Bioorg. Med. Chem. 109 (2024) 117791

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Review article

Bioisosteric heterocyclic analogues of natural bioactive flavonoids by scaffold-hopping approaches: State-of-the-art and perspectives in medicinal chemistry

Gabriele La Monica, Alessia Bono, Federica Alamia, Antonino Lauria, Annamaria Martorana *

Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, University of Palermo, Viale delle Scienze, Ed. 17, I-90128 Palermo, Italy

ARTICLE INFO

Keywords:
Natural flavonoid
Small molecules
Heterocycles
Scaffold-hopping
Bioisosteric substitution
Biological activity
SAR
Lead optimization process
Drug discovery

ABSTRACT

The flavonoid family is a set of well-known bioactive natural molecules, with a wide range of potential therapeutic applications. Despite the promising results obtained in preliminary in vitro/vivo studies, their pharmacokinetic and pharmacodynamic profiles are severely compromised by chemical instability. To address this issue, the scaffold-hopping approach is a promising strategy for the structural optimization of natural leads to discover more potent analogues.

In this scenario, this Perspective provides a critical analysis on how the replacement of the chromon-4-one flavonoid core with other bioisosteric nitrogen/sulphur heterocycles might affect the chemical, pharmaceutical and biological properties of the resulting new chemical entities. The investigated derivatives were classified on the basis of their biological activity and potential therapeutic indications. For each session, the target(s), the specific mechanism of action, if available, and the key pharmacophoric moieties were highlighted, as revealed by X-ray crystal structures and in silico structure-based studies. Biological activity data, in vitro/vivo studies, were examined: a particular focus was given on the improvements observed with the new heterocyclic analogues compared to the natural flavonoids. This overview of the scaffold-hopping advantages in flavonoid compounds is of great interest to the medicinal chemistry community to better exploit the vast potential of these natural molecules and to identify new bioactive molecules.

1. Introduction

Natural products and their analogues have contributed significantly to the discovery of new molecules in medicinal chemistry. Due to their wide range of biological activities, scaffold diversity and structural enantioselectivity, they represent one of the most fruitful and rich sources for drug development.[1,2] In this context, the flavonoid family (including flavones, flavonols, flavanonols, flavanones, anthocyanines and isoflavonoids, general structure in Figure 1) is probably one of the most investigated classes of natural biologically active compounds. With

Abbreviations: AD, Alzheimer disease; ALL, acute lymphoblastic leukemia; ALR, aldose reductase; AML, acute myeloid leukemia; AMR, antimicrobial resistance; ANF, α-naphthoflavone; ARHI, age-related hearing impairment; BBB, blood brain barrier; CC_{50}, half-maximal cytotoxic concentration; CDK, cyclin dependent kinase; COX, cyclooxygenase; CNS, central nervous system; CPX, ciprofloxacin; CYP, cytochrome; EC_{50}, half-maximal effective concentration; EGFR, epidermal growth factor receptor; EPI, efflux pump inhibitor; ER, estrogen receptor; GABA, gamma-amino butyric acid; GI_{50}, half-maximal growth inhibitory concentration; HGF, hepatocyte growth factor; HIV, human immunodeficiency virus; HRV, human rhinovirus; hTopIIα, human topoisomerase II α; HUVEC, human umbilical vein endothelial cells; IC_{50}, half-maximal inhibitory concentration; IFD, induced fit docking studies; IGF-1R, insulin-like growth factor receptor-1; LOX, lipoxygenase; LPS, lipopolysaccharide; MDR, multidrug resistance; MIC, minimum inhibitory concentration; MMP, matrix metalloprotease; MPC, minimum potentiating concentration; M^{pro}, main protease; MRSA, methicillin-resistant staphylococcus aureus; NA, neuraminidase; NCI, National Cancer Institute; NTM, nontuberculous mycobacteria; NO, nitric oxide; NSCLC, non-small cell lung cancer; PD, Parkinson's disease; PDB, Protein Data Bank; P-gp, glycoprotein P; PK, pharmacokinetics; pK_d, dissociation constant; PPAR, pan-peroxisome proliferator activated receptors; PTX, paclitaxel; ROS, reactive oxygen species; SAR, structure-activity relationship; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SuMD, supervised molecular dynamic simulation; TBNC, triple-negative breast cancer; TNF-α, tumor necrosis factor α; TP, thromboxane prostanoid receptor; TPSA, total polar surface area; TRAP1, TNF receptor associated protein 1; TrkA, Tropomyosin Receptor kinase A; VEGF, vascular endothelial growth factor.

* Corresponding author.
E-mail address: annamamaria.martorana@unipa.it (A. Martorana).

https://doi.org/10.1016/j.bmc.2024.117791
Received 7 March 2024; Received in revised form 13 May 2024; Accepted 5 June 2024
Available online 8 June 2024

their peculiar polyphenolic chromone scaffold, they exert a broad spectrum of biological activities and modulate physio/pathological processes related to cancer, inflammation, infection, oxidative stress, metabolic and neurological diseases.[3–5]

Despite the promising results obtained in preliminary in vitro/vivo studies, several physicochemical, pharmacokinetic, and dynamic issues prevented these compounds from moving into the more advanced phases of the drug discovery process. The scarce solubility in physiological medium and the poor cell permeability are responsible for an insufficient intestinal absorption, thus deficient oral bioavailability.[6–9] The presence of the endocyclic oxygen as well as free aromatic hydroxyl/alkoxy groups determines low metabolic stability, due to the rapid oxidation and conjugation in the intestine and liver. In addition, regarding the pharmacodynamic challenges, the high target promiscuity (off-target interactions) is the cause of the limited selectivity of action, frequently detected for many flavonoid compounds.[6–9].

Against this background, many attempts have been made to improve these properties, to obtain more stable compounds, with more chance to reach clinical trials. In addition to several studies in the field of drug delivery and carrier systems,[6,10-14] numerous medicinal chemistry papers have been published in the literature dealing with the decoration of the central phenylchromen-4-one core. O-glycosylation, O-alkylation/acylation, introduction of hydrophilic base chains, chelation with transition metals, modification of the carbonyl group (imine formation, selenation, sulfuration) are just a few examples of the approaches pursued in the last decades, and several published reviews have extensively analyzed these chemical modifications.[5,15-18].

On the other hand, an interesting approach to lead-optimization is the scaffold-hopping, also known as lead hopping, which consists of the modification/replacement of the core of an existing bioactive molecule with a bioisosteric one similar in size to create a new compound with comparable/improved features in a novel chemical space. The potential of the scaffold-hopping approaches in drug discovery process is well established in the literature, and their application on the optimization of bioactive natural products represents an invaluable tool to obtain more effective molecules, that are easier to synthetize and free from patent issues.[1,19-22] Patenting of natural products is often prohibited by legal frameworks; instead, intellectual property can be claimed for scaffold-hopped analogues whose structure is reasonably different from that of the natural lead.[1,19-22].

Despite this, there has been no critical analysis of how these lead compound optimization strategies might affect the physicochemical, pharmacodynamic and kinetic properties (the so-called P3 properties) of natural flavonoids.

In the proposed study, we aim to provide an analytical perspective on how the replacement of the chromon-4-one flavonoid core by other bioisosteric heterocycles could affect the chemical, pharmaceutical and biological properties of the newly obtained chemical entities. In this review the attention will be focused on nitrogen and, to a lesser extent, sulfur heteroaromatic cores, which resulted in the most fruitful and significative examples of scaffold-hopping on natural flavonoids. In detail, in Figure 2 the chemical structure of all these heterocyclic scaffolds is reported, in comparison to the natural chromone one. Bicyclic aza/thioflavonoids, such as quinolone, quinolinone, quinoline, thiochromenone and thiochromanone represent the simplest examples of scaffold-hopping, where the O1 is replaced by the corresponding heteroatom (N o S). In addition, regarding the nitrogen systems, several relevant examples of diaza bioisosteric scaffolds, where a second nitrogen is introduced, need to be mentioned: quinazolinone, dihydroquinazolinone, naphthyridinone, pyrido[1,2-*a*]pyrimidinone, quinazoline.

Furthermore, according to their biological activity, the studied compounds are divided into different classes, such as antibiotic, anticancer, antiviral, antioxidant, anti-inflammatory, and neuroprotective agents, as analyzed in detail in the following sections.

