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Polyalkoxyflavonoids as inhibitors of cell division

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Being structural analogues of natural microtubule-destabilizing cytostatics, polyalkoxyflavonoids represent a promising class of compounds for anticancer drug design. The review covers synthetic routes to various polyalkoxyflavonoids and the results of biological assays *in vitro* on human cancer cells and *in vivo* using sea urchin embryos as a model. Mechanisms of action and structure – relationship activity for polyalkoxyflavonoids are discussed.
The bibliography includes 151 references.

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I. Introduction

Inhibitors of cell division (cytostatics), *i.e.*, compounds with antiproliferative action, are widely used in cancer chemotherapy. The 'List of vitally essential and most important medicines' approved in 2003 in the Russian Federation includes 47 cytostatics for cancer chemotherapy. In the USA, the list of approved anticancer agents includes 50 drugs, five of which are so-called mitotic poisons vinca alkaloids and taxoids. The mechanism of their action is associated with the disruption of microtubule structure and dynamics. However, chemotherapeutic agents display undesirable side effects, such as neuro- and cardiotoxicity, inhibition of hematopoiesis, immune suppression, dysfunction of the gastrointestinal tract and kidneys. An important parameter of anticancer agents is a therapeutic window defined as the interval between the minimal dose (concentration) of the agent that causes the suppression of cancer cell growth and the initial dose causing toxic effects. Another limitation of the use of modern chemotherapeutic agents is the development of the multidrug resistance in cancer cells. This effect may be associated with enhanced production of P-glycoprotein, which is a transmembrane protein that promotes efflux of foreign molecules from the cell, as well as with various alterations of genes and proteins, including those related to apoptosis, the process of programmed cell death. Therefore, there is a continued interest in design of new effective anticancer agents with reduced systemic toxicity and a wide therapeutic window, which will have a selective effect on malignant cells and can overcome multidrug resistance.

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II. Polyalkoxyphenyl moiety as a pharmacophore group of natural antimitotics

When searching for anticancer agents, considerable attention is paid to natural compounds isolated from higher and lower plants, fungi, marine invertebrates, bacteria and so on. Natural compounds and their analogues account for 79.8% of anticancer drugs approved between 1981 and 2010.[1]

Many natural cytostatics and their semisynthetic and synthetic analogues contain a polymethoxyphenyl moiety.[2–6] These compounds have powerful antiproliferative action due to the inhibition of tubulin polymerization and alteration of the microtubule structure and functions. Microtubules are cytoskeletal elements abundant in all eukaryotic cells. In nondividing interphase cells, microtubules are responsible for the maintenance of the overall cell shape, serve as tracks for transport of intracellular organelles and macromolecules and are the main components of cilia and flagella that function as sensory and motility organelles. The preparation for cell division is accompanied by the rearrangement of interphase microtubules to form the mitotic spindle responsible for the correct orientation of chromosomes and their separation into the daughter cells. Cytostatics that selectively bind to tubulin/microtubules, disrupt the structure and function of the mitotic spindle and arrest the cell cycle at the mitotic phase. Polymethoxyphenyl-containing microtubule-destabilizing agents bind to the colchicine site of the tubulin molecule.[2,3,7] It was found that the antimitotic activity of these compounds depends on the number and positions of these substituents.[4,5] Figure 1 presents the structural formulas and biological activity of colchicine, steganacin, podophyllotoxin, combretastatins, and noscapine.

Colchicine was first isolated from the autumn crocus *Colchicum autumnale*[10] growing in Europe and North America and later from other plants of the genera *Colchicum*, *Merendera* and *Gloriosa* of the *Liliaceae* family.[11,12] However, because of high toxicity, colchicine is not used in cancer therapy.[3] Steganacin was isolated by extraction from the bark and wood of the carrot tree *Steganotaenia araliacea* growing in Ethiopia and Cameroon, and its structure was determined by X-ray diffraction.[13] This compound exhibits high cytotoxicity *in vivo* against P-388 mouse leukemia cells and *in vitro* against KB human cervical cells;[13] however, the cytotoxicity of steganacin tested on 11

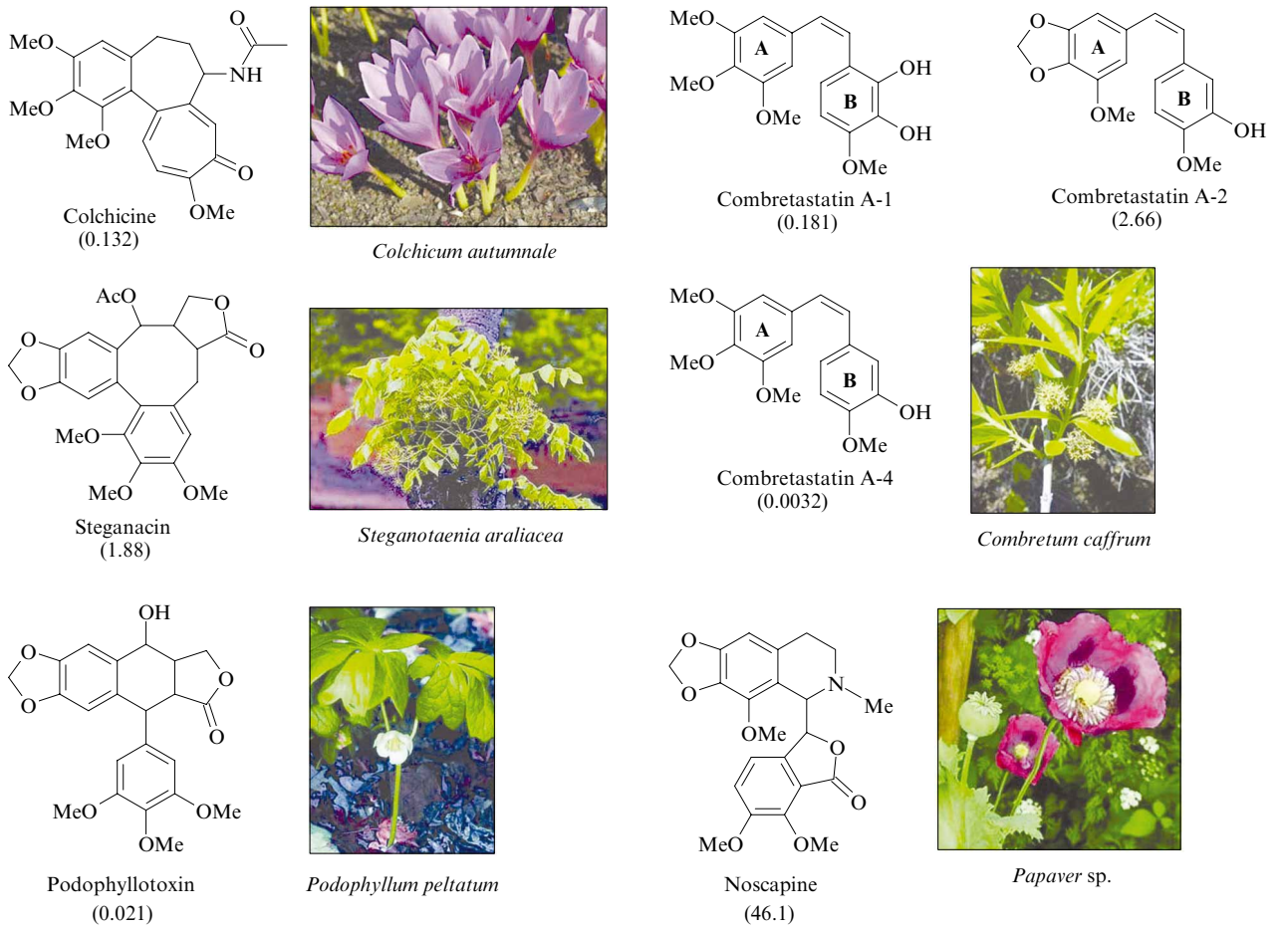


Figure 1. Natural microtubule-destabilizing cytostatics containing the polymethoxyphenyl moiety. Items enclosed in parentheses are the average concentrations (in μmol litre^{-1}) that arrest cell growth by 50% (IC_{50}) for 60 (colchicine, combretastatins A-1 and A-4, podophyllotoxin, noscapine; NCI Anti-cancer Screening Programme (NCI is National Cancer Institute, USA), http://dtp.nci.nih.gov/index.html), 10 (steganacin)[8] and 7 (combretastatin A-2)[9] human cancer cell lines. The figure presents photographs of the plants from which these compounds were isolated.

human cancer cell lines appeared to be approximately two orders of magnitude lower compared to colchicine.[8] Apparently, this is why steganacin is not used in medicine.[3] Steganacin inhibits tubulin polymerization through the binding to the colchicine site[14] resembling colchicine in its effect on guanosine triphosphate (GTP) hydrolysis of the β subunit of tubulin.[15]

Podophyllotoxin (PT) isolated from the South American plant *Podophyllum peltatum* and other representatives of the genus *Podophyllum* is a powerful tubulin polymerization inhibitor and acts *via* the colchicine site.[7,16] Due to the ability to disrupt the structure and dynamics of microtubules, PT and its derivatives exhibit anticancer, antiviral and insecticidal activities.[4,17–22] However, PT by itself is not used in clinical practice because of high toxicity.[4] Semisynthetic derivatives of PT modified at the hydroxy group, such as etoposide, teniposide and etopophos, are employed in the cancer treatment. These compounds suppress cancer cell growth due to the inhibition of the enzyme topoisomerase II involved in the DNA biosynthesis.[23]

Combretastatins A-1, A-2 and A-4 (CA1, CA2 and CA4, see Fig. 1) were isolated from the bark of the South African willow tree *Combretum caffrum*.[24,25] They have a powerful antimitotic action due to tubulin polymerization inhibition and disruption of the structure and function of the mitotic spindle.[2,3,26,27] These molecules contain two aromatic rings (**A** and **B**) located at a particular distance and dihedral angle between each other and are linked together by an ethylene bridge. The *cis* configuration of combretastatin molecules is required for the optimal binding to tubulin.[5,26,28] Currently, the water-soluble phosphorylated derivative CA4P (it contains the OPO_3Na_2 group instead of the OH group in CA4)[29] is under clinical trials as an anticancer drug (http://www.oxigene.com/pipeline/zybrestat_in_oncology).[30,31]

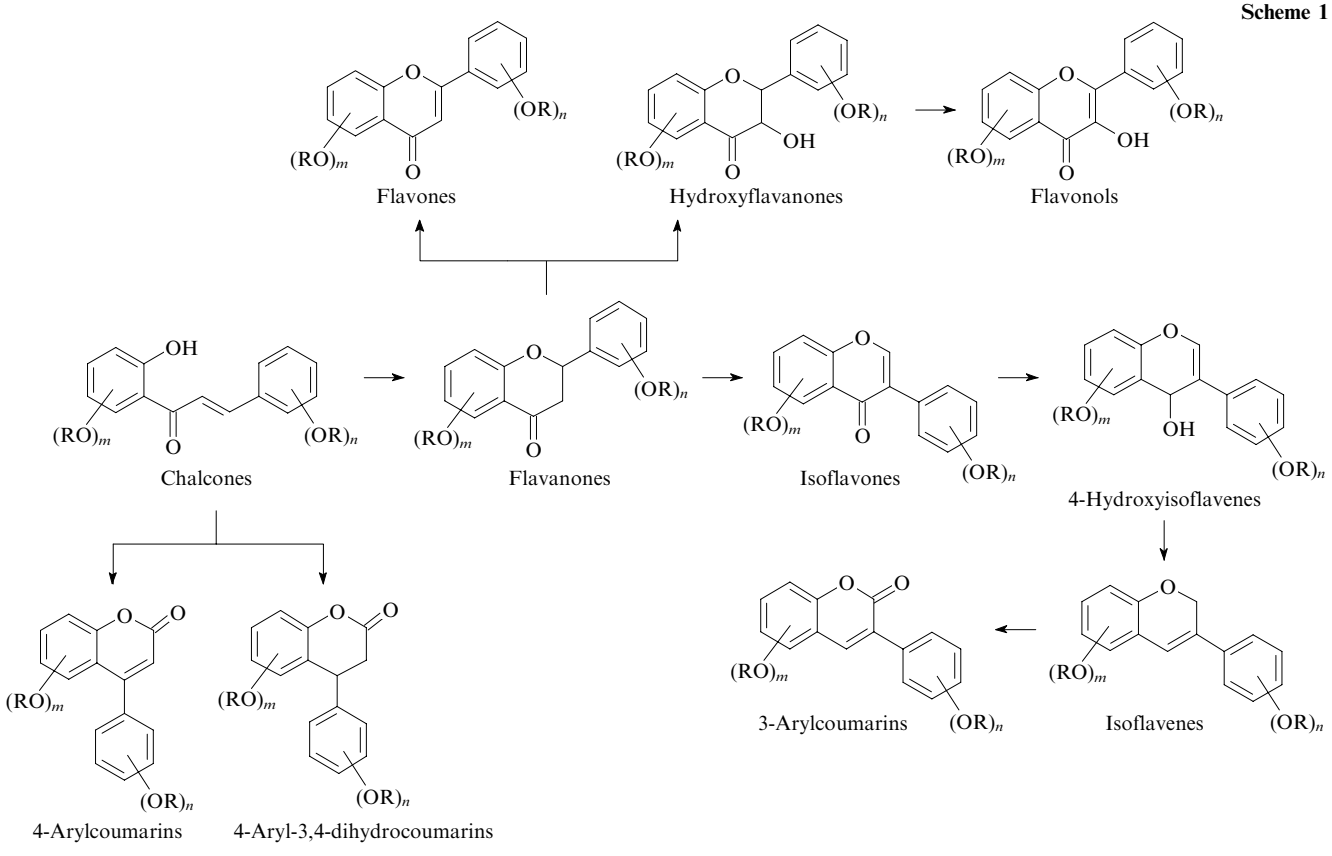
Noscapine, which was first isolated from the poppy *Papaver* sp. in 1817,[32] binds to tubulin at the colchicine site,[33] disrupts the dynamics of microtubules, but does not cause their depolymerization.[34] Despite low antimitotic activity, noscapine has low systemic toxicity, is capable of overcoming multidrug resistance and is soluble in water. Therefore, this compound holds promise as a drug in cancer chemotherapy.[35] Noscapine has passed the phase I clinical trials in multiple myeloma patients (http://clinicaltrials.gov/show/NCT00912899).

III. Prospects for the use of polyalkoxyflavonoids as cytostatics

The above-mentioned natural antimitotics are found mainly in plants growing outside the Russian Federation. The exception is colchicine, which was isolated from the autumn crocus cultivated in our gardens. Natural flavonoids containing the poly(methoxy/hydroxy)phenyl moiety are much more abundant (Scheme 1).

More than 6500 natural flavonoids are known. They are plant cell metabolites and occurr in the leaves, roots and fruits of various plants. Flavonoids are involved in photosynthesis and fulfill a protective function.[36] Under abiotic stresses, flavonoids protect cells against excessive white

Scheme 1



light and UV radiation, interact with reactive free radicals and form stable complexes with heavy metal ions. Under biotic stresses (infection of plants with bacteria or fungi), flavonoids exhibit antibacterial and fungicidal properties and are involved in the formation of a barrier against infections.[36, 37] Chalcones are biosynthetic precursors of abundant flavonoids. The biosynthesis of different types of flavonoids (see Scheme 1) by the dehydrogenation and oxidation of chalcones is comprehensively covered in many publications (see, for example, the reviews [38, 39]).

