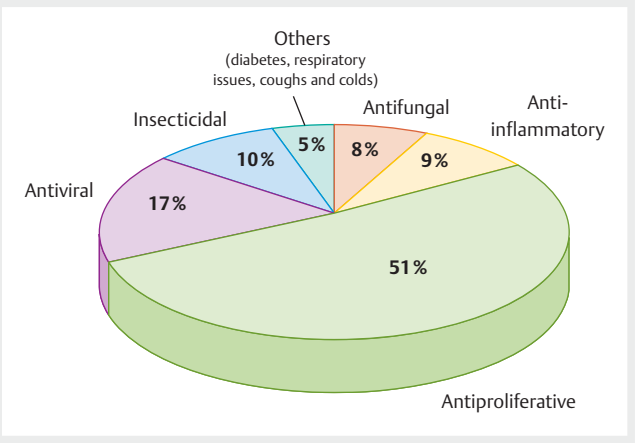
In addition to episilvestrol (5‴-episilvestrol) (**9**), a small number of additional structural modified variants of silvestrol (**8**) have

been reported from *Aglaia* species. In 2010, as a result of a large-scale recollection of the stem bark of *A. foveolata* from Kalimantan, Indonesia, the compounds 2‴-episilvestrol and 2‴,5‴-diepisilvestrol were obtained as trace constituents, with both having reduced cytotoxic potencies when compared to silvestrol [8]. As a result of this work, which utilized specialized large-scale isolation facilities available at NCI, a sufficient amount of silvestrol (ca. 100 mg) was purified from the recollected plant material to conduct extensive biological testing at OSU and elsewhere [34, 54, 55]. An et al. in 2016 reported aglapervirisin A (**17**) as a new silvestrol analog with an acetate group at the C-6‴ position of the dioxanyl ring side chain (▶ **Fig. 2**) [47]. Cytotoxic profiling of aglapervirisin A (**17**) in 4 human cell lines (HT-29 colon cancer, HepG2 hepatocarcinoma, HL-60 leukemia, MCF-7 breast cancer) showed that it had comparable potencies to silvestrol (**8**) and episilvestrol (**9**), with IC_{50} values ranging from 8–14 nM. Further mechanistic evaluation of **17** against HepG2 cells uncovered the ability of this mono-acetylated molecule to lower the expression levels of tyrosine phosphatases, Cdc2, and Cdc25, thereby causing apoptosis by arresting cells at the G2/M phase [47].

In 2019, our group at OSU reported 5 new cyclopenta[*b*]benzofuran analogs (**18–22**) from the leaves of *A. perviridis* collected in the Nui Chua National Park in Vietnam [53]. Of these compounds, **18–21** were observed to have a fused dihydrofuran ring to both the dioxanyl and aryl rings of the rocaglate core (▶ **Fig. 2**) and an amide moiety at C-2. Compound **22**, elucidated as a 2‴-hydroxy derivative of episilvestrol (**9**) with an amide moiety at C-2, was isolated as an enantiomeric mixture (▶ **Fig. 2**). Of these isolates, only compound **22** exhibited low micromolar cytotoxic potency against the human colon (HT-29) and prostate cancer (PC-3) cell lines. However, this study provided some structure-cytotoxic activity information, in that a hydroxy group at C-2‴ and the rigidity in structure between the dioxanyl and CPBF core might be detrimental to the cytotoxic activity of these flavaglines [53].

In the most recent report on *A. perviridis* by Kong and colleagues [48], a novel rocaglate derivative was described, (±) aglapernin (**23**), which did not show cytotoxicity against cancer cell lines but exhibited weak antibacterial activity (125 µM) against *Porphyromonas gingivalis*.