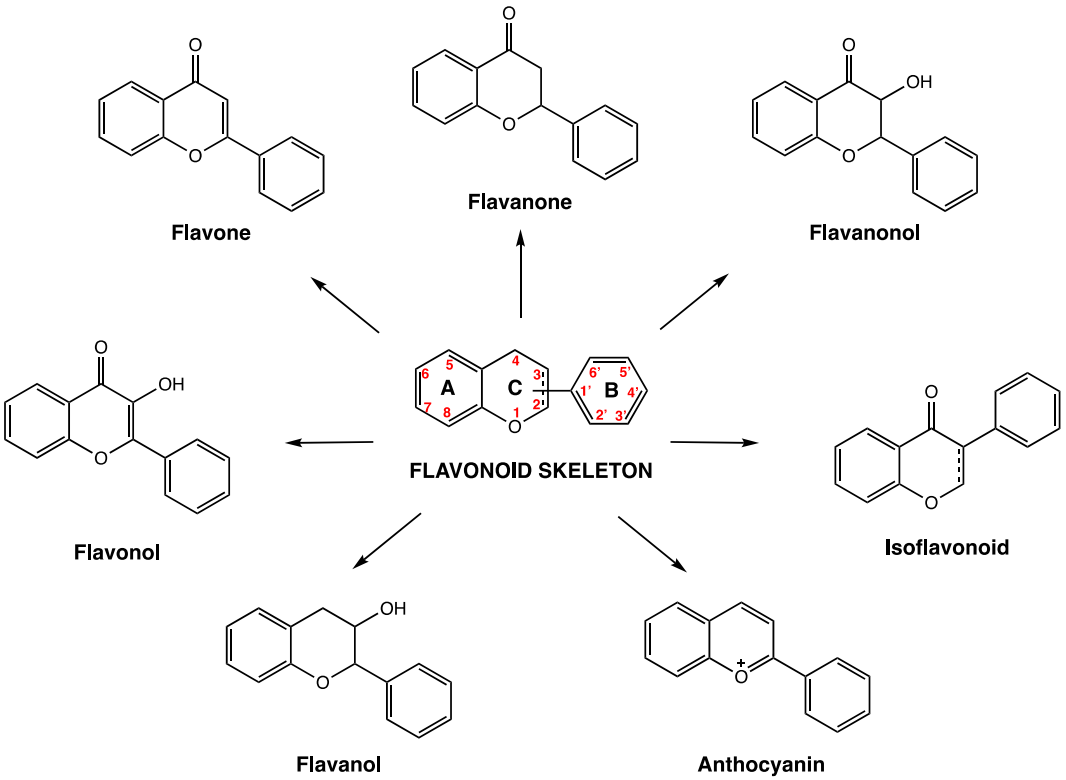


Figure 1. General skeleton of the class of natural bioflavonoids and the most important subclasses.

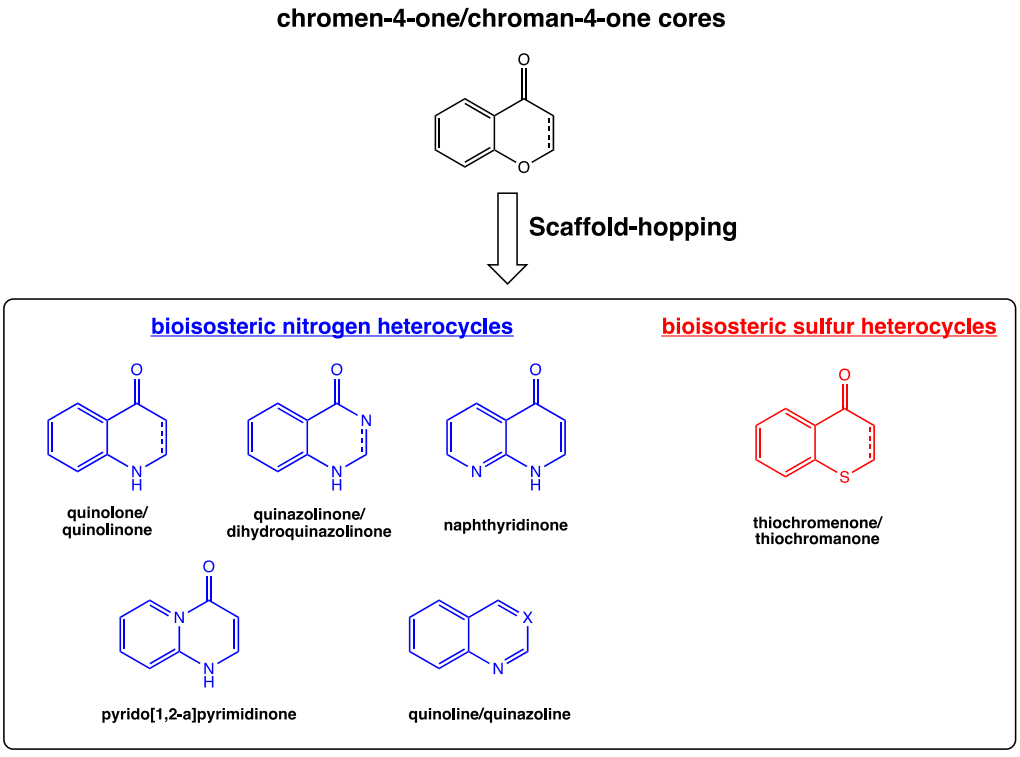


Figure 2. Chemical structures of chromones, the central cores of flavonoid compounds, and of the related bioisosteric nitrogen and sulfur heterocycles herein discussed.

2. Bioisosteric heterocyclic analogues of flavonoids with biological activity by scaffold-hopping approach

2.1. Antibiotic potentiating activity

Many natural and semisynthetic flavonoid derivatives have been found to block the bacterial signaling pathways responsible for resistance, offering potential application in addressing the emerging challenge of antimicrobial resistance (AMR). In particular, they act as antibiotic potentiators/adjuvants by restoring the chemosensitivity of multidrug-resistant bacterial strains (e.g.: methicillin-resistant *staphylococcus aureus,* MRSA; *pseudomonas aeruginosa*; *Escherichia coli*; *non-tuberculous mycobacteria*, NTM) to known classes of antibacterial drugs.[23,24]

Inhibition of bacterial efflux pumps (EP) is one of the most recurrent mechanisms of action to tackle the development and spread of AMR. Indeed, flavonoid compounds exhibited interesting inhibitory activity against NorA and MAV/MAC efflux pumps, which are overexpressed in the high resistant bacterial strains MRSA and NTM, respectively.[25–28].

Considering the outstanding results obtained in the field of benzo-fused heterocyclic antibacterial agents (e.g.: quinolones,[29] quinazolones,[29] quinolines,[30] sulfur-containing bicyclic scaffolds),[31] scaffold-hopping approaches for the bicyclic structure of flavonoids have been pursued to improve their performance as efflux pump inhibitors and thus as antibiotic potentiators.

2.1.1. Flavonoid derivatives with inhibitory effect on NorA efflux pump

Several flavonoid compounds have aroused the interest of the researchers for their capability to inhibit NorA efflux pump and increase the antibacterial effect of known antibiotics against MRSA. For example, compound **1**, with a 2-phenyl-4*H*-chromen-4-one (Figure 3), emerged from an in vitro screening of natural and semisynthetic products for its capability to potentiate the activity of berberine, an antibacterial alkaloid, against MRSA (MIC, i.e. minimum inhibitory concentration, of 0.4 μg/mL with subinhibitory concentration of berberine). As evidenced by a preliminary structure–activity relationship (SAR) analysis, the presence of a lipophilic alkyl substituent on the 2-phenyl ring (as OPr) was a leitmotiv in the most active derivatives of this series.[32]

In the light of these results, Sabatini and co-workers pursued a scaffold-hopping approach as lead-optimization strategy on **1**, in order

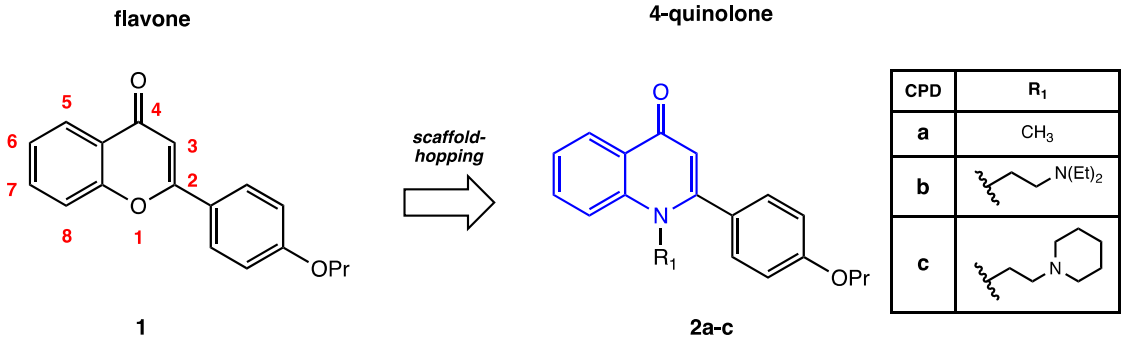


Figure 3. Scaffold-hopping from the flavone **1** to the 4-quinolone derivatives **2a-c** in the design of more effective NorA inhibitors.

to investigate whether the switch from the central chromone core to other heterocycles was ameliorative for the biological activity.[33] In particular, the bioisosteric replacement of the endocyclic O1 with a nitrogen atom was the most interesting and successful substitution and led to the heterocyclic analogues 2-(4-propoxyphenyl)-quinol-4-ones **2a-c** (Figure 3). Efflux pump inhibitory (*EPI*) activity was measured on the MRSA strain SA-1199B (which overexpresses the efflux pump NorA) by assessing the capability of the compounds to inhibit ethidium bromide (EtBr, a NorA substrate) efflux at a concentration of 50 μM. The analysis of the data underscores the relevance of the O substitution by an N atom: compound **2a**, indeed, showed a level of activity slightly higher than the reference flavonoid **1**, with *EPI* values of 33.9 % and 32.4 %, respectively; on the other hand, compounds **2b,c**, with N1-alkylamine side chain showed a much better inhibitory activity than the parent flavonoid, with *EPI* values of 57.3 % and 75.1 %, respectively.[33] From this first series of compounds, it emerged as the scaffold-hopping dramatically enhanced the NorA inhibitory activities of the new compounds. In particular, the possibility of a SAR exploration in 1 position, which was not feasible for the flavonoid lead, guaranteed an appropriate combination of both hydrophobic and electrostatic effects, affording compounds with a 2/3-fold increase in *EPI* inhibitory activity. However, none of these compounds exhibited a noteworthy synergism with common antibiotics substrates of NorA, such as the quinolone ciprofloxacin (CPX).