Flavonoids exert different effects on the human body.[36] These compounds have anti-inflammatory activity and vitaminizing effect, help strengthen blood vessel walls, normalize fat and protein metabolism in the cells, maintain skin health and youthful appearance, prevent premature ageing and are involved in a number of drugs. The 'Vitamin P' complex used in medical practice to reduce capillary permeability and fragility is a mixture of flavonoids containing quercetin and hesperidin. The citrus polymethoxylated flavones tangeretin and nobiletin are active ingredients of the drug Sytrinol ™, which is used to normalize cholesterol and prevent cardiovascular diseases.

Various representatives of flavonoids, both natural and synthetic, are able to prevent the cancer development or suppress malignant tumour proliferation.[39–41] Different mechanisms of anticancer action of flavonoids were described: the inhibition of protein kinases, including kinases involved in the cell cycle regulation; the inhibition of enzymes involved in the formation of active oxygen species; the effect on the carcinogen metabolism; the suppression of multidrug resistance development, in particular, the inhibition of the transport protein P-glycoprotein and, as a consequence, the suppression of foreign compound efflux from cells; antioxidant properties, the binding of reactive oxygen species; the suppression of angiogenesis in the tumour; the induction of apoptosis and the cell cycle arrest.[39, 41] The relationship between the flavonoid content of food and a decrease in the risk of the development of certain malignant tumours was revealed.[39] This underlines an important role of bioflavonoids in the prophylaxis of cancer diseases.

Among flavonoids, compounds with three or more alkoxy or hydroxy groups in one of the aromatic rings are worthy of notice. These compounds contain a bioisosteric polymethoxyphenyl moiety of combretastatins or podophyllotoxin. Therefore, some of such flavonoids would be expected to be active cytostatics with a similar mechanism of action. The present review considers the principal schemes of the synthesis and data on the antiproliferative activity (cytotoxicity, mechanisms of action, cellular targets) of such polyalkoxyflavonoids.

IV. Sea urchin embryos as a model for investigation of antimitotic activity

Generally, the antiproliferative activity of compounds is studied on different human cancer cell lines. However, experimental results obtained in different laboratories vary markedly due apparently to contamination, misidentification, and degeneration of cultured cells.[42, 43] Comprehensive investigations, including the use of other model systems,[44–47] facilitate the acquisition of more adequate data. One of promising approaches in searching for anticancer agents is based on the use of sea urchin embryos,[48] which are included in the list of model organisms for biomedical research and are widely used as a test system for the screening of compounds with antiproliferative activity. The phenotypic sea urchin embryo test of chemical compounds gives well reproducible results, does not require expensive equipment and reagents and allows for the rapid detection of molecules that suppress cell division due to disruption of the mitotic spindle structure and function.[49] Microtubule-destabilizing compounds cause a characteristic change in the mobility of embryos, resulting in the settlement on the bottom of the vessel and rapid rotation (spinning) around the animal–vegetal axis. The indirect evidence of the microtubule-destabilizing action of compounds is a specific tuberculate shape of arrested sea urchin eggs. The videos showing the swimming behaviuor of sea urchin embryos are available at http://www.chemblock.com. Tests based on sea urchin embryos and conventional models (cancer cell cultures, *in vitro* pure tubulin polymerization) gave similar results.[50–52] The typical alterations of cell division (cleavage) of sea urchin embryos induced by combretastatin A-4, which is clearly visible under a microscope, are shown in Fig. 2. The antimitotic properties of polyalkoxyflavonoids assessed in *Paracentrotus lividus* sea urchin embryos are taken from the Chemical Block database (www.chemblock.com), unless otherwise stated.

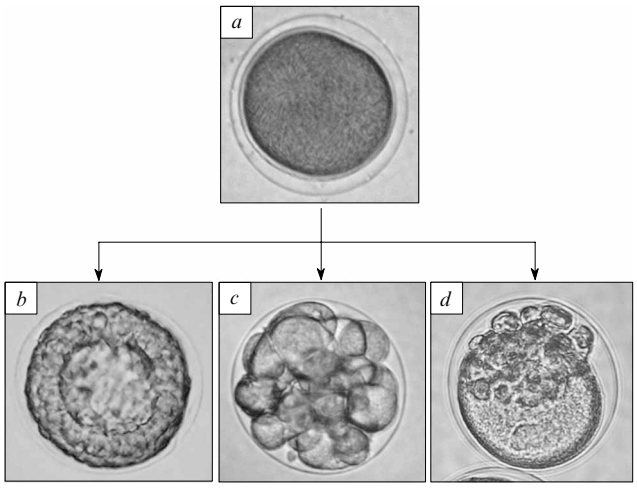


Figure 2. Abnormal development of *Paracentrotus lividus* sea urchin embryos after the treatment of fertilized eggs (*a*) with combretastatin A-4 at concentrations of 0.005 μmol litre^{-1} (*c*, abnormal cleavage) and 0.01 μmol litre^{-1} (*d*, tuberculate arrested egg); (*b*), intact blastula. The micrographs were taken 6 h after fertilization; the average embryo diameter is 115 μm. Unpublished micrographs based on the results of the study.[53]

V. Natural raw materials for the synthesis of polyalkoxyflavonoids

The isolation of biologically active flavonoids from plants, particularly from wild plants, is inefficient from engineering and economic points of view. Therefore, the development of methods for the synthesis of natural flavonoids and their

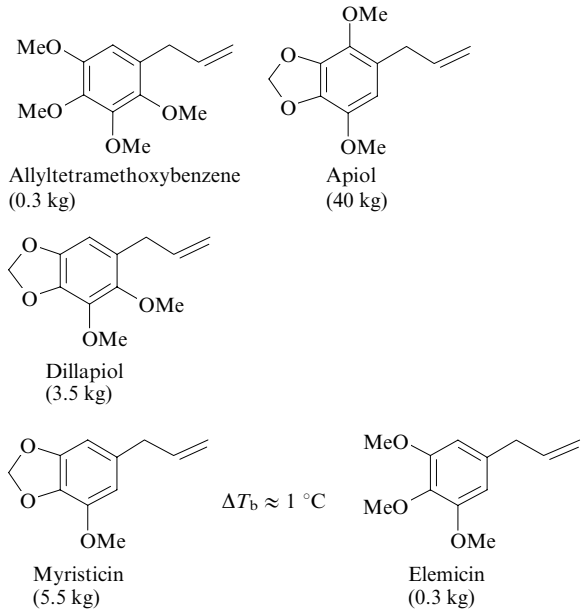
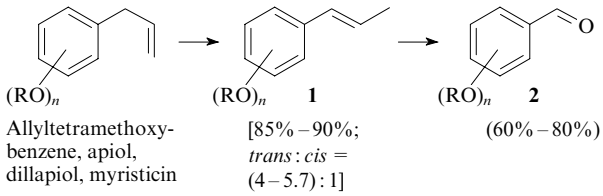


Figure 3. Allylpolyalkoxybenzenes from seeds of umbelliferous plants (see the text).[55]



Figure 4. Distillation of allylpolymethoxybenzenes on a pilot plant at the ZIOC RAS.

Scheme 2



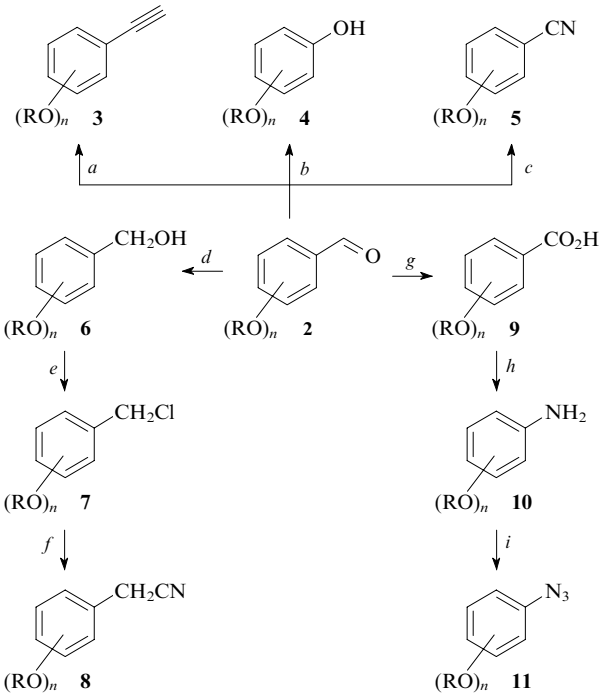
analogues is an important problem. A key issue is the preparation of accessible and cheap starting material, *viz.*, polyalkoxybenzenes with various functional groups.

Polymethoxyflavonoids can be synthesized from metabolites of crop plants, which are cultivated in the Russian Federation, are produced on a large scale and have a well-developed technology for planting and harvesting. An analysis of the current literature[54] and databases (http://www.ars-grin.gov/duke/) of biochemical compositions of more than 30 000 plants shows that umbelliferous plants (parsley, celery, dill, caraway, *etc.*) contain considerable amounts of various allylpolyalkoxybenzenes (Fig. 3). Analysis of CO_2 seed extracts of 15 parsley and dill varieties from different regions of Russia showed that essential oil from seeds of the turnip-rooted parsley Sakharnaya contains >70% of apiol. The curly-leaf parsley Astra is suitable for the production of myristicin (46% in essential oil). Besides, essential oils from seeds of most of domestic parsley varieties contain up to 15% of allyltetramethoxybenzene, whereas essential oils from Indian and Uzbekistan dill varieties contain 30%–35% of dillapiol.[55] The technology of CO_2 extraction developed at the plant of the Karavan Company Ltd (Krasnodar) gives ~100 litres of essential oil from 2500 kg of parsley and dill seeds.[56] Allylpolyalkoxybenzenes (allyltetramethoxybenzene, apiol, dillapiol, myristicin and elemicin) were isolated with a purity of 98%–99% in an amount of 5–40 kg (each compound) by highly efficient rectification on a pilot plant at the ZIOC RAS (Figs 3 and 4).[55] Myristicin can be separated by highly efficient rectification from elemicin despite their close boiling points ($\Delta T_b \approx 1$ °C).

Allyltetramethoxybenzene, apiol, dillapiol, myristicin and elemicin are easily isomerized to the corresponding propenylbenzenes **1** in the presence of solid KOH (Scheme 2). The highly efficient synthesis of aldehydes **2** is performed using a specially designed ozonator equipped with an IR detector, allowing for the introduction of exactly equimolar amounts of ozone into the reaction. Under these conditions, side reactions, such as the oxidation of aldehydes to acids, their Baeyer–Villiger rearrangement to phenols and subsequent oxidation of phenols to biaryl systems, almost do not proceed.[53, 55, 57] Key structures **3**–**11** for the synthesis of polyalkoxyflavonoids are produced from the corresponding aldehydes **2** in high yields (70%–95%) at loadings of 30–100 g (Scheme 3).[53] Aldehydes **2** are easily oxidized to polyalkoxybenzoic acids **9** with a 1 : 1 urea–hydrogen peroxide complex in water.[53] Polyalkoxybenzonitriles **5** can be synthesized from aldehydes by the reaction with iodine in aqueous ammonia[53] according to a method described in the study.[58]

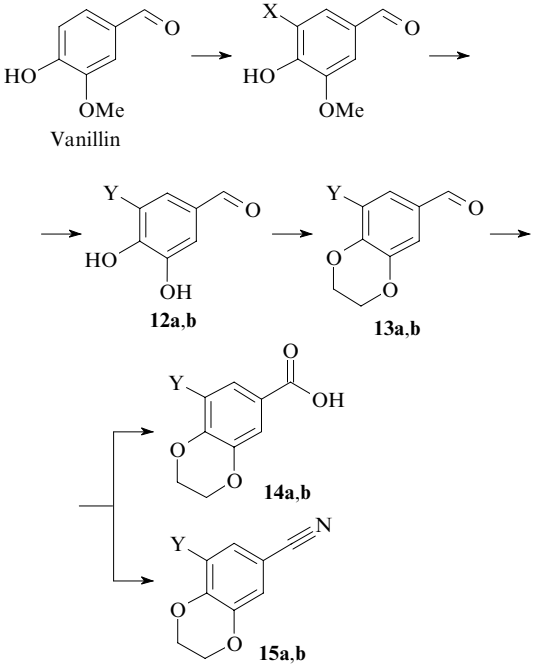
The metabolically stable ethylenedioxybenzene moiety is present in a number of drugs. Building blocks with this moiety were synthesized using the cheap natural product vanillin. The synthesis of 3,4-dihydroxy-5-methoxybenzaldehyde (**12a**) (Scheme 4) is well reproduced on a large scale[59] according to a known method.[60] The alkylation of aldehyde **12a** and 3,4-dihydroxy-5-nitrobenzaldehyde (**12b**) (see Scheme 4) with 1,2-dibromoethane proceeds under standard conditions in DMF to form the corresponding ethylenedioxybenzaldehydes **13a**,**b**.[59] Acids **14a**,**b** and nitriles **15a**,**b** are synthesized by analogy with the procedures shown in Scheme 3.[53]

Scheme 3



(*a*) $\bar{N}=\overset{+}{N}=C(Ac)P(O)(OMe)_2$; (*b*) $H_2O_2-HCO_2H$;
(*c*) $NH_3\cdot H_2O$ (28%)$-I_2$; (*d*) 5% Pd/Sibunit;
(*e*) $SOCl_2$; (*f*) KCN, 18-crown-6; (*g*) $H_2O_2-C(O)(NH_2)_2$;
(*h*) $SOCl_2-NaN_3$; (*i*) 1) $NaNO_2$, H^+; 2) NaN_3

Scheme 4

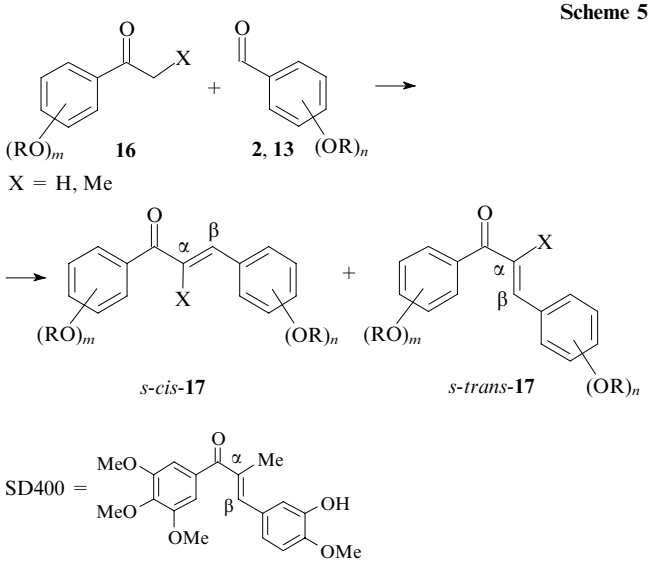


X = Br, NO_2; Y = OMe (**a**), NO_2 (**b**)

VI. Polyalkoxychalcones and indanones

The Claisen–Schmidt condensation of aldehydes **2**, **13** and ketones **16** (Scheme 5)[61,62] easily affords polyalkoxychalcones **17**, which can be considered in a simplified way as analogues of combretastatins (see Fig. 1) containing an acrolein moiety instead of the double bond that links benzene rings.[63,64] The ratio of conformers of product **17** depends on the reaction conditions.