Another recent development worthy of mention is the silvestrol-based antibody-drug conjugates (ADCs), developed in 2017 by Genentech, Inc. [56]. ADCs are target-specific prodrugs, with a warhead (the cytotoxic drug) connected to a specific antibody via a linker [57–59]. The warhead bioactive moiety of the ADC provides the biological activity to the macromolecule in antibody-targeted cells. The Genentech team synthesized various silvestrol-ADC analogs in their patent, incorporating a dioxanyl ring system with different antibodies. These ADCs were then evaluated both *in vitro* and *in vivo* in breast cancer cells and B-cell malignancies. Silvestrol-ADCs connected to cysteine-modified CD22 antibodies demonstrated promising results against a CD22-expressing xenograft mouse model [56].



▶ **Fig. 3** Graphical representation of publications on *Aglaia* spp. and rocaglate derivatives, concerning different disease states, 2014–2020 (n = 77, primary research articles).

Therapeutic Potential of Rocaglates (Update 2014–2020)

Since the initial report of rocaglamide (**2**) from *A. elliptifolia* as an antileukemic agent in the early 1980s [12], the therapeutic potential of CPBFs has been evaluated and reported by several research groups. The diverse range of biological activities evaluated for these compounds has included antineoplastic, insecticidal, anti-inflammatory, neuroprotective, and, more recently, antiviral properties [2, 22, 23, 60]. ▶ **Fig. 3** gives a graphical representation of the published work on these compounds from 2014 to 2020. Continued modifications of the functional groups at specific positions have contributed to a better understanding of the structure-activity relationships of rocaglates as potential therapeutic agents. Substantial work has been conducted in establishing their biological targets, of which 2 have been explored to the greatest extent, namely, eIF4A [29, 30, 61] and PHB1/2 [32, 62, 63].

The initial studies exploiting the cellular mechanistic action of silvestrol (**8**) were published just a few years after its isolation, where it was observed to influence the interaction between eIF4A and RNA [29, 30]. This work was followed by demonstrating that synthesized biotinylated 5‴-episilvestrol selectively inhibits eIF4AI/II [26].

Over the last few years, several studies have been published from our collaborative work on the effects of various rocaglate derivatives against several neurofibromatosis-associated tumors and pediatric sarcomas, with the biological testing experimental work performed at the Nationwide Children's Hospital, Columbus, Ohio, USA. In an initial study using silvestrol (**8**), it was shown that the eIF4F components, including eIF4A, are potential therapeutic targets in MPNSTs and vestibular schwannomas [64]. Genetic depletion of eIF4A using short-hairpin RNAs and pharmacological inhibition using the natural eIF4A inhibitor silvestrol potently suppresses the growth of MPNSTs and schwannomas by decreasing the levels of multiple mitogenic signaling molecules, including AKT, ERKs, Aurora A, and cyclins, essential for tumor growth. The

decrease of tumor growth was correlated with elevated phosphohistone H3 and with G2/M arrest and apoptosis observed in the tumor cells treated with silvestrol [64].

The inhibition of the overexpressed eIF4F components in meningiomas was also investigated using a panel of 23 natural products, inclusive of representatives of the cucurbitacin, diarylheptanoid, rocaglate, simaroubolide, stilbenoid, sesquiterpene lactone, and xanthone structural classes [65]. Of the compounds examined, silvestrol (**8**) and episilvestrol (**9**) were the 2 most growth-inhibitory compounds, with silvestrol being more potent (IC_{50} 10 nM) than episilvestrol (IC_{50} 32 nM) against *NF2*-deficient meningioma Ben-Men-1 cells. As in MPNSTs and schwannoma cells, silvestrol (**8**) treatment induced G_2/M arrest in the meningioma cells. These data suggested that inhibiting protein translation is a potential treatment approach for MPNSTs, schwannomas, and meningiomas, including those associated with neurofibromatosis [64, 65].