For these reasons, these preliminary insights served as the basis for an extensive SAR exploration on both the quinolone core and the side substituents aiming at exploiting better the *EPI* properties of this class. A turning point in the lead-optimization process, of which the most significative steps are herein briefly discussed, was represented by the switching of the protonable alkylamine side chain from N1 to C4 position. This further structural refinement led to O-substituted 2-phenyl-4-ethoxyquinoline series of compounds, where the quinolone core was replaced by the bioisosteric quinoline ring (iterative scaffold-hopping strategy), as reported in Table 1.33 Firstly, derivative **3a** (Table 1) with an aminoethoxy side moiety at the C4 resulted the most interesting, exhibiting a threefold increase in inhibition of the EtBr efflux (93.4 %) compared with flavonoid **1** and the aza-analogues **2a-c**, an IC_{50} value in the range of 7–10 μM and no appreciable antibacterial activity of its own was detected (MIC > 100, ideally *EPI* should have no direct antibacterial effect). Considering these promising results, the synergism with CPX was measured by determining the *n*-fold minimum potentiating concentration (MPC_n), i.e. the minimum concertation of efflux pump inhibitor capable of restoring *n*-time the sensitivity of a given bacterial strain to an antibiotic to which it is resistant. In this regard, it was capable to restore, in a concentration-dependent manner, the susceptibility of the NorA-overexpressing MRSA strain SA-1199B (norA++/A116E GrlA) tested to the quinolone antibiotic ciprofloxacin (CPX), with MPC_{16} = 3.13 μg/mL.[33]

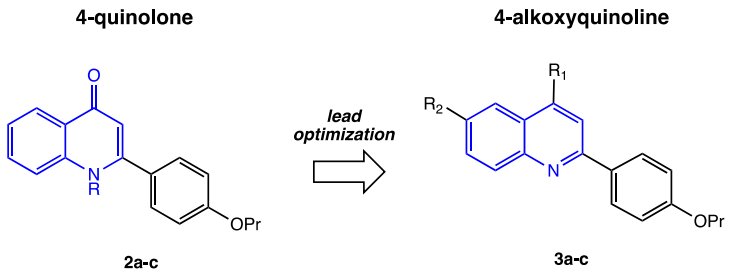
These observations guided the subsequent extensive SAR studies performed by the same authors on the **3a** structure. In particular, they observed the variation of biological activity as a result of the structural modification at C4 and C6 positions, while the 2-(4-propoxyphenyl)-4-ethoxyquinoline remained unchanged. A ligand-based pharmacophore modeling approach was applied to screen a variety of alkylamines at the C4 position. Compounds **3b,** with a 2′-piperazinyl portion showed comparable *EPI* potency to the parent compounds and also a strong synergism with CPX by reducing CPX MIC 16-fold (Table 1).[34]

Even the C6 position was extensively studied for possible structural modifications.[35–37] These efforts led to compounds **3c** (Table 1) with improved *EPI* activity and generally more favorable toxicological and PK profiles compared to the derivatives without C6 substitution. In detail, compound **3c**, with a 6-OCH_3 showed: a potent and selective inhibition of NorA efflux pump, without any nonspecific depolarizing effect on bacterial membrane (inhibition of EtBr efflux of 97.6 %); a marked synergism with CPX against SA-1199B (MPC_8 = 0.78 μg/mL); no-toxicity on human cells (CC_{50} significantly higher than the concentration required to synergize with CPX) and a favorable PK profile (good metabolic stability and lack of inhibition of cytochromes).[35]

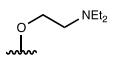
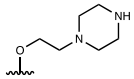
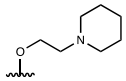
In general, from a structural point of view, the quinolone/quinoline cores, a protonable nitrogen at the C2′ position of the side chain and the propoxy group (OPr) at the C4′ position of the 2-phenyl ring are essential for maintaining the biological activity.

Recently, thanks to the publication of the first two crystallographic 3D structures of the NorA efflux pump (PDB codes 7LO7 and 7LO8),[38] structured-based molecular docking studies and supervised molecular dynamic simulations (SuMD) have been performed for the most representative compounds of these series, to gain insight into the possible binding mechanism and interactions at the catalytic site and to provide

Table 1
Chemical structure, EtBr Efflux Inhibition (%), NorA IC_{50} and MPC_n values measured on *Staphylococcus Aureus* strains SA-1199B (norA++/A116E GrlA) for the optimized 4-alkoxyquinolines **3a-c**.



2a-c **3a-c**

CPD	R_1	R_2	EtBr efflux inh. at 50 μM (%)	IC_{50} (μM)	MPC_n CPX MIC (μg/mL) §	Ref.
3a		H	93.4	8.9	MPC_{16} = 3.13	33
3b		H	93.4	8.3	MPC_{16} = 3.13	34
3c		OMe	97.6	4.2	MPC_8 = 0.78	35

§*n*-fold Minimum Potentiating Concentration (MPC_n) of the CFX MIC (synergism with CPX).

valuable information for the design of more effective molecules. Figure 4 shows the 3D structure of NorA and the 2D protein–ligand interaction diagram of the complex with **3c** as a representative example for the entire series. The remarkable biological activity of **3c** is closely related to the extensive and robust network of interactions in the binding site, which was maintained throughout the simulation period. In particular, the hydrophobic aromatic 4′-propoxyphenyl moiety (derived from lead compound **1**) is deeply inserted into the inner hydrophobic part of the channel where it forms an H-bond with the crucial Asn^{340} and is surrounded by many hydrophobic residues (Thr^{336}, Thr^{113}, Met^{109}, Ile^{136} and Phe^{16}); the central quinoline core forms π − π and hydrophobic interactions with Phe^{140} and Ile^{19}; the protonated piperidine portion, projected toward the outer part of the channel, turned out to be one of the most important pharmacophoric moieties, being involved in many contacts with pivotal residues (salt bridges with Asp^{307} and Glu^{222}; H-bond with Glu^{222}; π-cation bond with Phe^{303}).[37,39]

2.1.2. Flavonoid derivatives with inhibitory effect on MAV/MAC efflux pump

In addition to the NorA inhibitory activity on MRSA, the 2-phenylquinoline compound **3a** (Table 1) also showed promising inhibitory activity on NTM strains with overexpressed MAV/MAC efflux pumps (*mycobacterium smegmatis* and *mycobacterium avium*) and promising synergism with macrolide antibiotics (clarithromycin, erythromycin, azithromycin, which are used in clinics to treat NTM infections) against NTM macrolide-resistant strains. These results suggest similar pharmacophoric requirements for inhibition of the NorA and MAV/MAC efflux pumps.[40]

With the aim of identifying new and more active analogues with a selective *EPI* for NTM therapy, the scaffold-hopping strategy was applied to the isoflavone derivative biochanin A, which has been described in the literature as EP inhibitor of *Mycobacterium smegmatis*,[41] (Figure 5). Compounds **4a,b** were then designed by switching from the isoflavone core to the bioisosteric 3-phenylquinolone core, taking into account the SAR information extrapolated from the 2-phenylquinolone series described above: removal of the C6 and C7 hydroxyl groups at the central core; replacement of the 4′-OMe group by and OPr at the C3 phenyl moiety; introduction of an alkylamino chain at N1 (ethyl-piperidine and ethyl-azepane, respectively). Considering the outstanding biological results on both wild-type and multidrug resistant *Mycobacterium avium* strains overexpressing efflux pumps, the 3-phenylquinolone appeared to be a suitable substitute for the natural isoflavone scaffold for future development.[42] Indeed, compared with the natural parent compound, quinolones **4a,b** showed: stronger efflux inhibitory activity, broader *EPI* activity, stronger synergism with macrolides and fluoroquinolones, with a 4 to 128 fold decrease in their MIC values, and a similar cytotoxicity profile to the natural reference compound. However, since NTM are intracellular infections, low toxicity to infected human cells, especially monocyte-derived macrophages, is a requirement for the development of new *EPI* inhibitors against NTM.[42] With the aim of reducing the still unacceptable toxicity of **4a,b**, an in silico supported optimization process was performed, and compound **4c**, with an iPr group at the C6 and an N,*N*-diethyl-amino-ethyl chain exhibited an higher tolerability and selectivity, i.e., increased capability to synergize with macrolide antibiotics, especially clarithromycin, at concentrations 6-fold lower than its CC_{50}, and higher activity also in *ex vivo* models of *M. avium* 104-infected human monocyte-derived macrophages.[43]

2.1.3. Antibiotic potentiators: An overview of the SAR studies on aza-flavonoids

Given that the aza-analogues above discussed exhibited better antibiotic potentiating properties than the parent flavonoid compounds, in this paragraph and in Figure 6 an overview of the SAR on scaffold-hopped analogues is provided. In particular, it emerged as the position of the side phenyl group is strictly related to the selectivity against a particular efflux pump and bacteria strain: *C*2-phenyl derivatives (2-phenylquinolones/quinolines, i.e. aza-flavones) were selective against NorA, thus useful against MRSA infections; on the other hand C3-phenyl derivatives (3-phenylquinolones, i.e.aza-isoflavones) exhibited a selective inhibitory activity against MAV/MAC pump, thus to be used in the treatment of NMT. In both series the presence of an alkylamine/alkyl