Scheme 5



Many natural and synthetic polymethoxychalcones can suppress cancer cell growth.[61–63,65–67] The mechanisms of antiproliferative action of these compounds and cellular targets can be different. The natural prenylated chalcone derricin (Fig. 5) extracted from the roots of the South American tree *Lonchocarpus sericeus* (legume family) inhib-

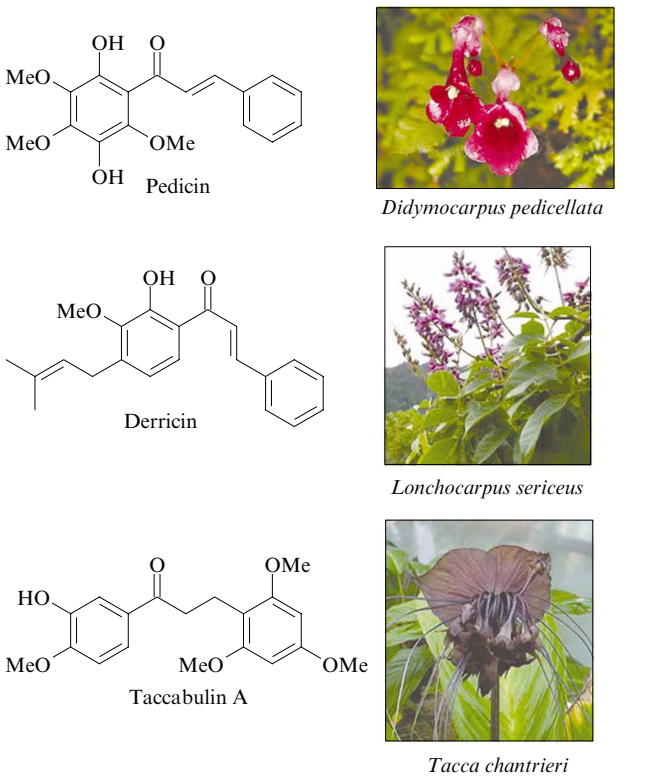
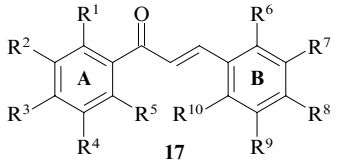


Figure 5. Natural methoxychalcones with antiproliferative activity and plants from which they are isolated.

its the growth of human leukemia cells CEM (IC_{50} = 40 μmol litre^{-1}) and alters fertilized egg division and blastula formation of the sea urchin *Lytechinus variegatus* presumably affecting the DNA and(or) protein biosynthesis.[68] Pedicin (see Fig. 5), which is found in some tropical plants, has cytotoxicity against cervical cancer cells KB (IC_{50} = 21 μmol litre^{-1}) and inhibits tubulin polymerization.[69] The dihydrochalcone taccabulin A (see Fig. 5) isolated from roots of the herbaceous plant *Tacca* sp. (Indo-China) binds to the colchicine site of tubulin, destabilizes microtubules and suppresses the growth of cervical cancer cells HeLa (IC_{50} = 0.58 μmol litre^{-1}) and ovarian cancer cells SK-OV-3 (IC_{50} = 0.81 μmol litre^{-11}).[70, 71]

Synthetic chalcones containing polymethoxy groups in the benzene rings **A** and **B** have pronounced cytotoxicity (Table 1), block the cell cycle at the mitotic phase (G2/M), destroy microtubules in the cells and inhibit tubulin polymerization through the binding to the colchicine site.[62, 66, 67, 75] The presence of three methoxy groups in positions 3, 4 and 5 of the ring **A** and the 3-OH and 4-OMe groups in the ring **B** (see Table 1, compound **17h**) are required for high antiproliferative activity of chalcones. This effect is enhanced upon the introduction of substituents (Me, OMe, OEt) in the α position at the double bond.[62] The most active α-methylchalcone SD400 (see Scheme 5) — a microtubule-destabilizing agent — suppresses the human cancer cell growth at nanomolar concentrations[67] and has a particularly strong effect on K562 leukemia cells (IC_{50} = 0.21 nmol litre^{-1}).[62, 75, 76] The mutual spatial arrangement of the carbonyl group and the double $C_\alpha-C_\beta$ bond in chalcone molecules plays an important role in the antimitotic microtubule-destabilizing properties of these compounds. The *s-trans* conformer of α-methylchalcones is more stable, which is considered as one of the factors responsible for the powerful antimitotic action of α-substituted chalcones.[61, 62] The molecular modelling (docking) revealed that α-substituted chalcones are located in the colchicine-binding site of the β subunit of tubulin similarly to podophyllotoxin, but in a way different from colchicine and CA4.[67]

Table 1. Cytotoxicity of polyalkoxychalcones **17**.



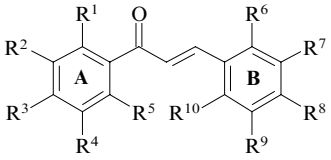
17

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	IC_{50}/μmol litre^{-1} (see [a])	Ref.
17a	OMe	H	H	H	OMe	OMe	H	H	H	OMe	40 [b]	66
											6 (CEM)	66
17b	OMe	H	H	H	OMe	OMe	H	OMe	H	OMe	14.0 [b]	66
											0.25 (SW48)	66
											0.45 (HCT116)	66
											0.65 (CEM)	66
17c	OMe	H	OMe	H	OMe	OMe	H	OMe	H	OMe	14.7 [b]	66
											0.8 (SW48)	66
17d	H	H	H	H	H	H	H	OMe	H	H	53 (TK-10)	65
											90 (MCF-7)	65
											54 (HT-29)	65
17e	H	H	H	H	OH	H	H	OMe	H	H	67 (TK-10)	65
17f	H	H	H	H	OH	H	OMe	OMe	H	H	90 (TK-10)	65
											95 (MCF-7)	65
17g	H	H	OMe	H	H	OMe	H	OMe	OMe	H	37.6 (HeLa)	72
											60.5 (SW-982)	72
											33.9 (MCF-7)	72
17h	H	OMe	OMe	OMe	H	H	OH	OMe	H	H	0.11 (MCF-7)	73
											0.0043 (K562)	62
17i	OMe	OMe	OMe	H	H	H	OH	OMe	H	H	0.01 (K562)	62
17j	H	H	OMe	H	H	H	OH	OMe	H	H	1.5 (K562)	62
17k	H	OMe	OMe	OMe	H	H	H	OMe	H	H	0.30 (K562)	62
17l	H	H	OMe	H	H	H	OMe	OMe	OMe	H	4.5 (K562)	62
17m	H	OMe	OMe	OMe	H	H	OMe	OMe	H	H	0.30 (K562)	62
17n	H	OMe	OMe	OMe	H	H	OMe	OMe	OMe	H	1.5 (K562)	62
17o	H	OCH_2CH_2O		H	H	H	OCH_2O		H	H	8.0 (K562)	62
CA4											0.0032 [c]	74

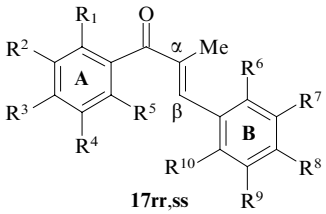
[a] Hereinafter, IC_{50} is the concentration required to suppress cancer cell growth by 50%; the human cancer cell lines are given in parentheses (CEM is lymphoblastic leukemia, SW48, HCT116 and HT-29 are colon cancer, TK-10 is kidney cancer, MCF-7 is breast cancer, HeLa is cervical cancer, SW-982 is synovial sarcoma, K562 is myeloid leukemia); [b] the average value for 11 human cancer cell lines; [c] the average value for 60 human cancer cell lines.

The assessment of antiproliferative activity of polyalkoxychalcones using the sea urchin embryo model (Table 2) made it possible to compare their activity and evaluate the mechanism of action. The antimitotic effect of chalcones **17h**,**hh**,**ii**,**rr**, which cause embryo spinning, is related to destabilization of microtubules, which is confirmed by the ability of compound **17h** to efficiently inhibit purified tubulin polymerization.[62] Compounds **17k**,**o**,**v**,**bb**,**ff**,**gg**,**jj**,**ss** can also be considered as microtubule-destabilizing agents, because the complete cleavage

Table 2. Effect of polyalkoxychalcones **17** on *P. lividus* sea urchin embryos (Chemical Block database, www.chemblock.com).



17p – **17qq**, **17tt** – **17ww**



17rr,**ss**

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	EC /μmol litre^{-1} (see [a])		
											cleavage alteration	cleavage arrest	embryo spinning
17p	H	H	H	H	H	OMe	OCH_2O		OMe	H	> 4	> 4	> 5
17q	H	H	H	H	H	H	OCH_2O		OMe	OMe	> 4	> 4	> 5
17r	H	H	Cl	H	H	OMe	OCH_2O		OMe	H	> 4	> 4	> 5
17s	H	H	F	H	H	OMe	OCH_2O		OMe	H	4	> 4	> 4
17l	H	H	OMe	H	H	H	OMe	OMe	OMe	H	2	> 4	> 4
17t	H	H	OMe	H	H	OMe	OMe	OMe	H	H	2	> 4	> 5
17u	H	H	OMe	H	H	OMe	H	OMe	OMe	H	4	> 4	> 5
17v	H	H	OMe	H	H	H	OCH_2O		OMe	H	0.5	2 [b]	> 5
17w	H	H	OMe	H	H	OMe	OCH_2O		OMe	H	2	> 4	> 4
17x	H	H	OEt	H	H	OMe	OCH_2O		OMe	H	1	> 4	> 4
17y	H	OMe	H	H	H	OMe	OCH_2O		OMe	H	2	> 4	> 4
17z	H	OEt	H	H	H	OMe	OCH_2O		OMe	H	4	> 4	> 4
17aa	H	H	OMe	H	H	H	OCH_2O		OMe	OMe	> 4	> 4	> 4
17bb	H	OMe	OMe	H	H	H	OCH_2CH_2O		H	H	0.1	4 [b]	> 5
17cc	H	OMe	OMe	H	H	OMe	OMe	OMe	H	H	4	> 4	> 5
17dd	H	OMe	OMe	H	H	OMe	OCH_2O		OMe	H	> 4	> 4	> 4
17ee	H	OCH_2O		H	H	OMe	OMe	OMe	H	H	2	> 4	> 5
17ff	H	OCH_2O		H	H	OMe	OCH_2O		OMe	H	0.5	4 [b]	> 5
17gg	H	OCH_2CH_2O		H	H	H	H	OMe	H	H	0.5	4 [b]	> 5
17o	H	OCH_2CH_2O		H	H	H	OCH_2O		H	H	0.5	2 [b]	> 5
17hh	H	OCH_2CH_2O		H	H	H	OMe	OMe	OMe	H	0.5	2	5
17ii	H	OCH_2CH_2O		H	H	H	OCH_2O		OMe	H	0.1	0.5	4
17jj	H	OCH_2CH_2O		H	H	OMe	OCH_2O		OMe	H	0.2	2 [b]	> 5
17kk	OMe	H	H	OMe	H	OMe	OCH_2O		OMe	H	> 4	> 4	> 5
17ll	H	H	OMe	H	OH	H	OMe	OMe	OMe	H	> 4	> 4	> 4
17mm	H	H	OMe	H	OH	H	OCH_2O		OMe	H	> 4	> 4	> 4
17nn	H	H	OMe	H	OH	OMe	OCH_2O		OMe	H	> 4	> 4	> 4
17oo	H	H	OMe	H	OH	H	OCH_2O		OMe	OMe	> 4	> 4	> 4
17n	H	OMe	OMe	OMe	H	H	OMe	OMe	OMe	H	> 4	> 4	> 4
17pp	H	OMe	OMe	OMe	H	H	OCH_2O		OMe	H	4	> 4	> 4
17qq	H	OMe	OMe	OMe	H	H	H	$OCH_2C(O)NH_2$	H	H	> 4	> 4	> 4
17k	H	OMe	OMe	OMe	H	H	H	OMe	H	H	0.2	1 [b]	> 5
17h	H	OMe	OMe	OMe	H	H	OH	OMe	H	H	0.02	0.2	0.5
17rr	H	OCH_2O		OMe	H	H	H	OMe	H	H	0.002	0.02	0.1
17ss	OMe	OCH_2O		OMe	H	H	H	OMe	H	H	0.5	2 [b]	> 5
17tt	H	OMe	OMe	H	Br	H	H	OMe	H	H	2	> 4	> 4
17uu	H	OMe	OMe	H	Br	H	OMe	OMe	H	H	4	> 4	> 4
17vv	H	OMe	OMe	H	Br	OMe	OMe	OMe	OMe	H	2	> 4	> 4
17ww	H	OMe	OMe	H	Br	H	OCH_2O		OMe	OMe	> 4	> 4	> 4
CA4											0.002	0.01	0.05

[a] Hereinafter, EC is the threshold concentration, at which the effect is observed; [b] arrested eggs acquire tuberculate shape characteristic of microtubule-destabilizing agents.

arrest resulted in the formation of tuberculate eggs. Generally, the antimitotic activity increases with an increase in the number of alkoxy groups in ring **A**. This is in agreement with the published data on the *in vitro* cytotoxic effect of chalcones on cancer cells.[61] The effect of compounds **17l**,**t**,**u**,**w**,**x**,**y** containing one alkoxy group in the ring **A** was weaker compared to compounds **17o**,**bb**,**ff**–**jj** bearing two alkoxy groups or the ethylenedioxy moiety (see Table 2). Interestingly, that compound **17k** containing three methoxy groups in ring **A** was 10 times more active than its total isomer **17l** featuring three methoxy groups in ring **B**. A similar relationship was observed in leukemia cells K562 (see Table 1).[62] Molecules **17v** and **17ii** bearing the myristicin moiety in ring **B** exhibited a more pronounced effect compared to analogues **17l** and **17hh** containing the trimethoxyphenyl group. α-Methylchalcones **17rr** and **17ss**, which are myristicin and apiol derivatives, respectively, proved to be the most active. Although having a weak effect on the cleavage, chalcones **17tt**–**ww** exhibited a general toxic effect because the post-hatching treatment with a concentration of 2–4 μmol litre^{-1} disrupted the development of sea urchin embryos. The results of assays on sea urchin embryos are in good agreement with the results obtained using cancer cells. The antimitotic activity depends on the following factors: 1) the activity increases with an increase in the number of alkoxy groups in ring **A**, the effect being particularly pronounced for 3,4,5-trimethoxy derivatives; 2) the 3-OH

Scheme 6

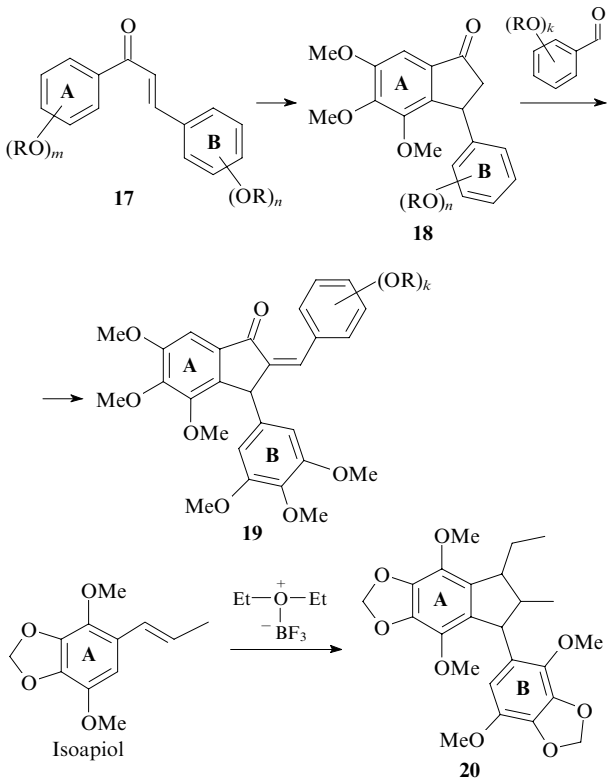
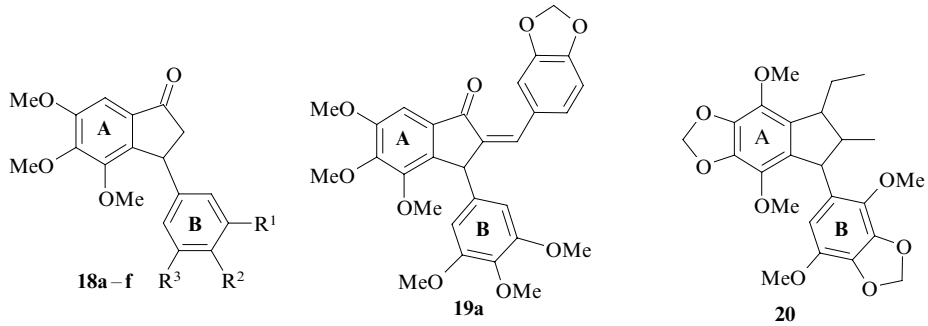


Table 3. Effect of polyalkoxyindanones **18a**–**f**, **19a** and **20** on human cancer cells (given in parentheses)[a] and *P. lividus* sea urchin embryos (Chemical Block database, www.chemblock.com).