However, silvestrol (**8**) has suboptimal drug-like properties, including a bulky structure, poor oral bioavailability [66], and sensitivity to MDR1 efflux [67]. Moreover, a toxicity study in larger animals conducted by our colleagues and collaborators at OSU and the NCI Developmental Therapeutic Program (DTP) revealed toxic effects of silvestrol in dogs' lungs [68]. Consequently, further development of silvestrol as a cancer therapy was suspended. To search for compounds with better drug-like properties, alternative rocaglate congeners to silvestrol (**8**) were sought as potential growth inhibitors of MPNST, schwannoma, and meningioma cells [68]. Upon side-by-side comparison of 10 rocaglates lacking the dioxanyl ring with silvestrol (**8**), both rocaglamide (**1**) and DDR (**3**) were found to exhibit growth-inhibitory activity comparable to silvestrol (**8**). Like silvestrol, rocaglamide (**1**) and DDR (**3**) arrested tumor cells at G_2/M and induced apoptosis and a DNA-damage response while decreasing the expression of multiple mitogenic kinases, consistent with translation inhibition. In collaboration with colleagues at the NCI DTP, rocaglamide (**1**) was observed to be 50% orally bioavailable and did not show any discernible pulmonary toxicity in dogs. Also, both rocaglamide (**1**) and DDR (**3**) were not sensitive to MDR1 inhibition, possibly due to the lack of a dioxanyl ring. Most importantly, when administered either intraperitoneally or orally, rocaglamide (**1**, NSC326408) potently inhibited tumor growth in an orthotopic MPNST model. In addition, rocaglamide (**1**) exhibited broad antitumor activity in PDX models for a Ewing sarcoma, an osteosarcoma, and an alveolar rhabdomyosarcoma. In a comparative *in vitro* study of 11 rocaglate congeners, including rocaglamide (**1**), methyl rocaglate (**2**), DDR (**3**), rocaglaol (**4**), and silvestrol (**8**), DDR (**3**, IC_{50} between 5 and 15 nM) was found to be the most potent compound, when tested against a panel of MPNST, a schwannoma, and a meningioma cell lines [68]. (−)-DDR (**3**) was obtained earlier in our work as a trace constituent from the combined leaves, twigs, and fruits of *A. perviridis* when collected in Vietnam and differs from rocaglamide (**1**) in possessing an amide unit instead of a dimethylamide functionality at the C-2 position of the CPBF core (▶ **Fig. 1**) [13]. In a follow-up investigation, chemically synthesized DDR (**3**) was also found to effectively block tumor growth in CDX and PDX models of osteosarcoma [69]. It was suggested that both rocaglamide (**1**) and DDR (**3**) are worthy of further evaluation as potential treatments for pediatric bone and soft tissue sarcomas.

Additional studies have been performed on the cellular mechanism of action of rocaglate family members as eIF4A inhibitors. Chu and colleagues used CRISPR-Cas9 as a tool for drug-target validation *in vivo*. They validated the rocaglate-eIF4A relationship by introducing an eIF4A1 mutant allele (F163L) into cells and showed that eIF4A1(F163L) retains helicase activity but was unresponsive to rocaglates, such as silvestrol (**8**) [70]. Iwasaki et al. reported that rocaglamide (**1**) does not repress translation of specific messenger RNAs by reducing cellular eIF4A availability but rather by clamping eIF4A onto the polypurine sequences in an ATP-independent manner [71]. This same group later elucidated the crystal structure of a complex of human eIF4A-rocaglamide-polypurine RNA and showed rocaglamide to target a bimolecular cavity between eIF4A and polypurine RNA [72]. Recently, Sidraharan et al. treated breast cancer stem cells (BCSCs) with rocaglamide (**1**), determining eIF4A as a valid molecular target for both BCSCs and bulk tumor cells. They further suggested that eIF4A inhibitors may be combined synergistically with existing chemo-, radio- and/or immunotherapies [73].