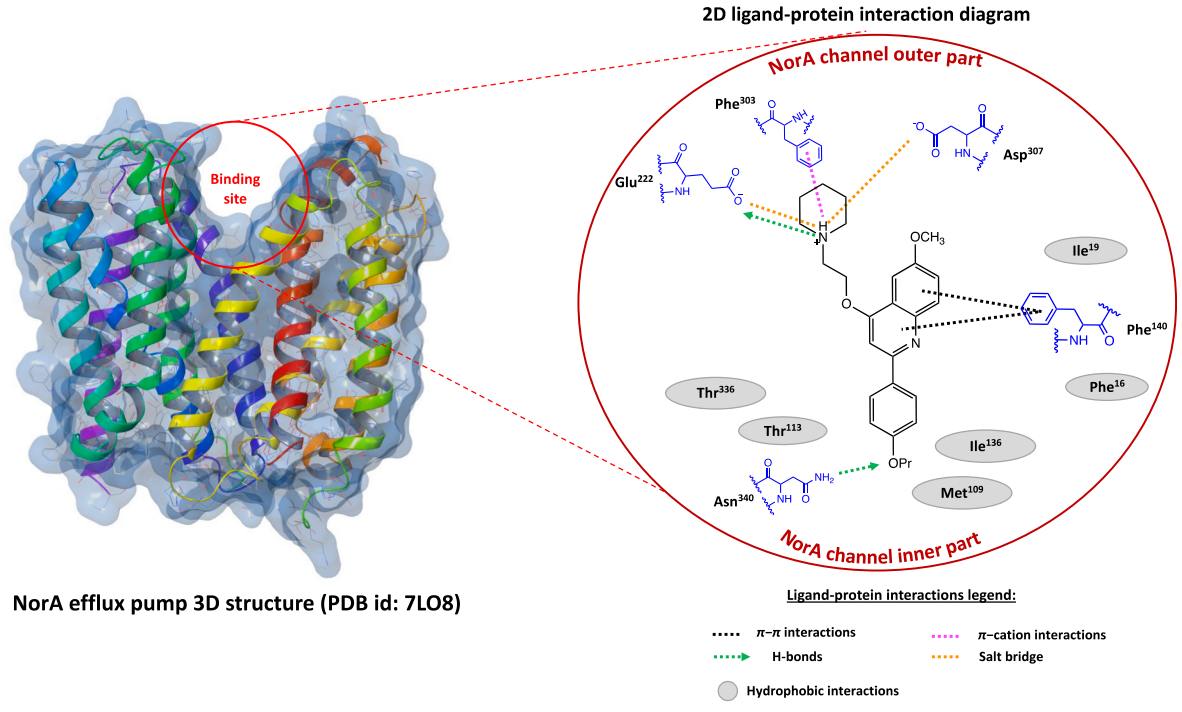


Figure 4. 3D cryo-EM structure of the NorA efflux pump of *Staphylococcus aureus* (PDB id: 7LO8 [38]) with a particular focus on the ligand binding site (left). The right box shows the 2D ligand–protein interaction diagram of NorA in complex with the 2-phenylquinoline derivative **3c**, highlighting the crucial interacting amino acids.

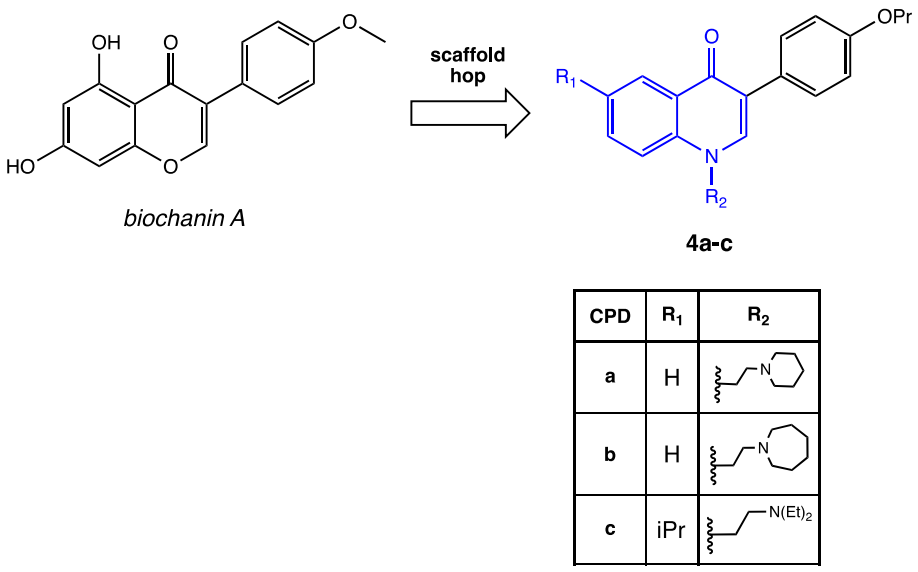


Figure 5. Aza-isoflavone compounds **4a-c** with *EPI* activity against NTM obtained by the scaffold-hopping strategy applied to the isoflavone biochanin A.

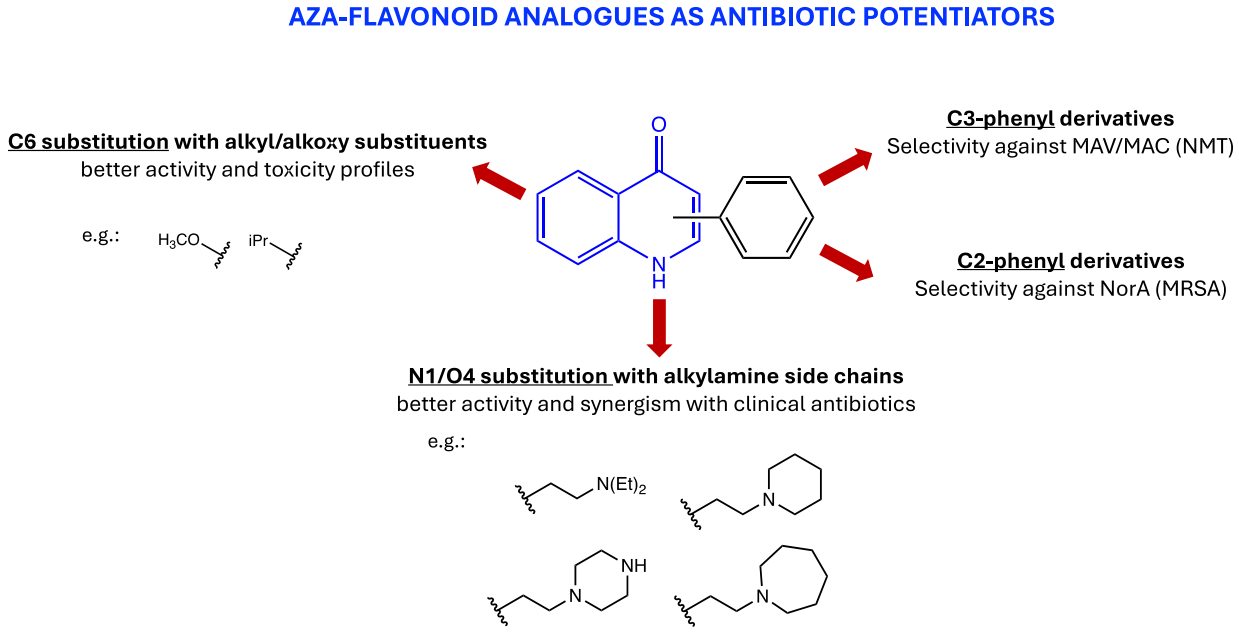


Figure 6. Overview of the SAR studies conducted on the aza-flavonoid analogues antibiotic potentiators.

side chain at N1/C6 or O4 positions was a key feature in the most active compound of the series.

2.2. Anticancer activity

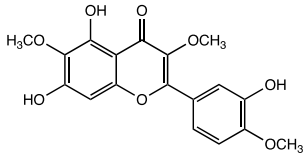
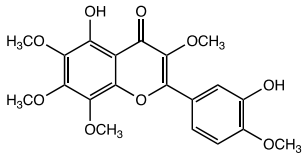
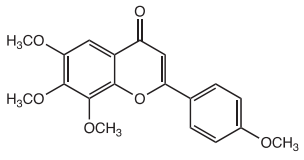
Flavonoids have been shown to possess a variety of anticancer effects against different types of human tumors.[44,45] The bioisosteric substitution of the chromone flavonoid scaffold has been particularly successful in optimizing the anticancer activity, affording compounds with both increased potency and selectivity.

2.2.1. Anti-tubulin activity

Several natural flavonoids have been identified and studied in the last decades for their capability to interfere with the microtubule assembly during the cell division. In particular, centaureidin and analogues **5a,b** (Table 2), with of poly-methoxylated flavonoid framework, attracted the attention of the researchers working in this field, since they showed encouraging antiproliferative activities in the micromolar range against a wide range of cancer cells as well as promising tubulin polymerization inhibitory activities.[46–49] In Table 2 the chemical structure and the preliminary biological results regarding both the antiproliferative activity and the inhibition of tubulin polymerization are reported for comparison with the aza-analogues discussed below.