Compound	R^1	R^2	R^3	IC_{50} for human cancer cells /μmol litre^{-1} (see [a])	Ref.	EC for sea urchin embryos /μmol litre^{-1}		
						cleavage alteration	cleavage arrest	embryo spinning
18a	H	OMe	H	0.49 (K562)	64	0.2	4[b]	>5
18b	OH	OMe	H	0.13 (K562)	64	–	–	–
18c	NO_2	OMe	H	0.08 (K562)	64	–	–	–
18d	F	OMe	H	0.10 (K562)	64	–	–	–
18e	OMe	OMe	OMe	2.2 (MCF-7)	77	>4	>4	>4
				75.9 (HCT)	77			
				55.4 (A549)	77			
18f	OCH_2O		OMe	–	–	>4	>4	>4
19a	OMe	OMe	OMe	0.01 (MCF-7)	77	–	–	–
				0.1 (HCT)	77			
				4.9 (THP-1)	77			
				11.0 (A549)	77			
20				–	>4	>4	>4	

[a] K562 is myeloid leukemia, MCF-7 is breast cancer, HCT is colon cancer, A549 is lung carcinoma, THP-1 is monocytic leukemia; [b] arrested eggs acquire tuberculate shape characteristic of microtubule-destabilizing agents.

and 4-OMe groups in ring **B** enhance the antimitotic action; 3) an increase in the number of methoxy groups in ring **B** to three leads to a decrease in this effect; 4) α-methylchalcones are more active than unmethylated analogues.

Indanones **18** synthesized by the Nazarov reaction from chalcones can be considered as isomeric analogues of chalcones **17** having the fixed *s-trans* conformation (Scheme 6).[64, 77–80]

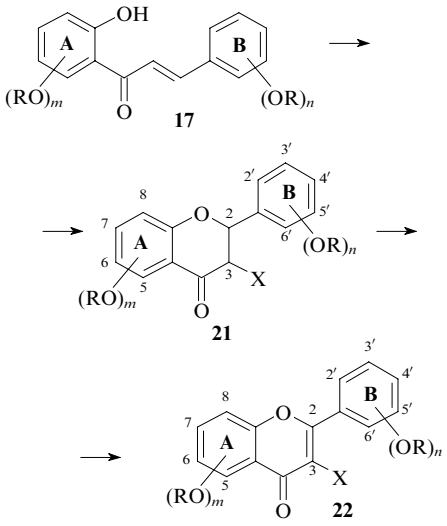
The cyclization of chalcones **17h** and **17k** to the corresponding indanones **18b** and **18a** leads to weakening of the cytotoxic effect on K562 leukemia cells, which is particularly pronounced for **17h**–**18b** pair (Tables 1 and 3). The MCF-7 breast cancer cell line proved to be the most sensitive to indanones. 2-Benzylideneindanones **19** are powerful antimitotics, among which compound **19a** [$(OR)_k$ = 3,4-OCH_2O, see Table 3] can be distinguished.[77] It was shown that indanones **18e** and **19a** inhibit tubulin polymerization.[77]

In the sea urchin embryo tests, the antimitotic effect remained virtually unchanged after the cyclization of chalcone **17k** to isomeric indanone **18a**, and this effect was comparable with the data published in the literature on the cytotoxicity of compound **18a** to K562 human myeloid leukemia cells (see Tables 2 and 3).[64] The appearance of tuberculate eggs after the treatment with compound **18a** at a concentration of 4 μmol litre^{-1} suggests that tubulin is the cellular target for this compound. An increase in the number of methoxy groups in ring **B** of indanones **18e**, **18f** and **20** led to the disappearance of antiproliferative activity against sea urchin embryos (see Table 3).

VII. Polyalkoxyflavanones

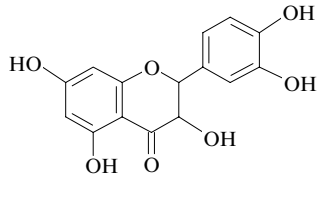
Flavanones (Fig. 6) are metabolic products of chalcones (see Scheme 1).[39] In plant cells, flavanones undergo dehydrogenation and oxidation to form flavones and flavonols (quercetin, apigenin, see Section VIII).[81] The natural antioxidant dihydroquercetin (taxifolin) (see Fig. 6) is the commonly known representative of flavanones. It is included in the public register of drugs and is approved as a food supplement for the use in food industry. Flavanones **21** can be easily synthesized by the cyclization of α-hydroxychalcones **17** (Scheme 7), although in some cases, the equilibrium is shifted toward the starting chalcone due to the reversibility of the reaction.[82]

Scheme 7

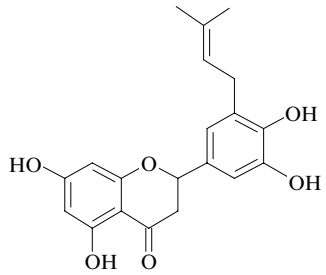


X = H, OH, OMe

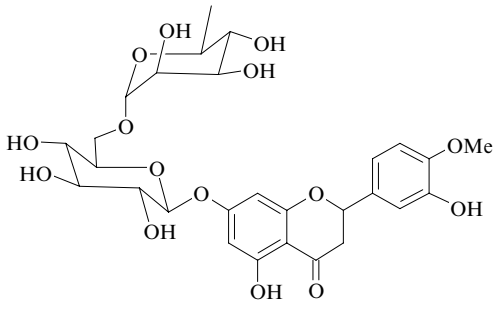
The antiproliferative activity of polymethoxyflavanones is poorly known. The flavanone glycoside hesperidin (see Fig. 6) found in the orange peel suppresses human cancer cell growth at micromolar concentrations (Table 4).[84] The mechanism of action of this compound is unclear. Presumably, the antiproliferative properties of hesperidin are related to its antioxidant activity.[84] Natural flavanones containing the isoprenoid group, *viz.*, sigmoidins A and B (see Fig. 6), isolated from the African medicinal plant *Erythrina sigmoidea* inhibit the cell division of *P. lividus* sea urchin embryos.[85] Dihydroquercetin has no effect either on the cancer cell growth or on the embryonic development of the sea urchin *P. lividus* (see Table 4). Polymethoxyflavanones **21b**,**c** also proved to be inactive.



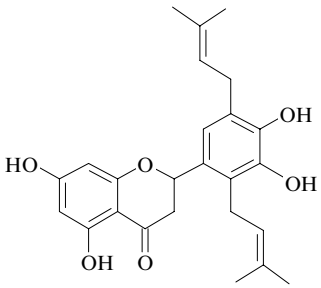
Dihydroquercetin



Sigmoidin B



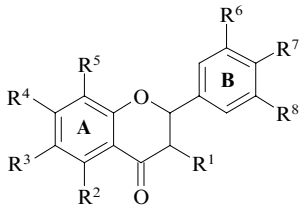
Hesperidin



Sigmoidin A

Figure 6. Natural flavanones.

Table 4. Effect of polymethoxyflavanones **21** and a series of natural flavones on human cancer cells (given in parentheses) and *P. lividus* sea urchin embryos (Chemical Block database, www.chemblock.com).



Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	IC_{50} for human cancer cellss/μmol litre^{-1} (see [a])	Ref.	EC for altered cleavage of sea urchin embryos /μmol litre^{-1}
Dihydroquercetin [b]	OH	OH	H	OH	H	OH	OH	H	> 160 (OVCAR-3)	83	> 10
Hesperidin [b]									2.74 (laryngeal cancer)	84	
									5.45 (cervical cancer)	84	
									6.83 (breast cancer)	84	
									7.5 (liver sarcoma)	84	
Sigmoidin A [b]									—	—	7.5 [c]
Sigmoidin B [b]									—	—	12 [c]
21a	H	OMe	OMe	OMe	OMe	H	OH	H	72 (KB)	40	—
21b	OH	OMe	H	OMe	H	H	OMe	OMe	—	—	> 10
21c	H	H	H	OMe	H	OMe	OMe	OMe	—	—	> 4

[a] OVCAR-3 is ovarian cancer, KB is cervical cancer; [b] the structure is shown in Fig. 6; [c] the concentration that causes cell division arrest of sea urchin embryos by 50%.[85]

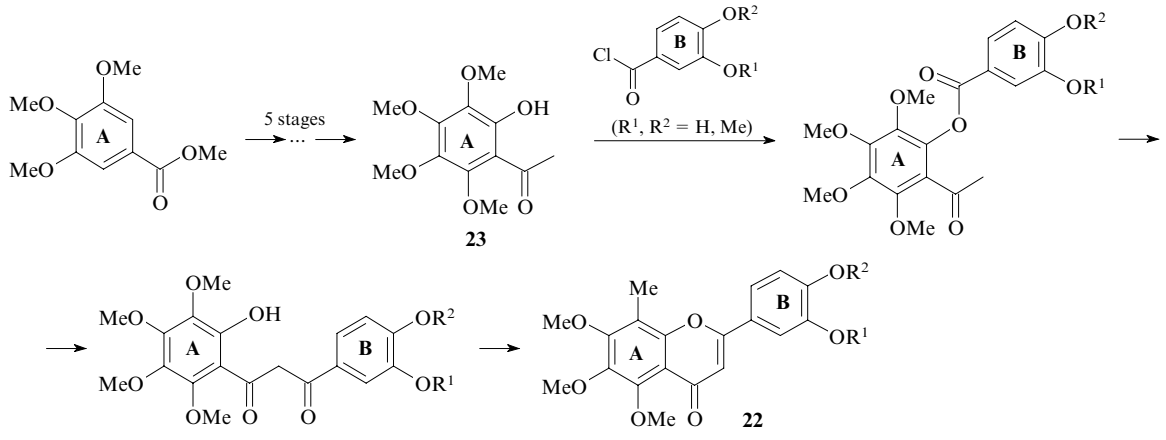
VIII. Polyalkoxyflavones

Flavones have various biological activities, in particular, they are antioxidants, prevent the development of many cancer types and can suppress cancer cell growth.[36, 39, 41, 86]

There are three approaches to the synthesis of polymethoxyflavones **22** and their analogues with antiproliferative properties: 1) by using the coupling of polymethoxybenzenes (oxidation, condensation, alkylation, acylation, cyclization, *etc*.) (Schemes 7 and 8);[82, 87] 2) an intermediate approach involving the degradation of natural flavone (tangeretin) to form polymethoxyphenol **23**, which is difficult to synthesize (Scheme 9, pathway *b*),[88] and the use of the latter for the preparation of polymethoxyflavones **22** according to known methods (see Schemes 7 and 8); 3) the modification of the skeleton of natural flavones by introducing alkoxy and amino groups (Schemes 9, pathway *a*, 10 and 11).[89, 90]

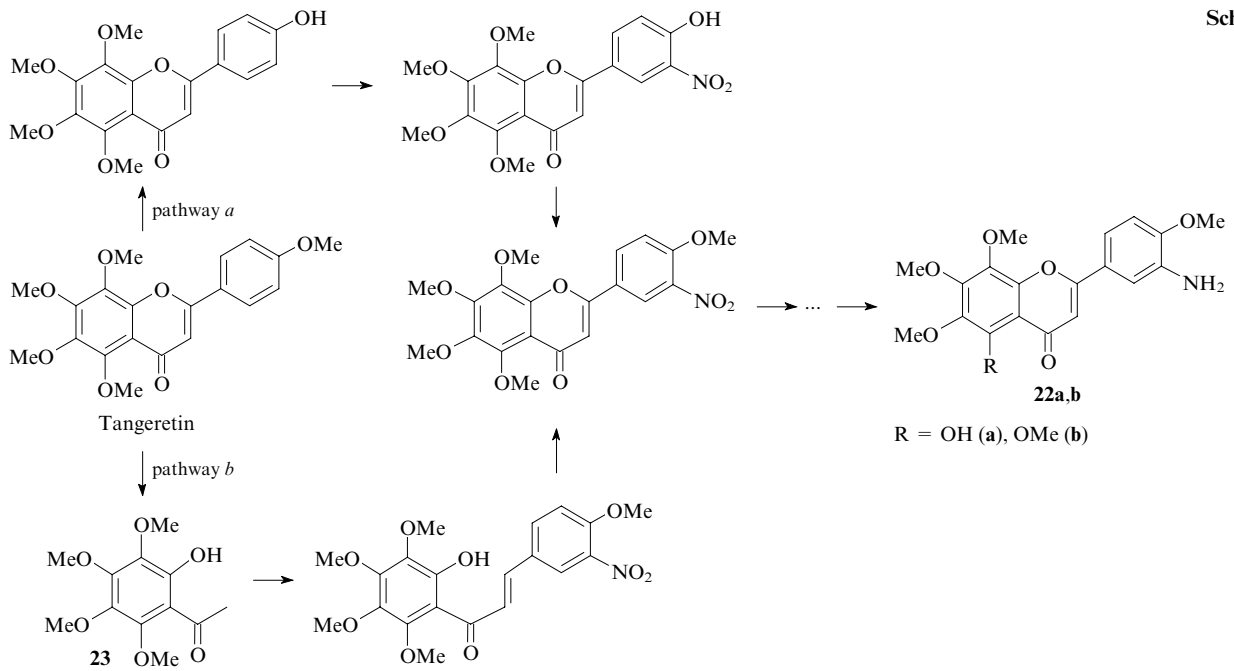
The initial tests of more than 200 flavonoids performed at the NCI in searching for new anticancer drugs revealed no relationship between the antiproliferative activity and the chemical structure of these compounds. The cytotoxicity against human cancer cells was observed at micromolecular concentrations (see below the data in Table 7). It was concluded that flavonoids hold no promise as anticancer compounds. However, it was hypothesized that some fla-