Extensive follow-up work has been done more recently since the initial documentation of rocaglates and their inhibitory effect on PHB1/2 [32]. This includes not only the investigation of their antineoplastic activity but also their potential antiviral effects. Liu and colleagues evaluated rocaglamide (**1**) and aglaroxin C (**5**) in HCV-infected human hepatoma cells [63]. HCV, responsible for hepatitis C and liver cancer in humans, enters human hepatocyte cells utilizing different membrane proteins, particularly based on the interaction between its glycoprotein E2 and PHB1/2 [74]. Rocaglamide (**1**) inhibits HCV entry into human hepatoma cells by targeting PHB1/2, which in turn inhibits the CRaf/RAS pathway, an integral component in cell proliferation and signaling [75]. While synthesized aglaroxin C (**5**, ▶ **Fig. 1**) was found to exhibit a weak effect on HCV replication or entry into cells, several further analogs of **5** were shown to be more effective HCV entry inhibitors, including **24** and **25**, in which the C-aryl group of the pyramidinone is differentially substituted [76, 77]. These 2 compounds exhibited low cytotoxicity (EC_{50} = 0.5 µM), 3-fold greater in comparison to aglaroxin C (**5**), against human hepatoma Huh-7.5.1 cells infected with HCV and were suggested to inhibit viral entry rather than replication as their mechanism of action [76]. Another similar study with EV71, responsible for hand, foot, and mouth disease in humans, demonstrated dependence on PHB for cell entry, with rocaglamide (**1**) used to investigate EV71 translation and entry inhibition [78]. An *in vivo* study of EV71-infected mice showed that mice survived longer, with lower viral loads in the brain and spinal cord, on treatment with rocaglamide (**1**, 0.25 mg/kg), as compared to those treated with vehicle (0.25% DMSO in olive oil). These data were supported by an *in vitro* study of EV71-infected motor-neuron NSC-34 cells, where a dose-dependent reduction in viral load was observed in cells treated with rocaglamide (**1**, 10–100 nM) [78].

In 2017, silvestrol (**8**) was evaluated *in vitro* for its antiviral activity against the EBOV [79]. This study by Biedenkof et al. demonstrated the ability of silvestrol (**8**) to inhibit EBOV infection at a low noncytotoxic concentration (10 nM). Additionally, they demon-

strated that reduction of EBOV propagation correlated with the disappearance of viral nucleoprotein (NP), which is comparable to translational inhibition of PIM1, a cellular kinase known to be affected by silvestrol (**8**) [79]. In another antiviral study, Slaine et al. examined the role of silvestrol (**8**) in blocking the replication of influenza A virus (IAV) [80]. They showed that silvestrol treatment during early IAV infection induced stress granule formation, inhibited viral protein synthesis, and blocked viral replication. Interestingly, the viral protein synthesis was "recovered" upon silvestrol (**8**) withdrawal, suggesting a reversible translation inhibition mode of action [80].

Several further evaluations of rocaglamide (**1**) and silvestrol (**8**) as potential antiviral agents have been performed against HEV [81, 82], corona- and picornaviruses [83], chikungunya virus [84], EBOV, Marburg virus [85], and zika virus [86]. All these studies were based on the assumption that efficient translation of the mRNAs of these viruses, which contain highly structured 5-UTRs, requires the DEAD-box RNA helicase eIF4A. (−)-CR-31-B (**26**), a synthesized rocaglate hydroxamate, was evaluated by Müller and colleagues for its antiviral activity against HEV, corona-, zika, Lassa, and Crimean Congo hemorrhagic fever viruses, in comparison with silvestrol [87]. It was found that (−)-CR-31-B (**26**) exhibited slightly more potent viral inhibition than silvestrol (**8**), with EC_{50} values in the low nanomolar range for most of the viruses examined. However, the inhibitory activity of (−)-CR-31-B (**26**) against HEV replication was somewhat weaker in comparison to silvestrol (**8**), suggesting a potential difference in the antiviral mode of action between these 2 rocaglates [87]. Recently, the synthetic rocaglate (−)-CR-1–31-B (**26**) was employed to show that eIF4A is a therapeutic target in PDA, and it suppressed tumor growth and extended the survival time in a genetically-engineered mouse PDA model [88].