Despite these interesting biological results, none of these compounds was deemed suitable to progress to advanced phases of drug development. On the other hand, they appeared an interesting starting point for lead optimization processes, especially through scaffold-hopping approaches. Indeed, in recent decades, many efforts have been directed towards the synthesis of aza-flavonoid analogues with improved antimitotic activity in comparison with the flavonoid leads of Table 2 and some of them reached also preclinical trials. In this section we give a brief overview of the most successful and recent results in this field.

Table 2
Chemical structure and biological activities of several natural flavonoid as antitubulin polymerization inhibitors.

CPD	Antiproliferative activity (mean IC_{50}, μM)	ITP (IC_{50})*	ITP %*	Ref.
 *centaureidin*	0.24	2.0	35	46–49
 **5a**	0.13	0.8	59	46–49
 **5b**	1.70	3.0	43	46–49

*ITP: inhibition of tubulin polymerization expressed as IC_{50} (μM) or as percent inhibition of colchicine binding at 5 μM.

Among the most representative studies, those carried out by Kuo, Hamel and co-workers represent undoubtedly a milestone in this area. They investigated a variety of modifications of the flavone nucleus and obtained very effective aza-flavonoid derivatives: 4-quinolones,[50–52] tetrahydro-4-quinolones,[53] 4-quinazolinones,[54] naphthyridinones.[55].

CHM-1, a 2-phenyl-4-quinolone derivative in which the hydroxy substituents are protected by a 6,7-methylenedioxy unit (Figure 7), is one of the most promising and studied compounds of the quinolone series. Preliminary in vitro biological screening showed remarkable antiproliferative activity, especially against colon, central nervous system (CNS), and small-cell lung NCI cancer cell lines (IC_{50} in the low-micromolar/nanomolar range against NCI-H226, HCT-116, OVCAR-3, SK-MEL-5, SF-268, SF-295, better than the flavonoid references in Table 2), and potent inhibitory effect on tubulin polymerization (IC_{50} = 0.85 μM), comparable to that of well-known inhibitors (colchicine, combretastatin A-4).[56] Due to its low solubility, the monosodium phosphate of CHM-1 was synthetized as a prodrug (phosphate group linked to the 4-O position of a quinoline), which exhibited increased bioavailability and retained potent antiproliferative and anti-tubulin activity.[57] Further studies on the potential applications of CHM-1 and its prodrug in the therapeutic treatment of cancer, demonstrated significant antiproliferative effects: 1) anti-invasive properties against hepatocellular carcinoma cells by inhibiting hepatocyte growth factor (HGF)/c-Met signaling and matrix metalloprotease 9 (MMP-9), and consequent mitotic arrest and apoptosis via a caspase-independent pathway;[58,59] 2) anti-breast cancer effect via inducing the expression of SIRT-2, a tubulin deacetylase, and of the mitotic checkpoint BubR1;[60] 3) in vitro and in vivo antitumor effects against CT-26 murine colorectal adenocarcinoma cells;[61] 4) strong anti-neovascularization effect in vitro and in vivo by inducing p53 up-regulation and apoptosis;[62] 5) anticancer effects in leukemia, ovarian cancer and glioblastoma multiforme xenograft mice models.[63–65]

Lead optimization processes of CHM-1 led to CVM-1125 and its monophosphate prodrug CVM-1128 (foslinanib, Figure 7), which also showed interesting antiproliferative and anti-tubulin activities, promoted apoptosis, and inhibited IGF-1R tyrosine kinase receptor and TRAP1.[66,67] In particular, CVM-1118 reached phase II clinical trials where it was evaluated against neuroendocrine tumors and hepatocellular carcinoma in combined treatment with sorafenib.[68,69]

Another promising compound developed by Kuo and coworkers is CSC-3436, in which the flavonoid scaffold was replaced by a 2-arylnaphthyridin-4-one one (Figure 7). This compound showed excellent results in preliminary in vitro antiproliferative assays, with a mean IC_{50} value in the low micromolar range against the NCI60 panel, especially against the highly tumorigenic cells K562 and SR (leukemia), NCI-522 (non-small cell lung cancer, NSCLC), SW-620 (colon cancer), M14 and MDA-MB-435 (melanoma). Mechanistic studies performed on hepatocellular carcinoma and NSCLC models evidenced the capability of CSC-3436 to

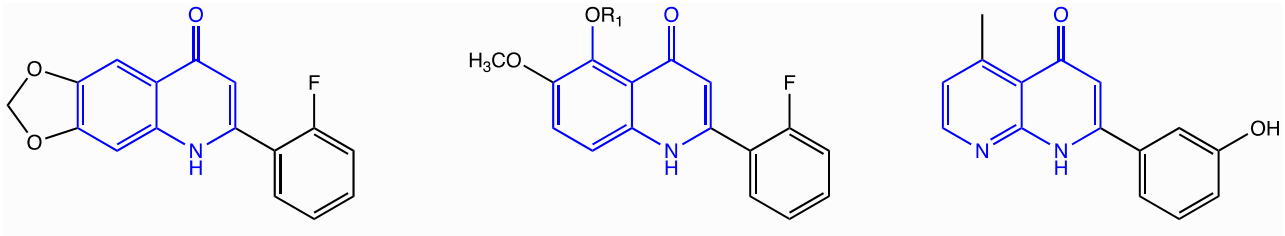


Figure 7. Chemical structures of the most representative anticancer aza-flavone derivatives developed as tubulin polymerization inhibitors.

exert a potent antimitotic activity (arresting cell cycle in G_2/M phase) by directly interfering with tubulin (IC_{50} of 1 μM on tubulin polymerization), disrupting microtubule dynamics, and then inducing apoptosis.[70,71] The in vitro results were confirmed by in vivo testing against hepatoma Hep3B xenograft nude mice models. CSC-3436 reduced tumor weight by 78.6 % at a dose of 40 mg/kg.[70] In addition, further studies indicated that CSC-3436 represent a new potential anti-breast cancer agent, particularly against the triple negative subtype (TNBC).[72,73]

In the same field, Shobeiri et al. performed a bioisosteric O to N substitution in the benzopyrone scaffold of centaureidin, to obtain a new series of quinoline compounds with improved anticancer properties.[74] Among all, quinoline compound **6** with trimethoxyphenyl and hydroxymethylene substituents at C2 and C4 positions (Fig. 8), respectively, was the most interesting molecule of the series, with antiproliferative activity in the micromolar range against both resistant breast and ovarian cancer cell lines (IC_{50} in the range of 8.1–16.3 μM against MCF-7, MCF-7/MX, A2780, A2780RCIS).[74]

Further investigations allowed to study the antimitotic anti-tubulin activity of quinoline **6**, which showed an inhibitory effect on the microtubule assembly comparable to CA-4, with cell cycle arrest in G2/M phase. Docking studies at the colchicine-binding site of tubulin provided further explanations for the inhibitory effect compared to the other compounds in the series: the alcohol group plays a crucial role in the formation of two hydrogen bonds with the key residues Asn^{249} and Ala^{250}, while the trimethoxyphenyl moiety, which recurs in many potent tubulin polymerization inhibitors (such as colchicine and CA-4), is deeply inserted into a hydrophobic pocket surrounded by apolar residues such as Ala^{316} and Ala^{354}.[74]

In addition, the aza-flavonol derivative **7**, with a fluorinated 2-phenyl-3-hydroxy-4-quinolinone core (Figure 8), was recently developed and biologically tested as an anticancer agent against several cancer cell lines, with IC_{50} values in the micromolar range (1.3–4.0 μM against CCRF-CEM, K562, HCT116, A549 and the chemo resistant cell lines CCRF-CEM-DNR, K562-Tax, HCT116p53-/-), no toxicity against the normal human skin fibroblast cell line BJ and arrest of cell cycle in mitosis by disrupting the mitotic spindle assembly.[75]

To selectively enhance the of anti-tubulin and antimitotic activity of the natural flavones, Ferlin et al pursued an original scaffold-hopping optimization strategy from the bicyclic chromone scaffold to different regioisomeric tricyclic pyrroloquinolone cores.[76–85] In general, the switch to this heterocycle was particularly successful, and it results evident from the comparison with the reference flavonoid compounds in Table 2, suggesting even in this case the beneficial effect of scaffold-hopping. In the following paragraphs a brief overview of the most interesting derivatives of this class is provided.