Scheme 8

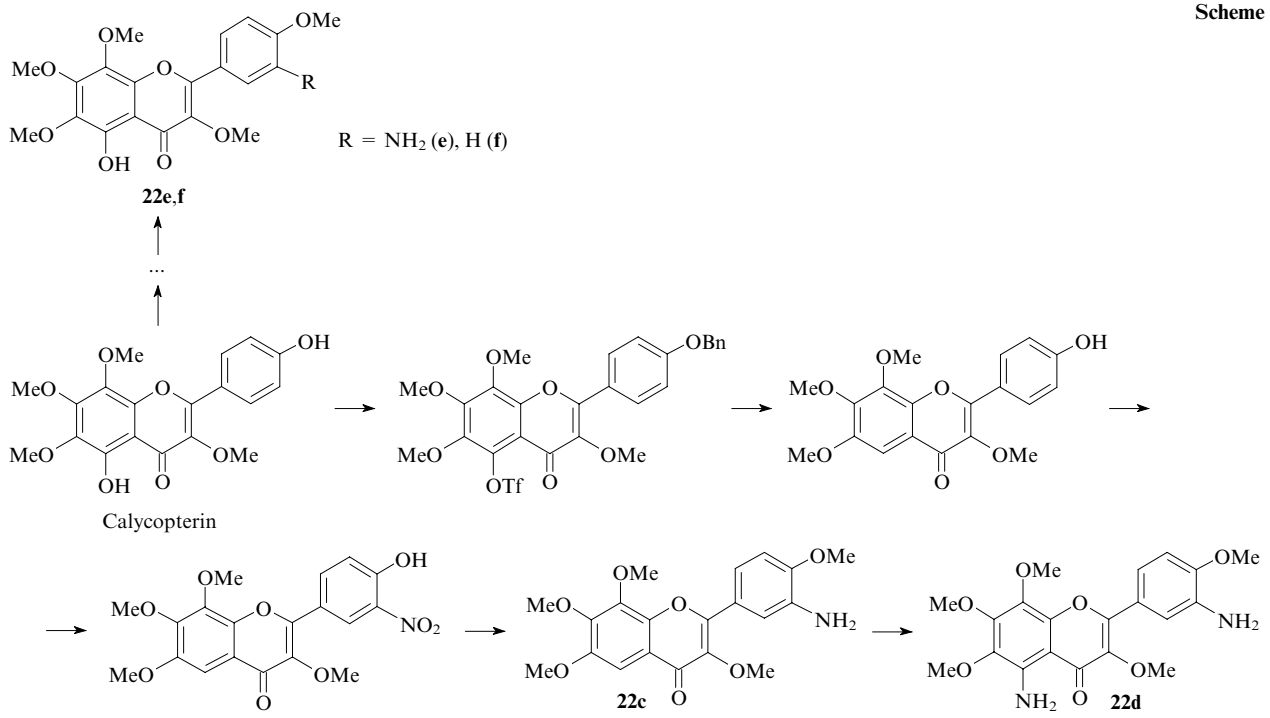


22: $R^1 = R^2 = Me$ (nobiletin); $R^1 = H$, $R^2 = Me$; $R^1 = Me$, $R^2 = H$

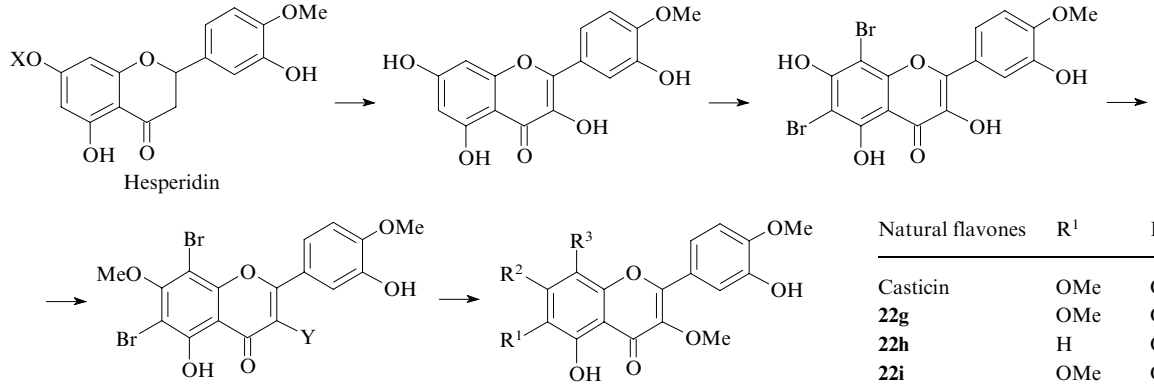
Scheme 9



Scheme 10



Scheme 11



Natural flavones	R^1	R^2	R^3
Casticin	OMe	OMe	H
22g	OMe	OMe	OMe
22h	H	OMe	OMe
22i	OMe	OH	OMe

X is rutinosyl; Y = OH, OMe

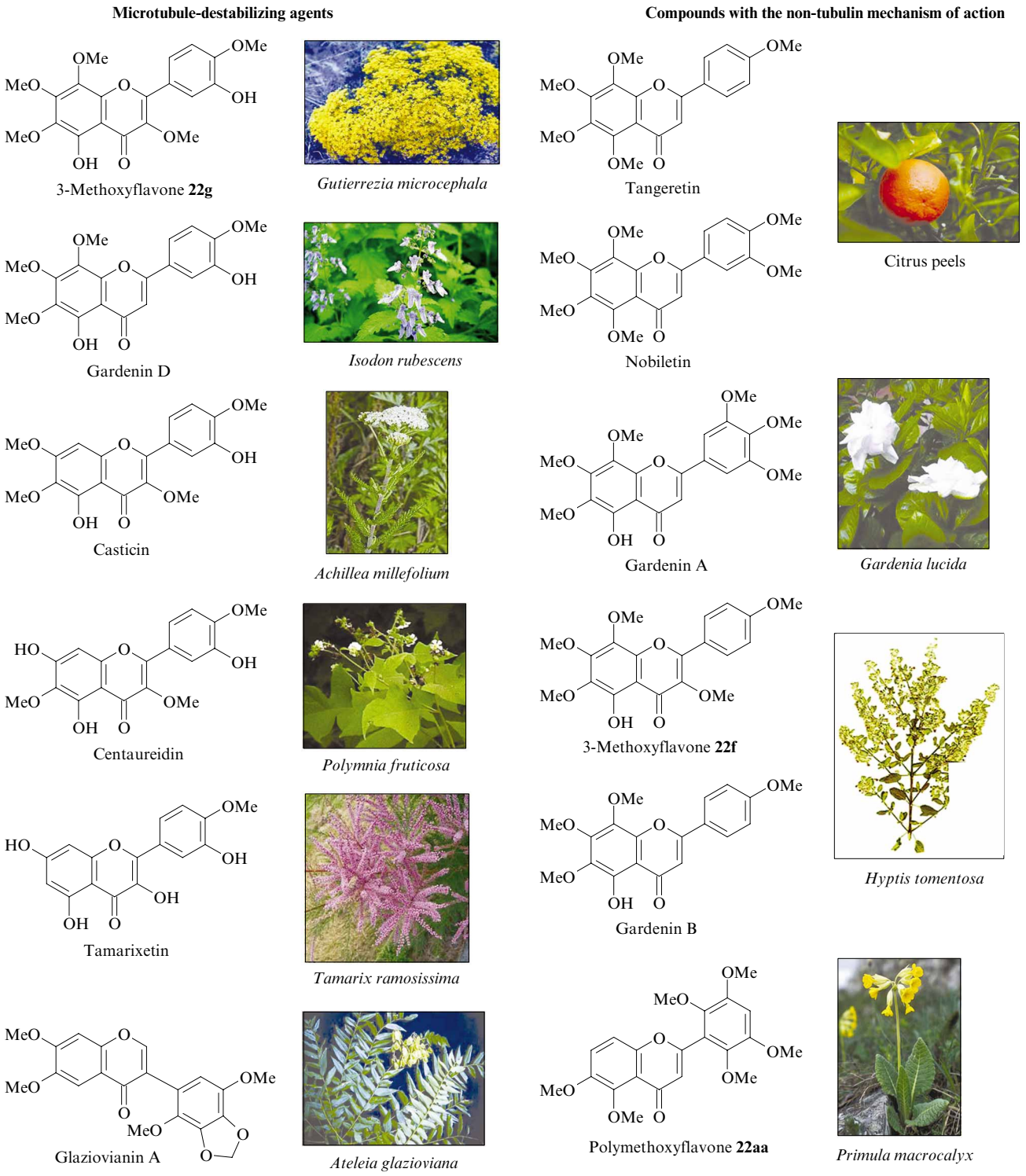


Figure 7. Natural polymethoxyflavones with antiproliferative activity and plants from which they are isolated.

vonoids can disrupt the mitotic spindle formation due to the presence of vicinal polyalkoxy groups and, consequently, these compounds deserve further investigation.[40] Actually, more recently, polymethoxyflavones with antiproliferative activity were isolated from some plants; these compounds have different mechanisms of action, including the effect on tubulin/microtubules similar to colchicinoid cytostatics (Fig. 7, Table 5).

3-Methoxyflavone **22g** was first isolated from the North American plant of the Asteraceae family *Gutierrezia microcephala* in 1986.[107] More recently, this compound was found in plants of the rutaceae family *Zieridium pseudobtusifolium* (New Caledonia) and *Acronychia porteri* (Malaysia)[91] and in the North American plant cleome *Polanisia dodecandra*.[92] Gardenins A,[108] B and D[109] were first isolated from the leaf buds of the Indian tree *Gardenia lucida*. Later on, in 2003,

Table 5. Cytotoxicity and the mechanism of action of natural polymethoxyflavones and the isoflavone glaziovianin A.[a]

Compound	IC_{50} /μmol litre^{-1} (see [b])	ITP IC_{50} /μmol litre^{-1} (see [c])	Mechanism of action	Ref.
3-Methoxyflavone **22g**	0.099 (KB)	12	inhibition of tubulin polymerization	91
(NCI ID 642321) [d]	0.13 [e]	0.83	inhibition of tubulin polymerization, binding to the colchicine site	92, 93
	0.008 (KB)	8.9	inhibition of tubulin polymerization, cell cycle arrest at G2/M phase	89, 90
Gardenin D	0.08 (KB)	86	inhibition of tubulin polymerization, apoptosis	88
Casticin	2 (MCF-7: sublines MN1 and MDD2)	–	inhibition of tubulin polymerization, disruption of microtubules in the cell, cell cycle arrest at G2/M phase, apoptosis	94
	0.097 (KB)	19	–	89
Centaureidin	–	3	inhibition of tubulin polymerization,	95
(NCI ID 106969) [d]	0.24 [e]	2	binding to the colchicine site	92, 93
Tamarixetin	7.5 (HL-60)	–	cell cycle arrest at G2/M phase, caspase-mediated	96
	5.5 (U937)	–	apoptosis, inhibition of tubulin polymerization	96
	7.5 (MOLT-3)	–		96
	6.1 (Raij)	–		96
	24.1 (K562)	–		96
	17.8 (MCF-7)	–		96
	23.1 (SK-MEL-1)	–		96
	22.0 (A549)	–		96
Glaziovianin A	0.66 [f]	–	cell cycle arrest at G2/M phase, disruption of	97
	0.29 (HL-60)	–	the mitotic spindle structure, no effect	98
	0.59 (HeLa S_3)	–	on interphase microtubules	98
	–	–	inhibition of tubulin polymerization, disruption of microtubule dynamics, binding to the colchicine site	99
Tangeretin	21.5 (HTB43) [g]	–	–	100
(NCI ID 53909) [d]	37.5 (COLO 205)	–	cell cycle arrest at G1 phase, inhibition of cyclin-dependent kinases 2 and 4, enhancement of expression of p23 and p27 cyclin-dependent kinase inhibitors	101
	40 (MDA-MB-435)	–	cell cycle arrest at G1 phase	102
	25 (MCF-7)	–		102
	33 (HT-29)	–		102
	48 (HT-29)	–	–	103
	53.1 [e]	–	–	–
Nobiletin	19.9 (HTB43) [g]	–	–	100
	66.2 (COLO 205)	–	–	101
	75 (MDA-MB-435)	–	cell cycle arrest at G1 phase	102
	50 (MCF-7)	–		102
	50 (HT-29)	–		102
	50 (HT-29)	–		103
Gardenin A	19.4 [e]	> 40	tubulin/microtubule	93
(NCI ID 94889) [d]			independent cytotoxicity	
3-Methoxyflavone **22f**	5 (KB)	–	tubulin/microtubule	104
	0.72 (KB)	–	independent cytotoxicity	90
Gardenin B	15.5 (KB)	–	–	104
	16.6 [e]	–	–	40
Polymethoxyflavone **22aa**	0.2 [h]	–	tubulin/microtubule independent cytotoxicity	105, 106

[a] The structures are shown in Fig. 7; [b] KB is cervical cancer, MCF-7 is breast cancer, HL-60 is promyelocytic leukemia, U937 is lymphoma, MOLT-3 is T-lymphoblastic leukemia, Raij is Burkitt lymphoma, K562 is myeloid leukemia, SK-Mel-1 is melanoma, A549 is lung carcinoma, Hela S_3 is cervical cancer, HTB43 is squamous cell carcinoma, COLO 205 is colorectal cancer, MDA-MB-435 is breast cancer, HT-29 is colon cancer; [c] ITP IC_{50} is the concentration of the compound required to inhibit 50% of the rate of tubulin polymerization; [d] NCI ID is the identification number of the compound in the NCI database; [e] the average value of IC_{50} for 60 cancer cell lines (NCI Anti-cancer Screening Program, http://dtp.nci.nih.gov/index.html); [f] the average value for 39 human cancer cell lines and the 3Y1 rat fibroblast cell line; [g] the concentration that causes the suppression of cancer cell growth by 70% – 72%; [h] the concentration that causes cleavage alteration and development of *P. lividus* sea urchin embryos.

gardenin D was extracted from the leaves of the herbaceous plant isodon of the mint family (*Isodon rubescens*).[110] Casticin was isolated from the nosebleed *Achillea millefolium* in 2006.[94] Centaureidin was extracted from the North American plant of the Asteraceae family *Polymnia fruticosa* in 1993.[95] Tamarixetin was first found in 1981 in the liana *Eupatorium odoratum* grown in the Bahamas.[111] Later on, this compound was isolated from the leaf extract of the tamarisk *Tamarix ramosissima* abundant in Central Asia, China, Mongolia, Iran and Southern Europe countries.[112] Glaziovianin A was isolated from the leaves of the Brazilian plant *Ateleia glazioviana* in 2007.[97] Tangeretin and nobiletin are found in the citrus peels,[113, 114] and their cytotoxic effect was described in 1991.[100] Methoxyflavone **22f** and gardenin B were isolated from the above-ground parts of the herbaceous plant *Hyptis tomentosa* of the Lamiaceae family in 1979.[104] Polymethoxyflavone **22aa** is found in the leaves of the macrosepalous primrose (*Primula macrocalyx*) abundant in the Crimea, the Caucasus, the southern Ural and in the south region of Western Siberia.[105, 106]

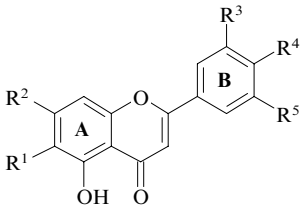
The mechanism of antiproliferative action of polymethoxyflavones substantially depends on the ratio and mutual arrangement of alkoxy and hydroxy groups. For example, all compounds containing the hydroxy group in the *meta* position of the benzene ring **B** (gardenin D,[88] flavone **22g**,[90–93] casticin,[94] centaureidin [92, 93, 95] and tamarixetin [96]) inhibit tubulin polymerization. Centaureidin [92, 93, 95] and flavone **22g** (the latter compound exhibits the highest cytotoxicity among all the isolated flavonoids) [88, 90, 91] interact with the colchicine site of the tubulin molecule.[92, 93] It is interesting that the cytotoxicity of the flavones tangeretin,[101, 102] nobiletin,[102] gardenin A [93] and compound **22f** lacking hydroxy groups in ring **B** is not associated with the effect on tubulin and(or) microtubules. Recently, it was found that tangeretin inhibits the membrane transport protein P-glycoprotein involved in the development of multidrug resistance in cancer cells.[115] The mechanisms of cytotoxic action and cellular targets for gardenin B and polymethoxyflavones **22f** and **22aa** remain unclear (see Table 5).