Two additional mechanistic targets of rocaglates have been suggested, namely, KRAS [89], a member of the RAS family of small GTPases, and DDX3, a DEAD-box RNA helicase [90]. RAS proteins are imperative for triggering multiple signaling pathways required for cell proliferation and survival [91]. Mutations in *KRAS* have been frequently found in several types of cancer, including pancreatic, lung, and colon cancers, and NSCLC [92]. According to Yurugi et al., flavaglines, particularly rocaglamide (**1**), potently inactivate RAS by inhibiting its GTP loading and deterring its nanocluster formation at the phospholipid-enriched sites on the plasma membrane [89]. In turn, Chen et al. [90] focused on DDX3, a highly conserved DEAD-box helicase involved in cell-cycle regulation, differentiation, survival, and apoptosis [93]. Rocaglamide (**1**) was discerned to clamp DDX3 on its polypurine sequences in an ATP-independent manner, and the glutamine at position 360 was found to be a critical residue for DDX3 binding by this rocaglate [90].

Other Biological Properties of Rocaglate Derivatives

In 1985, Chiu published an initial report of the antifeedant activities against 3 agricultural pests of an acetone extract of *A. odorata* [94]. This was followed up in 1993 by the work of Ishibashi and colleagues showing that 2 CPBF constituents, rocaglamide (**1**) and methyl rocaglate (**2**), from this plant demonstrated potent insecticidal activities against the larvae of the variegated cutworm (*Peridroma saucia*) [16]. Subsequently, several studies have evaluated the potential insecticidal effects of rocaglamide and its analogs. Although the exact mechanism of action for the insecticidal property of CPBFs is as yet unknown, phytochemists and medicinal chemists have obtained several congeners to evaluate their structure-activity relationships. For instance, the free hydroxy groups at both C-1 (*R*) and C-8b were essential for mediation of the insecticidal activity of rocaglamide (**1**) (▶ **Fig. 1**), when evaluated against the pest insect *S. littoralis* [95, 96]. Moreover, favorable modifications by chemical synthesis at C-2 or C-4′ for insecticidal activity are a hydroxamide and halogen (Br or Cl) functional group, respectively. Such derivatives were well-tolerated compared to rocaglamide (**1**). They exhibited LC_{50} values ranging from 3 to 12.5 mg/L against an array of pests and beetles inclusive of *Diabrotia balteata, Heliothis vierescens, Plutella xylostella*, and *S. littoralis* [97].

Treatment of cerebral malaria, caused by infection of *Plasmodium falciparum*, has proven to be a continued challenge. Despite the widely available synthetic analogs of the plant-derived sesquiterpene lactone, artemisinin, resistance to this compound class by the causative organisms has been observed [98, 99]. Langlias and associates recently suggested the possibility of using rocaglates as potential therapeutic intervention agents for malaria. They showed the synthetic rocaglate derivative (−)-CR-31-B (**26**) to exhibit antiplasmodial activity. According to their report, owing to its potential to inhibit eIF4A, (−)-CR-31-B (**26**) not only inhibited *Plasmodium* protein synthesis at low nanomolar levels (ranging between 0.9 and 2.8 nM) *in vitro* but also showed similar effects in a dose-dependent manner in mice infected with *Plasmodium berghei* [100]. Additionally, their study highlighted the anti-inflammatory activity of (−)-CR-31-B (**26**) by suppressing the production of IRF1, a pro-inflammatory transcription factor essential for the expression of critical inflammatory factors, like GBP2 and CXCL10 [100]. Complemented by a previous study that established good pharmacokinetic properties of this synthetic rocaglate [101], Langlais et al. proposed that (−)-CR-31-B (**26**) warrants further evaluation as a potential therapy for cerebral malaria, either as a single agent or in combination with artemisinin [100].