In particular, compound **8** (Figure 9), with a 2-phenylpyrrolo[2,3-*h*]-quinolin-4-one core, was the first derivative discovered and showed a strong in vitro antiproliferative activity (IC_{50} in the range 0.7–7 μM) especially against steroid hormone-responsive cell lines (ovarian, liver, breast and adrenal gland adenocarcinoma), as well as capability to specifically block cell cycle in G2/M phase, disrupt microtubule structure and induce cell death via apoptosis pathway.[86]

In in vivo models, it showed anti-hepatocarcinoma activity in Balb/c mice model, with no detectable toxicity (no significant variation in body weight in treated mice). In addition, compound **8** inhibits aromatase in a dose-dependent manner, probably due to its similarity with the 7,8-benzoflavone scaffold of known potent aromatase inhibitors.[76] The compounds from this series also showed antiangiogenic activity, with the ability to halt HUVEC cell proliferation (pro-apoptotic effect), endothelial cells migration and capillary formation both in vitro and in vivo.[86]

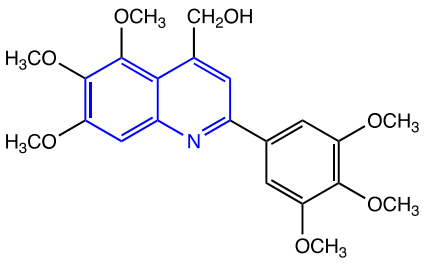
Starting from this lead, further structural refinements were conducted and were mainly focused on moving the pyrrole ring to different position of the quinolone core in order to evaluate whether such modification could increase the selectivity towards the anti-tubulin mechanism of action. In particular, a second scaffold-hopping led to the regioisomer 7-phenyl-3H-pyrrolo[3,2-*f*]-quinolin-9-one derivative **9a** (Figure 9), the first pyrrolo-azaflavone as a selective microtubule polymerization inhibitor and without any off-target activity. This compound showed interesting IC_{50} values against several cancer cell lines in vitro (in the range of 0.4–8 μM) and activity in vivo in a syngeneic hepatocellular carcinoma model transplanted into Balb/c mice (inhibition of cancer growth by 83.2 % after a daily intraperitoneal injection of 40 mg/kg).[77].

These encouraging results supported a process of lead optimization on the central scaffold of **9a**, to design new, more effective derivatives and few of them are worthy to be mentioned in this overview (Table 3). Alkylation of pyrrole with lipophilic substituents led to 100-fold more active compounds, such as the 7-phenyl-3H-pyrrolo[3,2-*f*]quinolin-9-one derivative MG-2477 (**9b**), which showed IC_{50} values in the low-micromolar/nanomolar range against multiple cancer cell lines, even against multidrug resistant subtypes and potent antimitotic activity, comparable to the reference compound combretastatin A-4 (Table 3).[78] The mechanism of action of **9b** was investigated in the NSCLC cell line A549. In addition to microtubule disruption, which consisted of a specific arrest in the G2/M phase of the cell cycle, with a concomitant accumulation of cyclin B, MG-2477 induced autophagy and down-regulation of the Akt/mTOR signaling pathway.[79] A similar mechanism of action was also found in neuroblastoma cells, in addition to a process of ROS-accumulation.[80].

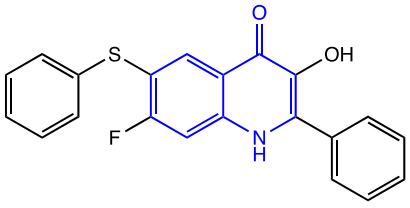
N-alkylation with butyric acid ethyl ester, compound **9c**, improved the pharmacokinetic profile and yielded a more selective and potent antiproliferative derivative (especially against the leukemic cell lines, IC_{50} values in the nanomolar range).[81]

Recent efforts led to compound **9d**, in which the 7-phenyl group was decorated with a fluorine, with even better anticancer and anti-tubulin activity both in vitro and in in vivo animal models (Table 3).[85]

The integration of molecular docking studies on the 3D structure of α/β-tubulin and the results extrapolated from classical SAR analysis allowed to identify the key pharmacophoric moieties required for selective antitumor/anti-tubulin activity of this class of compounds.[76,78-85,87] Figure 10 provides a graphical overview of the crucial interactions between the amino acids in the tubulin binding site and the



6



7

Figure 8. Chemical structure of compounds **6,** an aza-analogue of centaureidin, and **7**, an aza-flavonol analogue with antitubulin polymerization activity.

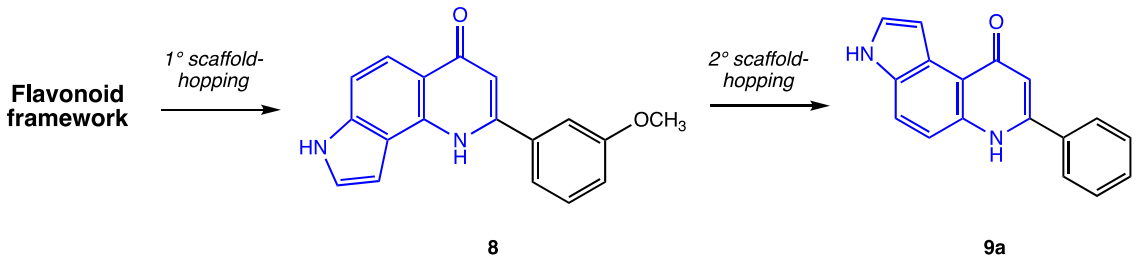
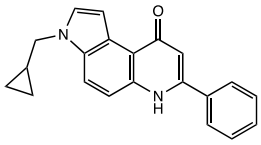
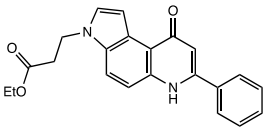
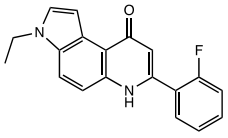


Figure 9. First efforts of scaffold-hopping from flavonoid framework to the pyrroloquinoline (i.e. pyrroloazaflavone) series.

Table 3
Chemical structures of optimized anti-tubulin pyrrolo-azaflavone derivatives **9b-d**, and related in vitro biological activities.

CPD	Antiproliferative activity (IC_{50} range, μM)*	ITP (IC_{50})#	ITP %#	Ref.
 **9b** (MG-2477)	0.02–0.10	0.90	83	78–80
 **9c**	0.0005 – 0.05	0.75	75	81
 **9d**	0.00009 – 0.24	0.96	77	85

*Cell lines tested: HeLa, HL-60TB, HepG2, H295R, OVCAR-3, MCF-7, Aro, A549, HT-29, PT-45, OE19, OE33, A549, MDA-MB-468, A375, Jurkat.
#*ITP: inhibition of tubulin polymerization expressed as IC_{50} (μM) or as percent inhibition of colchicine binding at 5 μM.

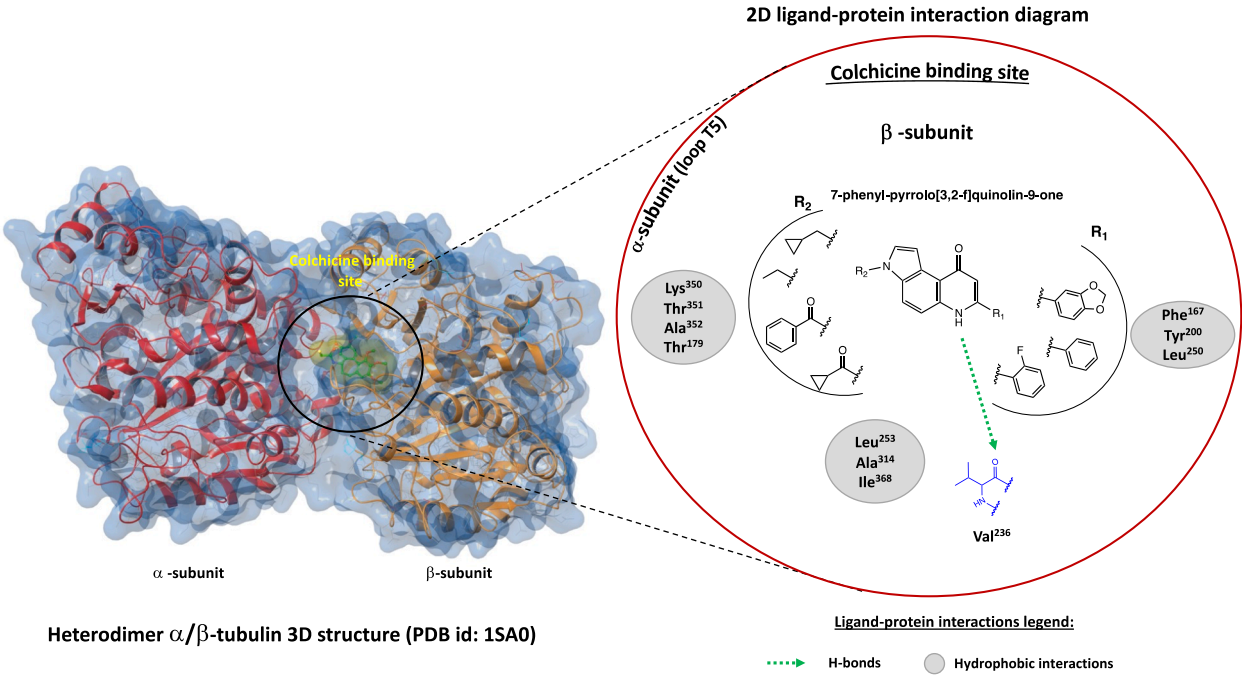


Figure 10. 3D crystal structure of α/β-tubulin (PDB id: 1SA0[88]) with a particular focus on the ligand binding site (right). The left box shows a 2D diagram of the ligand-α/β-tubulin interactions for the pyrrolo-azaflavone class of anti-tubulin agents.

pyrrolo-azaflavone series.

In details, the central tricyclic pyrrolo[3,2-*f*]quinolin-9-one core has the best angular geometry and optimal steric/electronic features to adopt a favorable and stable orientation into the hydrophobic colchicine binding site (β-tubulin subunit). In fact, with other angular geometries (h or g) the insertion in the colchicine binding site was not as deeper as for the f one, and this is reflected also in the detrimental effect on biological activity observed. Regarding the interactions: the H-bond between Val^{236} and the NH of the quinolone scaffold and the numerous hydrophobic contacts with crucial residues (e.g.: Leu^{253}, Ala^{314}, Ile^{368}, shared with the most common tubulin polymerization inhibitors, such as colchicine), play a central role in the stabilization of many ligand–protein complexes.