Several research teams attempted to find the relationship between the antiproliferative activity of flavones and their molecular structure. Cytotoxic poly(hydroxy/methoxy)flavones found in the leaves of the widespread ornamental lantana (*Lantana montevidensis*) (Table 6) inhibit cancer cell growth at micromolar concentrations.[116] It was noted that ambiguous effects were observed in different cell lines; however, it was hypothesized that polar substituents in rings **A** [5,7-$(OH)_2$] and **B** [3′,4′-$(OH)_2$ or 3′-OH-4′-OMe] play an important role in the cytotoxicity.[116] The values of IC_{50} for a number of polymethoxyflavones are given in Table 7.

Flavones **22c–g** containing the 3-OMe group in the pyran moiety and three OMe substituents in the positions 6, 7 and 8 of the ring **A** have the highest antiproliferative microtubule-destabilizing activity.[89, 90] Similarly to the CA4 molecule,[90, 93] the presence of the 3′-OH and 4′-OMe groups in ring **B** (**22g**) play an important role; the removal of the OH group (compound **22f**) or the replacement of this group by the NH_2 group (**22e**) leads to a decrease in activity.[90] The

Table 6. Effect of flavones from leaves of lantana (*Lantana montevidensis*) on human cancer cells.[116]

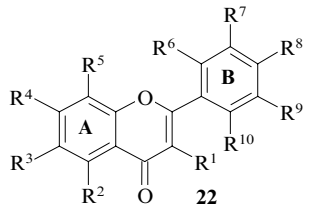




Flavone	R^1	R^2	R^3	R^4	R^5	IC_{50}/μmol litre^{-1}	
						HeLa [a]	MK-1 [b]
Baicalein	OH	OH	H	H	H	30	26
6-Hydroxyluteolin	OH	OH	OH	OH	H	30	26
Hispidulin	OMe	OH	H	OH	H	17	83
Pectolinarigenin	OMe	OH	H	OMe	H	10	115
Eupafolin	OMe	OH	OH	OH	H	6	29
Demethoxycentaureidin	OMe	OH	OH	OMe	H	9	24
Jaceosidin	OMe	OH	OMe	OH	H	33	27
Eupatilin	OMe	OH	OMe	OMe	H	35	55
Isodemethocentaureidin	OH	OMe	OH	OMe	H	55	55
Isoeupatilin	OH	OMe	OMe	OMe	H	73	73
Iso-5′-methoxycirsiliol	OH	OMe	OMe	OH	OMe	44	33
Cirsiliol	OMe	OMe	OH	OH	H	21	18
Eupatorin	OMe	OMe	OH	OMe	H	15	58
Cirsilineol	OMe	OMe	OMe	OH	H	203	17
5′-Methoxycirsiliol	OMe	OMe	OH	OH	OMe	14	22
5′-Methoxycirsilineol	OMe	OMe	OMe	OH	OMe	>267	>267

[a] HeLa is cervical cancer; [b] sMK-1 is gastric adenocarcinoma.

Table 7. Cytotoxicity of polymethoxyflavones **22**.



22

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	IC_{50} /μmol litre^{-1} (see [a])	Ref.
22a	H	OH	OMe	OMe	OMe	H	H	OMe	NH_2	H	0.16 (KB)	117
22b	H	OMe	OMe	OMe	OMe	H	H	OMe	NH_2	H	6% (KB) [b]	117
22c	OMe	H	OMe	OMe	OMe	H	H	OMe	NH_2	H	0.078 (KB)	90
22d	OMe	NH_2	OMe	OMe	OMe	H	H	OMe	NH_2	H	0.128 (KB)	90
22e	OMe	OH	OMe	OMe	OMe	H	H	OMe	NH_2	H	0.025 (KB)	90
22f	OMe	OH	OMe	OMe	OMe	H	H	OMe	H	H	0.72 (KB)	90
											5 (KB)	104
22g	OMe	OH	OMe	OMe	OMe	H	H	OMe	OH	H	0.008 (KB)	90
											0.13	93
22h	OMe	OH	H	OMe	OMe	H	H	OMe	OH	H	9% (KB) [b]	89
22i	OMe	OH	OMe	H	OMe	H	H	OMe	OH	H	10% (KB) [b]	89
22j	Cl	OH	OMe	OMe	OMe	H	H	OMe	NH_2	H	0.14 (KB)	88
22k	OMe	OH	OMe	OMe	OMe	H	H	OMe	OMe	H	7.1	93
22l	OMe	OH	OMe	H	OMe	H	OMe	OMe	OMe	H	19	93
22m	OMe	OH	OMe	OH	OMe	H	OMe	OMe	OMe	H	54	93
22n	OH	H	H	H	H	H	OMe	OMe	OMe	H	5.9	93
22o	H	OH	OMe	OMe	OMe	H	H	OMe	OMe	H	17	93
22p	H	OH	OMe	OMe	OMe	H	H	OH	OH	H	4	93
22q	H	OH	OMe	OH	H	H	H	OMe	H	H	9.5–79.5	40
22r	H	OH	OMe	OMe	H	H	H	OMe	OH	H	5.8–11.6	40
22s	H	OH	OMe	OMe	OMe	H	H	OH	H	H	2–290	40
22t	H	OMe	OMe	OMe	OMe	H	OMe	H	OMe	H	7.5–75	40
22u	H	OMe	OMe	OMe	OMe	OMe	H	H	OMe	H	9.9–29.8	40
22v	H	OMe	OMe	OMe	OMe	H	OMe	OMe	OMe	H	1.2–16.2	40
22w	OH	H	H	H	H	OMe	H	H	H	OMe	3.4–53.6	40
22x	OH	H	H	H	H	H	OMe	OMe	H	OMe	15.2–30.5	40
22y	OMe	OH	H	OH	H	H	H	OMe	H	H	6.1	40
22z	OMe	OH	OMe	OH	H	H	H	OMe	OH	H	1.1–8.3	40

[a] KB is cervical cancer; if the cancer cell line is not specified, the average values or the range of IC_{50} for 60 cancer cell lines are given (NCI Anticancer Screening Program, http://dtp.nci.nih.gov/index.html); [b] the degree of suppression of cell growth upon the treatment with polymethoxyflavone at a concentration of 1 μmol litre^{-1}.

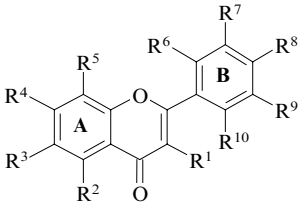
3-OMe substituent in the pyran moiety is required for the interaction with tubulin.[93]

The flavonol quercetin — a component of vitamin P — with anti-edematous, spasmolytic, antihistamine, anti-inflammatory and antioxidant activities is of particular notice. The trimethoxyphenyl moiety is absent in this compound (Table 8); however, quercetin shows, although weak, microtubule-destabilizing effect and has antimitotic activity.

By interacting with the colchicine site,[118] quercetin inhibits purified tubulin polymerization by 40% at a concentration of 40 μmol litre^{-1} (Ref. 119) and by 50% at a concentration of 54 μmol litre^{-1}.[118] Under the same conditions, the effect of colchicine was much stronger (IC_{50} = 1.5 μmol litre^{-1}). At concentrations of 80–100 μmol litre^{-1}, quercetin destroys microtubules and blocks the cell cycle at the mitotic phase (G2/M) in bovine aortic endothelial (BAE) cells [120] and cervical cancer cells (HeLa).[121] According to the NCI data, the average value of IC_{50} of quercetin for 60 human cancer cell lines is 59.9 μmol litre^{-1} (http://dtp.nci.nih.gov/index.html; NCI ID 9219). The cytotoxicity of quercetin to prostate cancer cells PC3 (LD_{50} = 23 μmol litre^{-1}; LD_{50} is the concentration causing the 50% cell death) is most likely associated with the destruction of microtubules.[119] Quercetin inhibits the HeLa cell growth at a concentration of 30–100 μmol litre^{-1} (IC_{50} = 80 μmol litre^{-1}) due to the induction of apoptosis *via* the mitochondrial pathway.[121]

The effect of natural polymethoxyflavones isolated from the primrose *Primula macrocalyx* **22aa**–**cc** [105,106] and synthetic analogues **22dd**,**ee** was studied on *P. lividus* sea urchin embryos (see Table 8). The natural flavonols quercetin and apigenin with antiproliferative action were used as reference compounds. The effect of apigenin is associated with the inhibition of topoisomerase I and a number of kinases involved in proliferation of malignant cells.[122] The average

Table 8. Effect of polyalkoxyflavones **22** on *P. lividus* sea urchin embryos (Chemical Block database, www.chemblock.com).



Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}	EC /μmol litre^{-1}		
											cleavage alteration	cleavage arrest	embryo spinning
Quercetin	OH	OH	H	OH	H	H	H	OH	OH	H	100	> 200	> 200
Apigenin	H	OH	H	OH	H	H	H	OH	H	H	50 [a]	> 50 [a]	> 50 [a]
22aa [b]	H	OMe	OMe	H	H	OMe	OMe	H	OMe	OMe	0.5	> 4	> 4
22bb [b]	H	H	H	H	H	OMe	H	H	OMe	H	1	4	> 5
22cc [b]	H	H	H	H	H	H	OMe	OCH_2O		H	4	> 4	> 4
22dd	H	H	NH_2	H	H	H	H	OMe	OMe	H	> 4	> 4	> 4
22ee	H	H	Cl	H	H	H	F	H	H	H	0.02	> 10	> 10

[a] See Ref. 49; [b] natural polyalkoxyflavones from leaves of macrocephalous primrose (*Primula macrocalyx*).[105, 106]

value of IC_{50} of apigenin for 60 human cancer cell lines is 27.9 μmol litre^{-1} (http://dtp.nci.nih.gov/index.html; NCI ID 83244). Quercetin and apigenin exert a weak antiproliferative effect on eggs, whereas the normal development is observed for embryos treated at later stages (after hatching). It was found that the antiproliferative activity of flavonoids depends on the quantitative ratio and the arrangement of the alkoxy and hydroxy groups in the benzene rings (see Table 8). Apigenin containing three OH groups proved to be two times more active [49] than quercetin with five OH groups. The effect of polymethoxyflavonoids **22aa**–**cc** isolated from primrose on sea urchin embryos was much stronger than that of apigenin. Compounds **22aa**–**cc**,**ee** altered sea urchin egg cleavage *via* the non-tubulin mechanism, because they did not cause embryo spinning. Halogen-containing analogue **22ee** exhibited the highest activity.

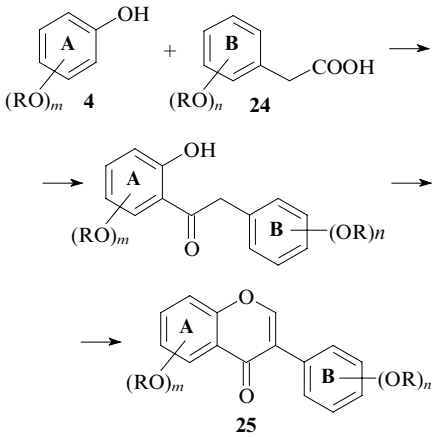
IX. Polyalkoxyisoflavones

Polyalkoxyisoflavones with antiproliferative action are less known. The main method for their synthesis is based on the Friedel–Crafts acylation of polyalkoxyphenols **4** at the *ortho* position with alkoxy-substituted phenylacetic acids **24** followed by cyclization to isoflavones **25** under the action of formic acid derivatives (Scheme 12).[123, 124]

Another method for the synthesis involves the oxidative rearrangement of *o*-benzoyloxychalcone **17xx** to dimethyl acetal **26** followed by cyclization to nitroisoflavone **25a** and the transformation to aminohydroxyisoflavone **25b** (Scheme 13).[117]

A synthetic method was developed for the preparation of the natural antimitotic compound glaziovianin A (see Fig. 7, Table 5) and its analogues **25d**–**h** modified at rings **A** (Scheme 14) and **B** (Scheme 15, pathway *a*) by the Suzuki reaction.[125, 126] However, the intermediates, particularly boron-containing polyalkoxybenzenes **27a**,**b**, which serve as the basis for the construction of ring **B**, are rather difficult to synthesize. A versatile method for the synthesis

Scheme 12

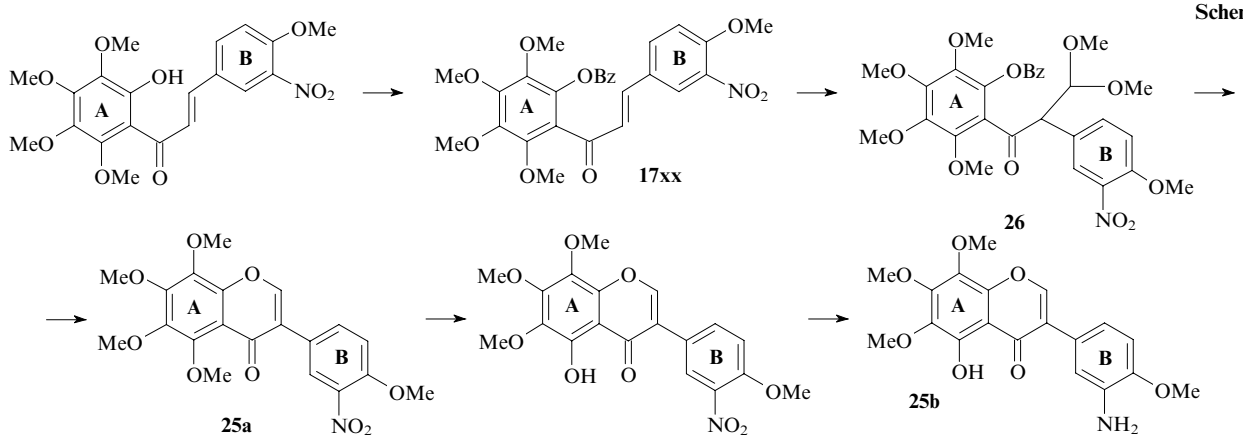


of glaziovianin A and its analogues has been developed recently by the authors of the present review. This approach is based on polyalkoxyaldehydes **2**, which can be easily prepared from the corresponding allylbenzenes (see Fig. 3) — the main components of parsley and dill seed extracts (Scheme 15, pathway *b*).[127]