In a recent publication, Wang et al. evaluated the potential neuroprotective effects of a 95% ethanol extract of *A. odorata* leaves [102]. This plant extract exhibited a neuroprotective effect in a middle cerebral artery occlusion (MCAO) rat model. Treatment with this extract reduced the number of apoptotic cells and increased mitochondrial membrane potential in oxygen-glucose deprivation/reperfusion (OGC/R)-induced PC12 cells. It was hypothesized that the extract exerts a neuroprotective effect against cerebral ischemia by suppressing the p53/Puma mediated signaling pathway [102]. While these biological results look interesting, the investigation was not supported by any detailed phytochemical work, but only with a preliminary chromatographic profiling method, indicating the presence of triterpenoids in the extract [102]. The actual active constituents may include rocaglate derivatives, as reported from *A. odorata* [16, 46].

Conclusions

Unlike many other structural classes of specialized metabolites from higher plants that have long been known, the novel rocaglate (flavagline) derivative (−)-rocaglamide (**1**) was first reported in 1982 from the leaves of *A. elliptifolia* [12]. At the time of its isolation, the structure and absolute configuration of this CPBF were determined by single-crystal X-ray crystallographic analysis, and it was shown to exhibit antileukemic activity (T/C value of 156 %) in a P388 murine leukemia *in vivo* assay at a nontoxic dose (1.0 mg/kg) [12]. Likewise, the antileukemic activity of the dioxanyl-ring containing CPBF (−)-silvestrol (**8**) was reported in 2004 from *A. foveolata*, and its full structure and stereochemistry were also determined by X-ray diffraction analysis [21]. Dioxanyl ring-containing CPBFs are rare constituents in *Aglaia* species, and, at present, they have been found in only 3 of the approximately 120 members of this genus [8, 21, 47, 51, 53]. Subsequently, and particularly over the last decade, the key cellular mechanism inhibition of eIF4A [29, 30] and PHB1/2 [32] has made rocaglamide (**1**) and silvestrol (**8**) of great interest to the biomedical community as standard protein translation inhibitors. Both these compounds are now available commercially from fine-chemical scientific suppliers for research use. As a potential means of increasing their supply, rocaglamide [103–106] and silvestrol [107, 108] have been subjected to total chemical synthesis. In addition, methods have been developed for synthesizing rocaglate analogs to establish further structure-activity relationship information [109, 110]. However, it should be reiterated that while silvestrol has proven to be a useful pharmacological tool, it has suboptimal drug-like properties and can cause pulmonary toxicity [68]. Therefore, this dioxanyl ring-containing natural product must be modified structurally for further development as a bioactive molecule drug lead.

Several review articles have appeared recently by various groups on the pharmacological activities of rocaglates and have dealt, in particular, with their antineoplastic [14, 25, 111], antiviral [111, 112], and miscellaneous biological effects [14]. In terms of drug development, work on a synthetic derivative of rocaglamide (**1**), eFT-226 (**27**, Zotatifin), which was elucidated to have good pharmacokinetic properties and potent antitumor effects, like rocaglamide (**1**) and DDR (**3**), seems promising. The potent eIF4A inhibitor, Zotatifin, is the first compound with this mechanism of action to have entered into a clinical trial as a potential treatment for patients with advanced solid-tumor malignancies [113].

Contributors' Statement

Conception: A. D. Kinghorn, G. Agarwal, D. D. Soejarto, L. S. Chang; design of work: A. D. Kinghorn, G. Agarwal, D. D. Soejarto, L. S. Chang; drafting the manuscript: A. D. Kinghorn, G. Agarwal, L. S. Chang, D. D. Soejarto; critical revision of the manuscript: L. S. Chang, A. D. Kinghorn, G. Agarwal, D. D. Soejarto.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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