In addition, the 7-phenyl group (formally derived from the flavonoid skeleton) was deeply inserted into a side hydrophobic pocket on the β-tubulin subunit, in the colchicine binding site, interacting with hydrophobic side chains of Phe^{167}, Tyr^{200} and Leu^{250}. Generally, derivatives with a phenyl ring or at least not excessively bulky and 8-phenyl regio-isomers (pyrrolo-aza-isoflavones) were less or completely inactive, suggesting that the C7 substitution was essential to reach the colchicine side pocket.

On the other hand, the substituents attached to the pyrrole nitrogen were positioned on the other side of the colchicine site, toward another hydrophobic cleft between the β and the T5 loop of the α-subunit and interacting with Lys^{350}, Thr^{351}, Ala^{352} and Thr^{179}. In contrast with the other parts of the molecule, modification on the N3 pyrrole resulted less restricted: lipophilic substituents of medium length, both linear/cyclic alkylic and aromatic (e.g.: cyclopropylmethyl; cyclopropylmethanon; benzamide; ethyl) characterized the most active compounds, whereas polar or charged portions led to inactive products.[76,78-85,87].

In general, in this section, it has been shown as the aza-flavonoid derivatives possess much more improved antitubulin activity than the parent flavonoids of Table 2. In Figure 11 an overview of the SAR of these compounds is provided: the presence of multiple methoxy and hydroxy groups, especially on ring A and B, is fundamental to afford potent antitubulin compounds. Furthermore, azaflavone derivatives (2-phenylquinolones) were better than azaisoflavone (3-phenylquinolone), since the in the formers the phenyl ring was deeper inserted into the colchicine binding pocket. The condensation of heterocycles, such as pyrrole, on ring A showed a notable positive effect on biological activities.

2.2.2. *MDR reversal activity*

Multidrug resistance (MDR) phenomena are a spreading issue even in the anticancer drug discovery field. Many flavonoid compounds are reported in the literature as anticancer-MDR reversal agents, thus with capability to restore the chemosensitivity of multidrug-resistant cancer cells to the clinical anticancer drugs.[89] In this context, several aza-flavonoid analogues have been developed as P-gp inhibitors by pursuing a scaffold-hopping strategy on natural flavonoids (Figure 12).

The 3-phenyl-4-quinolone derivative **10** (aza-isoflavone analogue) (Figure 12), for example, showed good activity in vitro as a P-gp inhibitor in the rhodamine-123 accumulation assay, in contrast to the corresponding isoflavone analogue, which was almost inactive. Compound **10** showed no cytotoxic activity, but enhanced the cytotoxicity of the anticancer drug daunorubicin, a substrate of P-gp, against the two resistant leukemic cell lines K562S and K562R with overexpressed P-gp.[90]

Moreover, the aza-flavone derivatives **11a,b** (Figure 12) with a 6-methoxy-2-arylquinoline scaffold showed remarkable P-gp inhibitory activity (1.3-fold and 2.1-fold more potent than the known P-gp inhibitor verapamil) when tested on the MDR-resistant gastric cancer cell line EPG85-257RDB (overexpressing P-gp) at non-cytotoxic concentrations. In addition, both compounds restored the sensitivity of the same cell line to daunorubicin, determining a drastic decrease in IC_{50}. According to docking studies, several key interactions play a central role in stabilizing ligand–protein complexes: the central aromatic quinoline scaffold and the side phenyl ring were involved in hydrophobic interactions and π-π contacts with aromatic residues (e.g.: Phe^{979}, Phe^{299}, Tyr^{306}, Phe^{339}), whereas the 6-methoxy group formed an H-bond with the side chain of Gln^{986}.[91]

Interestingly, Dong and co-workers tried to optimize the biological and physicochemical properties of nobiletin, a flavonoid compound with potent P-gp inhibitory and MDR reversal activity, characterized by both low solubility and synthetic availability. The aza-analogue of nobiletin **12** (Figure 12) exhibited outstanding biological properties such as capability to reduce the IC_{50} of paclitaxel (PTX) against PTX-resistant human intestine cancer cell line HCT8/T by 87-fold and to enhance the pro-apoptotic effect and the G2/M arrest induced by PTX. In addition, thanks to both the heterocyclic nitrogen and the basic hydrophilic dimethyl-amino-ethyl side chain on the N1, the water solubility was 280-fold higher than that of nobiletin. When tested in in vivo models, compound **12** showed improved anti-tumor effects in A549/T NSCLC xenograft when co-administered with PTX (57 % reduction at an of 50 mg/kg dose of **12** + a 15 mg/kg dose of PTX), while also increasing the PTX levels in tumor cells by 4.82-fold compared to the PTX group alone (vs 2.93-fold for nobiletin). Docking and molecular dynamics studies shed light on the possible binding mode in the P-gp binding site, represented in the 2D interaction diagram in Figure 13. The extended aza-flavone aromatic framework interacts with the target protein not only through the numerous H-bonds (Gln^{721}, Tyr^{306}), but even with hydrophobic and π-π interactions (Phe^{724}, Phe^{332}); in addition the N1 dimethyl-amino-ethyl side chain allowed to form additional Van der Waals interactions (with Phe^{339} and Ile^{302}) responsible for stronger binding compared to the reference flavone compound.[92] In this case, the scaffold-hopping to nitrogen heterocycle has opened up the possibility of

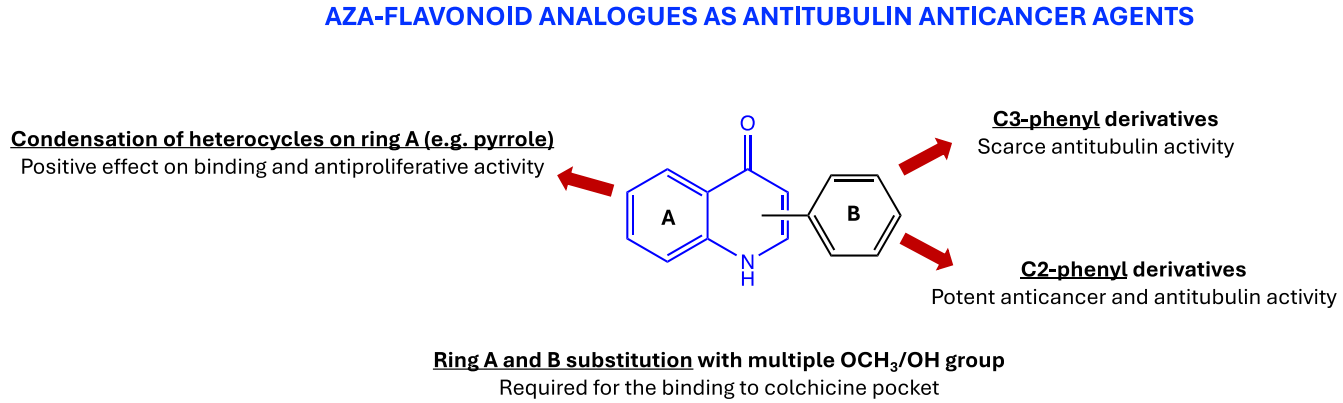


Figure 11. SAR insights on the azaflavonoid analogues with antitubulin activity.

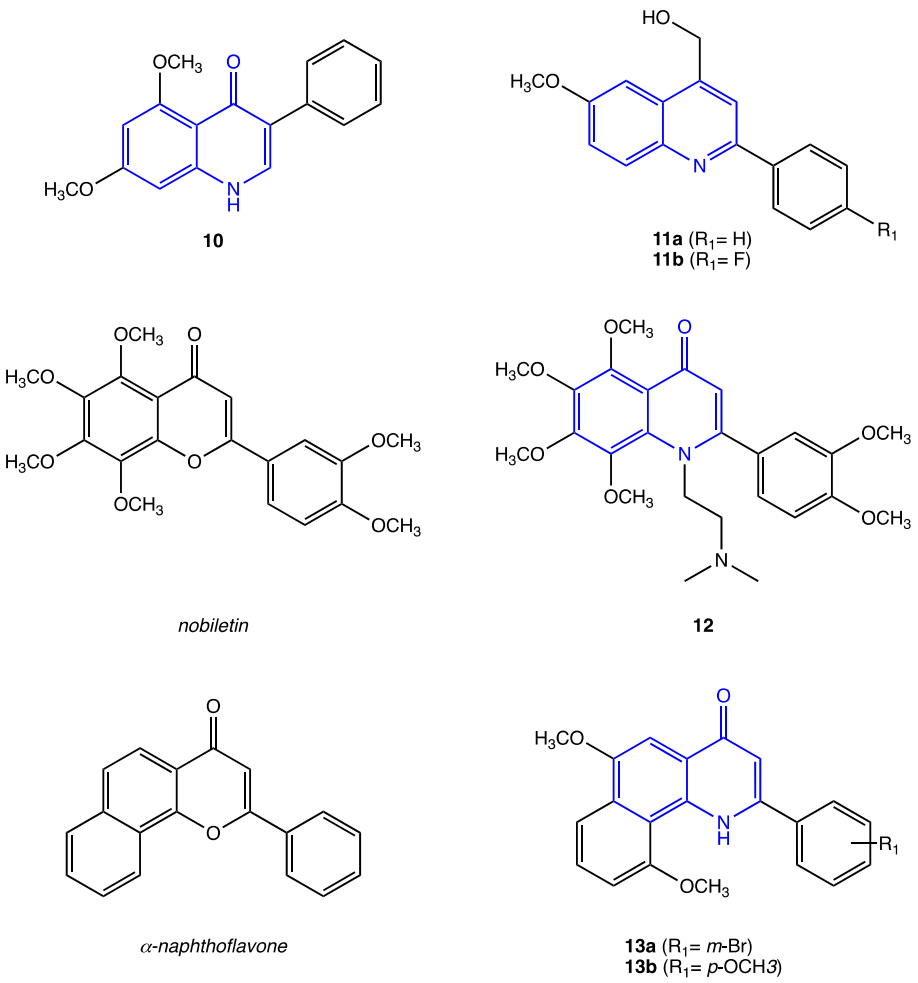


Figure 12. Chemical structure of aza-flavonoid analogues with MDR reversal activity for cancer chemotherapy and the corresponding flavone leads: **10**–**12**, P-glycoprotein inhibitors; selective CYP1B1 inhibitors **13a,b**.