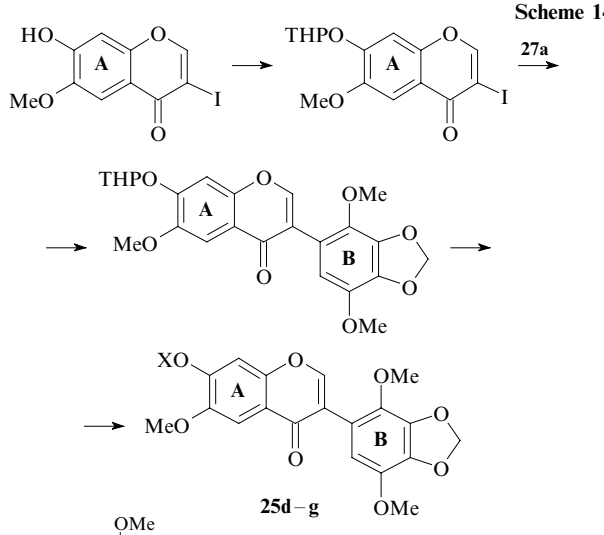
Glaziovianin A shows high cytotoxicity against human cancer cells, has microtubule-destabilizing activity, disrupts microtubule dynamics and the mitotic spindle structure and blocks mitosis (see Table 5).[97–99] Leaves of *Ateleia glazioviana* containing glaziovianin A cause abortions, cardiac failure and the central nervous system injuries in bovines.[97] The antimitotic microtubule-destabilizing properties of glaziovianin A were confirmed by the sea urchin (*P. lividus*) embryo tests. This compound causes egg cleavage alteration and arrest at threshold concentrations of 0.05 and 0.5 μmol litre^{-1}, respectively. Embryo spinning after the treatment with glaziovianin A (4 μmol litre^{-1}) at the swimming blastula stage indicates tubulin-targeting effect and destabilization of microtubules.[127]

Studies of the structure–activity relationships showed that the antimitotic microtubule-destabilizing properties of

Scheme 13



Scheme 14



27a = [$(RO)_2$ = $OCMe_2CMe_2O$]; X = H (**d**), Bn (**e**), $CH_2C{\equiv}CH$ (**f**), All (**g**); THP is tetrahydropyran-2-yl

glaziovianin A depend on the nature of the alkoxy substituents in the position 7 of the ring **A** and the apiol moiety (ring **B**) (Table 9).[98,126] Synthetic analogue **25g** containing an OAll substituent in the position 7 proved to be most toxic against human cervical cancer cells HeLa S_3 and blocked mitosis at a lower concentration.[98] Using one pair of analogues, it was shown that isoflavones are less active than flavones containing the same substituents. A comparison of 3′-amino-substituted isoflavone **25b** (see Table 9) and its full analogue flavone **22a** (see Table 7) using cervical cancer cells KB as a model showed that isoflavone **25b** is inactive up to the concentration of 1 μmol litre^{-1}, whereas flavone **22a** taken at the same concentration inhibits cell growth by 96% with IC_{50} = 0.16 μmol litre^{-1}.[117]

X. Polyalkoxy-4-aryl-3,4-dihydrocoumarins and polyalkoxy-4-arylcoumarins

More than 1300 natural coumarins devoid of aromatic substituents in the pyran ring are found in plants, bacteria and fungi.[129] These compounds have various biological activities, including the inhibition of cancer cell prolifera-

Scheme 15

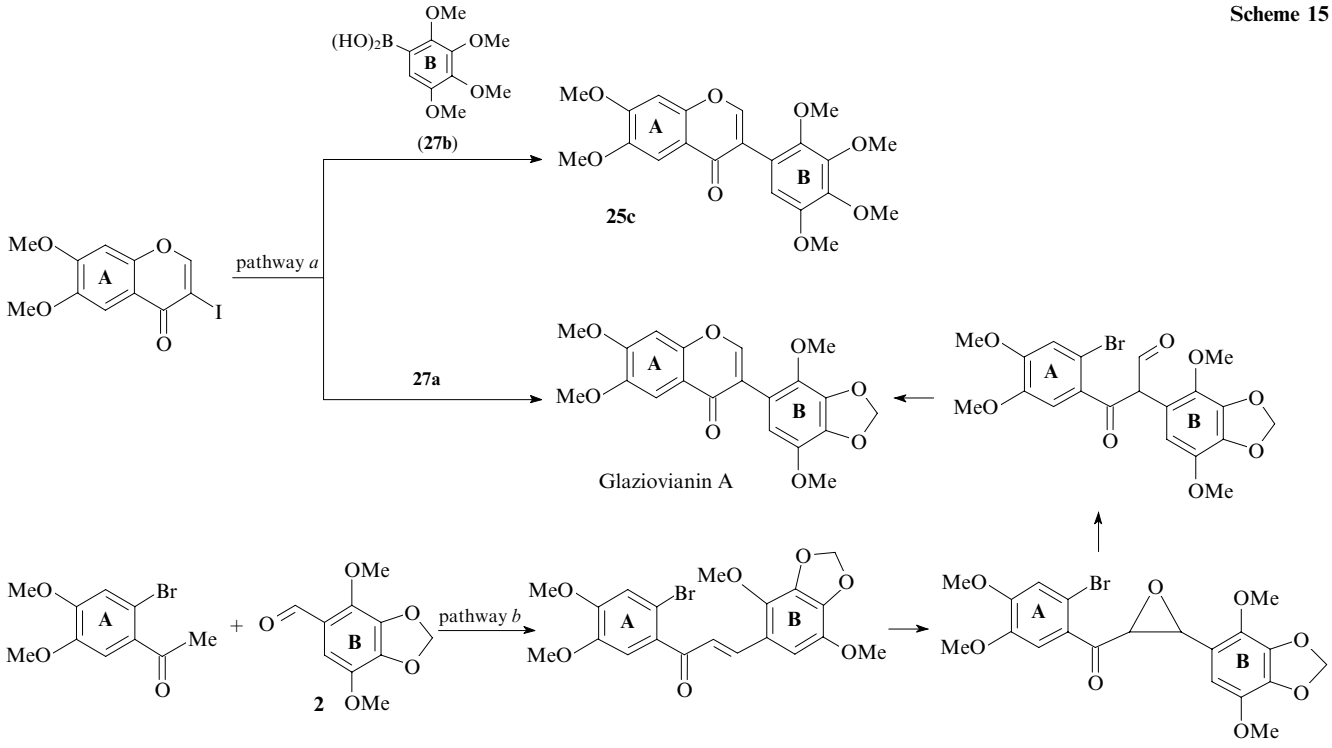
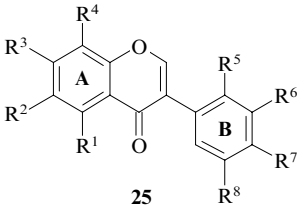


Table 9. Cytotoxicity of polyalkoxyisoflavones **25** and glaziovianin A.



25

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	IC_{50}/μmol litre^{-1} (see [a])	Ref.
25b	OH	OMe	OMe	OMe	H	H	OMe	NH_2	0% (KB) [b]	117
25c	H	OMe	OMe	H	OMe	OMe	OMe	OMe	22 (HeLa S_3)	98, 126
Glaziovianin A	H	OMe	OMe	H	OMe	OCH_2O		OMe	0.59 (HeLa S_3)	98, 126
25d	H	OMe	OH	H	OMe	OCH_2O		OMe	>100 (HeLa S_3)	98, 126
25e	H	OMe	OBn	H	OMe	OCH_2O		OMe	0.75 (HeLa S_3)	98, 126
25f	H	OMe	$OCH_2C{\equiv}CH$	H	OMe	OCH_2O		OMe	0.74 (HeLa S_3)	98, 126
25g	H	OMe	OAll	H	OMe	OCH_2O		OMe	0.19 (HeLa S_3)	98, 126
25h	H	OMe	OMe	H	H	OCH_2O		H	56.2 (HeLa S_3)	98, 126
25i	OMe	OMe	OMe	H	H	H	OMe	H	99 [c]	40
25j	H	OMe	OMe	H	H	H	H	H	91.3% (HCT116) [d]	128
25k	H	OMe	OMe	H	H	OMe	OMe	H	85.3% (HCT116) [d]	128

[a] KB, HeLa S_3 are cervical cancer, HCT116 is colon cancer; [b] inhibition of cell growth at a concentration of 1 μmol litre^{-1}; [c] the average value of IC_{50} for 60 cancer cell lines; [d] the suppression of clonogenic activity of cells at a concentration of 40 μmol litre^{-1}.

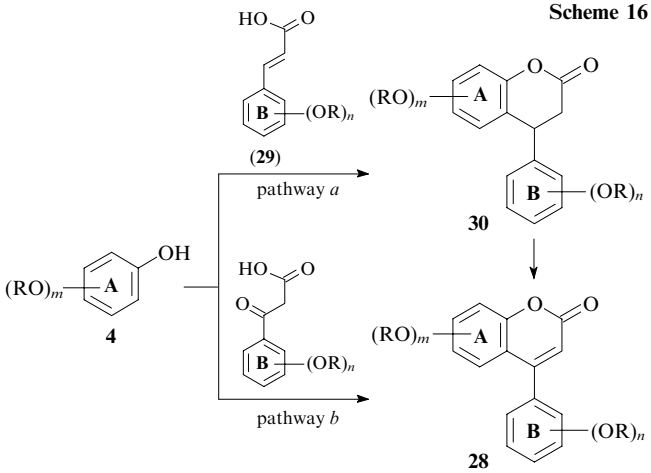
tion and migration.[129–131] Coumarins containing three or four alkoxy groups in the benzene ring **A** and the unsubstituted pyran ring are rarely found in nature.[81, 132–134] It is known that these compounds can suppress the proliferation and stimulate differentiation of U937 human leukemia cells.[130, 131]

4-Arylcoumarins were isolated from the plants of the families *Guttiferae* (garcinia), *Rubiaceae* (madder), *Leguminosae* (legumes), *Compositae* (Asteraceae), *etc.*[135]

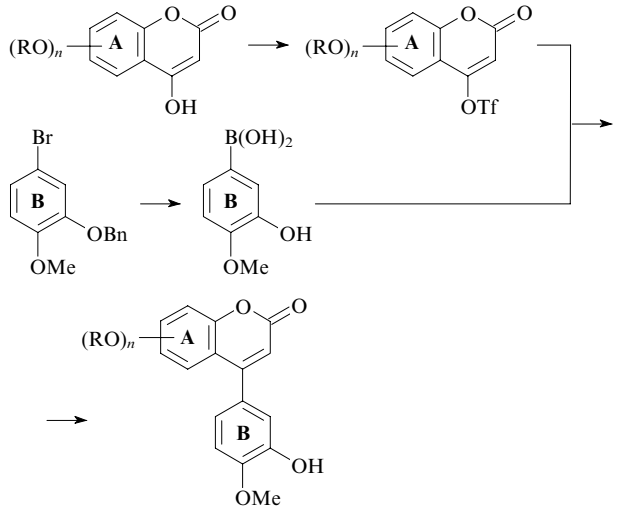
The arrangement of the benzene rings A and B in polyalkoxy-4-arylcoumarins 28 (Schemes 16 and 17) allows for the consideration of these compounds as bioisosteric analogues of CA4 and its synthetic derivative iso-CA4 — a highly active antimitotic agent with microtubule-destabilizing action (Table 10).[141, 142] 4-Arylcoumarins **28** are structurally similar to other powerful microtubule-destabilizing agents, *viz.*, oxapodophyllotoxins (oxa-PT) with pronounced cytotoxicity (see Table 10).[143] The role of the lactone ring of the PT molecule and its analogues in the antimitotic microtubule-destabilizing effect is unclear. In some cases, the replacement of the lactone ring by other heterocycles or the cyclopentane moiety,[4] as well as the cleavage of lactone by bases,[144] results in very active structures. It was reasonable to expect that the removal of the lactone ring in oxa derivatives of PT would yield promising and more synthetically accessible antimitotics.

The reactions of phenols with cinnamic acids **29** (see Scheme 16, pathway *a*) easily afford 4-aryl-3,4-dihydrocoumarins with the polyalkoxy-substituted ring **B30**.[140, 145] The latter are considered as analogues of PT devoid of the lactone ring. Analogue **30a** proved to be very active.[136] A method was developed for the synthesis of enantiomers of these compounds at the C(4) atom; however, data on the comparative biological activity are absent.[145] A method for the oxidation of 4-aryl-3,4-dihydrocoumarins **30** to the corresponding 4-arylcoumarins **28** (see Scheme 16) was reported.[140] The latter compounds

Scheme 16

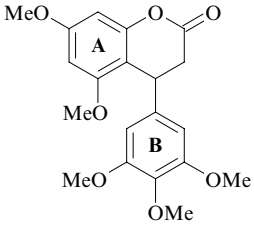


Scheme 17

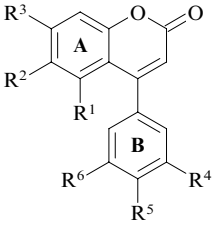


28a,**b**,**d**–**i** (83%–94%)

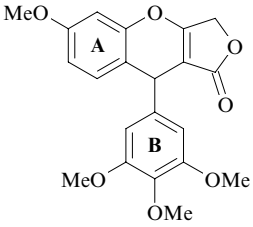
Table 10. Cytotoxicity of polymethoxy-4-arylcoumarins.



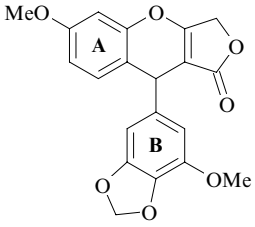
30a



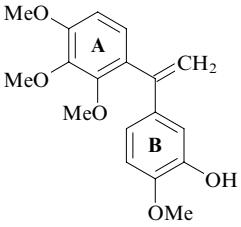
28a – u



oxa-PT I



oxa-PT II



iso-CA4

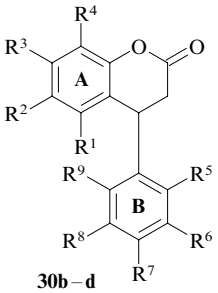
Compound	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} /μmol litre^{-1} (see [a])	Ref.
30a	OMe	H	OMe	OMe	OMe	OMe	9.4 (Caco-2)	136
							120.8 (HepG2)	136
							24.2 (KB)	136
							214.8 (MCF-7)	136
28a	OMe	H	OMe	OH	OMe	H	0.087 (HBL100)	137
							0.088 (HBL100)	138
							0.083 (CEM)	139
							0.12 (CEM)	140
28b	OMe	OMe	OMe	OH	OMe	H	0.42 (HBL100)	137
							0.433 (HBL100)	138
							0.52 (CEM)	139
28c	OMe	OMe	OMe	OMe	OH	H	inactive	137
28d	H	H	H	OH	OMe	H	0.084 (HBL100)	138
28e	OMe	H	H	OH	OMe	H	0.182 (HBL100)	138
28f	H	OMe	H	OH	OMe	H	0.039 (HBL100)	138
28g	H	H	OMe	OH	OMe	H	0.606 (HBL100)	138
							1.13 (CEM)	140
28h	OMe	OMe	H	OH	OMe	H	0.439 (HBL100)	138
28i	H	OMe	OMe	OH	OMe	H	0.714 (HBL100)	138
28j	OMe	OMe	OMe	H	OH	H	47 (CEM)	139
28k	OMe	OMe	OMe	OMe	OH	H	54 (CEM)	139
28l	OMe	OMe	OMe	OMe	OH	OMe	62 (CEM)	139
28m	OMe	OMe	OMe	OCH_2O		H	> 50 (CEM)	139
28n	OMe	H	OMe	H	OH	H	16 (CEM)	139
28o	OMe	H	OMe	OMe	OH	H	10.6 (CEM)	139
28p	OMe	H	OMe	OMe	OH	OMe	> 75 (CEM)	139
28q	H	H	OMe	H	OMe	H	> 300 (CEM)	140
28r	H	H	OH	H	OH	H	> 400 (CEM)	140
28s	H	H	OMe	OMe	OMe	OMe	> 300 (CEM)	140
28t	OMe	H	OMe	OAc	OMe	H	0.29 (CEM)	140
28u	H	H	OMe	H	H	H	73.3 (CEM)	140
CA4 (NCI ID 613729) [b]							0.0032 [c]	74
iso-CA4							0.0022 (K562)	141
							0.004 (H1299)	141
							0.0025 – 0.005 [d]	142
PT (NCI ID 24818) [b]							0.0209 (60 cell lines)	see [c]
oxa-PT I (NCI ID 760222) [b]							0.631 (60 cell lines)	143
oxa-PT II (NCI ID 758032) [b]							0.072 (60 cell lines)	143

[a] Caco-2 is colon cancer, HepG2 is hepatocellular carcinoma, KB is cervical cancer, MCF-7, HBL100 is breast cancer, CEM is lymphoblastic leukemia, K562 is myeloid leukemia, H1299 is lung carcinoma; [b] NCI ID is the identification number of the compound in the NCI database; [c] the average value of IC_{50} for 60 cancer cell lines (NCI Anti-cancer Screening Program, http://dtp.nci.nih.gov/index.html); [d] the range of values of IC_{50} for 4 human cancer cell lines.

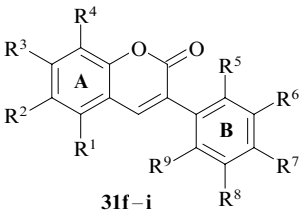
can also be synthesized in one step by the Pechmann reaction of phenols with benzoylacetic acids (see Scheme 16, pathway *b*).[82] Besides, polyalkoxy-4-arylcoumarins **28a**,**b**,**d – i** can be prepared in high yields by the Suzuki reaction (see Scheme 17).[138]

Some polyalkoxy-4-phenylcoumarins containing several methoxy and hydroxy groups in rings **A** and **B** proved to be active cytostatics (see Table 10). These compounds suppress cancer cell growth,[137 – 140, 146, 147] arrest the cell cycle at the mitotic phase (G2/M) (com-

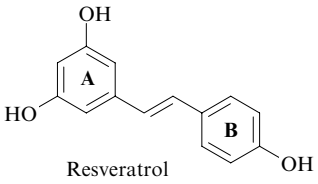
Table 11. Effect of polyalkoxy-substituted 4-aryl-3,4-dihydrocoumarins **30** and 3-arylcoumarins **31** on *P. lividus* sea urchin embryos.



30b–d



31f–i



Resveratrol

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	EC /μmol litre^{-1}			Ref.
										cleavage alteration	cleavage arrest	embryo spinning	
30b	H	H	OMe	H	H	OMe	OCH_2O		H	0.1	1 [a]	> 5	see [b]
30c	H	H	OMe	H	H	OMe	OCH_2O		OMe	> 4	> 4	> 4	see [b]
30d	H	H	OMe	H	OMe	OMe	OCH_2O		H	> 4	> 4	> 4	see [b]
oxa-PT II [c]										0.002	0.01	0.1	143
31f	OMe	OMe	OMe	H	H	H	OMe	H	H	0.2	4 [a]	> 5	57
31g	OMe	OCH_2O		H	H	H	OMe	H	H	4	> 4	> 4	57
31h	OMe	OCH_2O		OMe	H	H	OMe	H	H	1	> 4	> 4	57
31i	OCH_2O		OMe	OMe	H	H	OMe	H	H	> 4	> 4	> 4	57
Resveratrol										43.8	–	–	148

[a] Arrested eggs acquire tuberculate shape characteristic of microtubule-destabilizing agents; [b] ChemicalBlock database, www.chemblock.com; [c] the structure is shown in Table 10.

pounds **28a**,**b**,**d–f**)[137,138] and induce apoptosis (compounds **28a**,**b**,**d–f**).[137,146] The mechanism of action of these compounds involves the inhibition of tubulin polymerization and destruction of microtubules in the cells.[137–139] Interestingly, compound **28a** inhibits P-glycoprotein and the BCRP (breast cancer resistance protein), which are membrane proteins responsible for efflux of foreign compounds from the cell. Therefore, **28a** can enhance the action of chemotherapeutic agents and overcome multidrug resistance in cancer cells.[138] The presence and the mutual arrangement of OH and OMe substituents in ring **B** play an important role in the antiproliferative activity of polyalkoxy-4-phenylcoumarins. The most active compounds **28a**,**b**,**d–i**, like CA4, chalcones and flavones, contain 3-OH and 4-OMe groups in ring **B**. The rearrangement of these groups in coumarin **28c** (4-OH and 3-OMe) leads to the loss of activity. The number and arrangement of methoxy groups in ring **A** are less important. Thus, derivative **28d** with the unsubstituted ring **A** can also suppress cancer cell growth at a rather low concentration (see Table 10).[137–140,147] The cytotoxic properties of polyalkoxy-4-phenylcoumarins are weaker than those of CA4, iso-CA4, PT and oxa-PT. Thus, the full analogue of the active antimitotic compound oxa-PT I, *viz*., coumarin **28s**, has no effect on the CEM leukemia cells (see Table 10).[140]

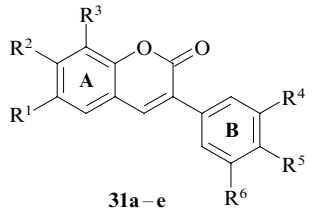
The cytotoxic properties of 4-aryl-3,4-dihydrocoumarins **30** are virtually unknown. The only characterized representative of this class of coumarins, *viz*., synthetic compound **30a** (see Table 10), suppresses human cancer cell growth at micromolar concentrations.[136] In the ZIOC RAS, 3,4-dihydro-4-arylcoumarins **30b–d** with the polyalkoxy-substituted ring **B** were synthesized based on parsley and dill extracts according to a method described in the study.[145] These compounds are structurally similar to the powerful antimitotic oxa-PT II with microtubule-destabilizing action.[143] These compounds were tested on the sea urchin embryo model (Table 11). Among these compounds, only myristicin derivative **30b** alters egg cleavage, although this effect is substantially weaker than that of oxa-PT II. The formation of tuberculate arrested eggs suggests that the antimitotic effect of compound **30b** is due to destabilization of microtubules.

XI. Polyalkoxy-3-arylcoumarins

Polyalkoxy-3-arylcoumarins **31** are bioisosteric analogues of *trans*-stilbenes, in which the 3,4-double bond of the coumarin moiety is fixed in the *trans* position by the pyran ring. The action of *trans*-stilbenes is not associated with tubulin, as opposed to natural stilbenes (combretastatins), the antimitotic microtubule-destabilizing action of which is greatly determined by the *cis* configuration of rings **A** and **B**.[5,28]

The natural *trans*-stilbene resveratrol (Table 12) occurs in the skin of the grape and other fruits. This compound exhibits antioxidant and tubulin/microtubule independent antiproliferative acitivity, arrests the cell cycle at the G0/G1 phase and induces apoptosis.[148,150] It was found that resveratrol alters the development of *Lytechinus variegatus* sea urchin embryos.[148] Polyalkoxy-3-arylcoumarins **31** were synthesized by the formylation of polyalkoxyphenols **4** followed by the Perkin condensation[151] of the latter compounds with phenylacetic acids **24** in the presence of carbonyldiimidazole (CDI) (Scheme 18).[57,149]

Table 12. Cytotoxicity of polyalkoxy-3-phenylcoumarins **31**.[149]

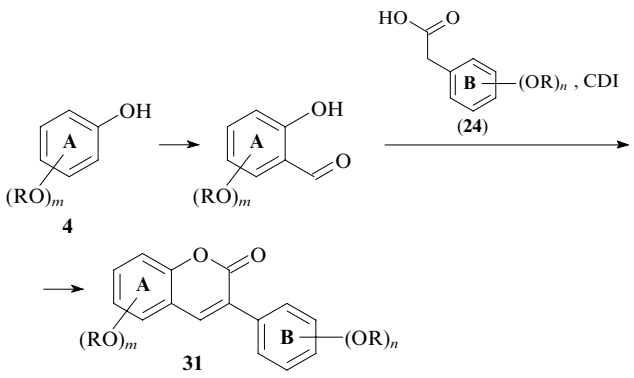


31a–e

Compound	R^1	R^2	R^3	R^4	R^5	R^6	IC_{50} /μmol litre^{-1}	
							HL-60 [a]	A549 [b]
31a	OMe	OH	H	H	OH	H	5.2	7.5
31b	OMe	OH	H	OMe	OH	H	24.3	21.4
31c	H	OH	H	OMe	OH	H	91.7	200
31d	OMe	OH	OMe	H	OH	H	25.1	45.8
31e	OMe	OH	OMe	OMe	OMe	OMe	72.2	> 200
Resveratrol (NCI ID 327430) [c]							49.8 [d]	

[a] HL-60 is promyelocytic leukemia; [b] A549 is lung carcinoma; [c] the structure is shown in Table 11 (the identification number of the compound in the NCI database is given in parentheses); [d] the average value of IC_{50} for 60 human cancer cell lines (NCI Anti-cancer Screening Programme; http://dtp.nci.nih.gov/index.html).

Scheme 18



Some polymethoxy-3-phenylcoumarins inhibit the growth of HL-60 human promyelocytic leukemia cells and A549 human lung carcinoma,[149] with compound **31a** being more active than resveratrol (see Table 12). Unlike resveratrol, coumarin **31a** arrests the cell cycle at the mitotic phase (G2/M), suggesting another mode of action for this compound. Experiments in HL-60 and A549 cells showed that the methoxy group as R^1 in ring A is essential for the cytotoxicity of polymethoxy-3-phenylcoumarins, whereas an increase in the number of methoxy groups in the ring **B** leads to a decrease in the effect (see Table 12).[149]

The antimitotic properties of polyalkoxy-3-(4-methoxyphenyl)coumarins **31f–i** were studied using *P. lividus* sea urchin embryo model.[57] Compounds **31f–h** alter the embryonic development at much lower concentrations compared to resveratrol (see Table 11). Among them coumarin **31f** containing three methoxy groups exhibits considerable antiproliferative activity which is likely related to destabilization of microtubules, since it causes the formation of tuberculate arrested eggs. This compomund inhibits cancer cell growth with IC_{50} = 3.98 μmol litre^{-1} (the average value for 60 cell lines).[57]

XII. Conclusion

Polyalkoxyflavonoids occur widely in nature, have various biological activities and are potent antioxidants. New biologically active flavonoids are isolated and identified every year from the previously unstudied plant species, particularly those applied in traditional medicine of different countries. Many of these compounds exhibit cytotoxicity against malignant cells. Polyalkoxyflavonoids can suppress cancer cell growth by interaction with different cellular targets. The most active representatives of this class inhibit cancer cell growth through the specific binding to tubulin/microtubules in the colchicine site, resulting in the damage of the mitotic spindle structure and function and in the cell division arrest followed by the induction of apoptosis — programmed cell death. Studies of the structure–activity relationships showed that the pharmacophore groups in various polyalkoxyflavonoids responsible for antimitotic microtubule-destabilizing activity are identical to those in combretastatin A4: the 3,4,5-trimethoxy-substituted benzene ring **A** and the 3-hydroxy-4-methoxy-substituted ring **B** (Fig. 8). Polyalkoxyflavonoids (tangeretin, 4-arylcoumarin **28a**) not only display microtubule-destabilizing activity but also inhibit membrane protein transporters responsible for efflux of foreign compounds from the cell. Therefore, these compounds are promising drug candidates for the application in complex cancer chemotherapy, particularly against multidrug resistant malignancies.

Although the antiproliferative effect of polyalkoxyflavonoids is often weaker compared to other natural mitotic poisons (colchicine, podophyllotoxin, combretastatins), they can be considered as promising compounds for the design of new anticancer agents. These compounds abundant in fruits and vegetables, are involved in diets, and their metabolic products are non-harmful for humans. Plant wastes from food and perfume industries, which are often disposed or used as cattle feed, can serve as available and cheap sources of flavonoids. There are relatively facile

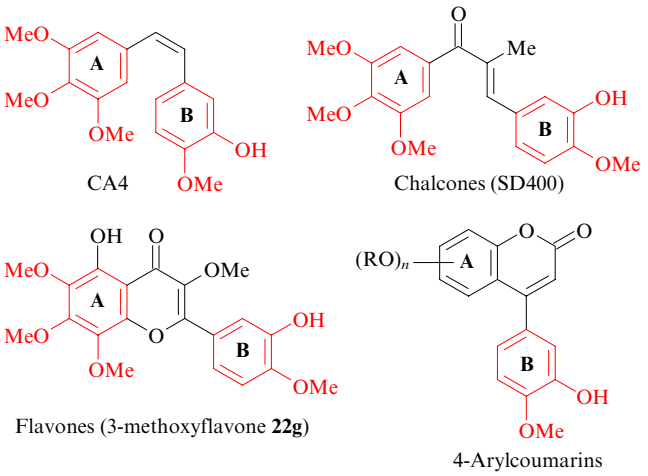


Figure 8. Pharmacophore groups of polymethoxyflavonoids responsible for antimitotic microtubule-destabilizing effect.

synthetic methods for the preparation of natural polyalkoxyflavonoids and their analogues, in particular, methods employing industrial CO_2 extracts of parsley and dill seeds as the starting material. The total synthesis of flavonoids is usually more simple compared to known natural colchicinoids. Due to the widespread occurrence of natural flavonoids and their structural diversity, it is possible to choose an appropriate skeleton to synthesize a series of analogues for further structure–activity relationship study and revealing antimitotics with a wide therapeutic window that best meet the requirements of medicine.

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