a deeper SAR exploration on the 1 position: the introduction of N1 alkylamino side chains, not feasible in the oxygen natural compound, resulted essential to improve the interactions within the target protein binding site and then to increase the biological activity.

Dong et al., instead, investigated the inhibitory effect of many natural flavone derivatives and synthetic analogues against another target involved in the development of resistance, the CYP1B1. This enzyme, which belongs to the cytochrome family, is involved in the activation of pro-carcinogenic xenobiotics as well as in the inactivation of anticancer drugs used in therapy (e.g.: docetaxel, doxorubicin, paclitaxel, and mitoxantrone).[93–95] In detail, aza-analogues of the synthetic oxygenated lead compound α-naphthoflavone (ANF, Figure 12), were synthetized to increase water solubility and selectivity to CYP1B1 over CYP1A2. The most promising compounds **13a,b**, with a dimethoxy substitution, displayed both higher potency and selectivity towards the desired target compared to ANF, with an IC_{50} of 3.6 nM and 4.1 nM, respectively, and an IC_{50} ratio between the two isoforms A2/B1 of 163.2 and > 1219.5 (for ANF, $IC_{50} = 5.6$ nM, and IC_{50} ratio 1A2/1B1 of 2.5). In addition, the higher solubility (for **13a,b** and clogP 4.4 and 3.5, respectively; for ANF clogP of 4.7) was responsible for a better performance in cellular assays in reversing CYP1B1-mediated docetaxel resistance in the breast cancer cell line MCF-7/1B1 (overexpressing CYP1B1); noteworthy, capability to restore the cytotoxicity of docetaxel to levels comparable to that measured in normal MCF-7 cells. Docking studies on the X-ray structures of the target protein elucidated the central role of the bioisosteric oxygen–nitrogen exchange in both improving the selectivity and the inhibitory effects on 1B1 cytochrome: the NH, in contrast to oxygen, was capable to selectively form a strong H-bond with the key residue Asp^{333} in the CYP1B1 active site, which is absent in the other isoform. In addition, an H-bond between the carbonyl oxygen and Asp^{326} and a strong $\pi - \pi$ interaction between the extended aromatic core and Phe^{231} side chain play also a key role in maintaining the high affinity against this isoform (Figure 14).[95]

2.2.3. *Protein kinase inhibitory activity*

Quinoline-, quinolone-, quinazoline- and quinazolone-based compounds have been widely described in the literature for their remarkable effects in inhibiting protein kinases for anticancer therapies.[96–99] Since several natural flavonoids showed interesting inhibitory properties on these targets, the scaffold-hopping approaches also led in this case to more effective heterocyclic analogues, with selective activity against various kinases (EGFR, CDKs, TrkA).

Starting from the isoflavone derivative genistein, with inhibition activity on EGFR, Traxler et al. developed the 3-phenyl-4(1H)-quinolones **14a,b** (Figure 15), which showed an enzymatic inhibitory effect 25–125-fold higher than the corresponding isoflavone derivative (IC_{50} of 0.008, 0.038 and 1.0 μM for **14a**, **14b** and genistein, respectively). The improved activity observed in the scaffold-hopped derivatives was explained and justified also by the in silico molecular docking simulations studies performed: the switch from the H-bond acceptor (O) of genistein to the H-bond donor group (NH) of **14a,b** enables additional H-interactions in the ATP-binding site of the protein (e.g. with Asp^{831} in the triphosphate binding part of the cavity), and stabilizes the ligand–-protein complex.[100] Indeed, the nitrogen heterocycle, due to its

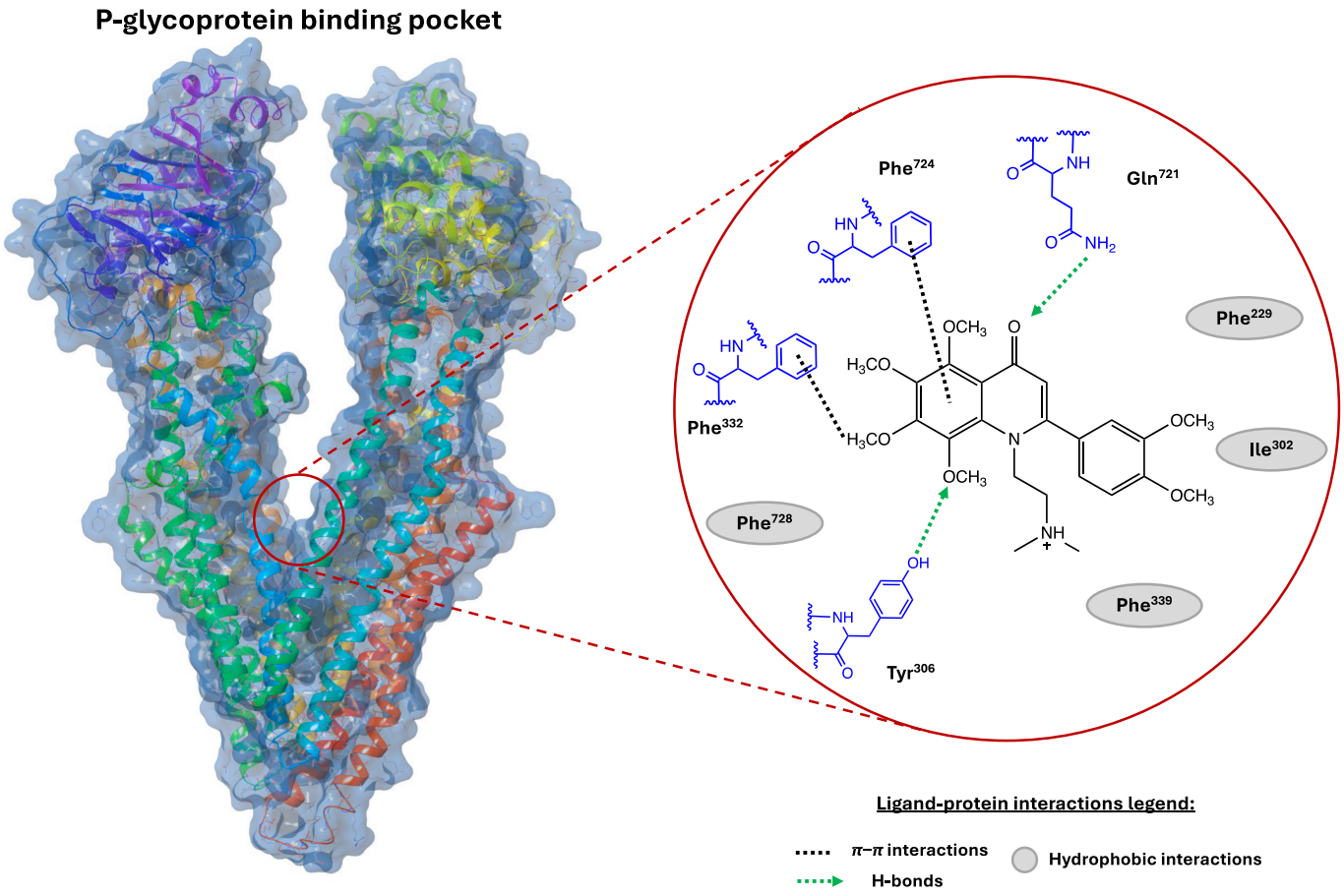


Figure 13. 3D crystal structure of P-glycoprotein (PDB id: 4M2S) and representation of the 2D interaction diagram of the complex between **12** and P-gp binding site, with the crucial interacting amino acids highlighted.

capability to better mimic the adenosine core of the natural substrate, establishes a great number of ATP-like interactions in comparison to the flavonoid compound.

To further explore the SAR of this class of compounds, Xiao and co-workers synthesized compounds **15a,b** (Figure 15) which showed potent cytotoxic activity against the cancer cell lines KB and HepG2, much higher than that of genistein, and no toxicity against the normal human cell line L02 (for **15a** IC_{50}-HepG2 = 0.05 ± 0.02 μM, IC_{50}KB = 0.06 ± 0.04 μM; for **15b** IC_{50}-HepG2 = 0.01 ± 0.009 μM, IC_{50}KB = 0.03 ± 0.02 μM; for genistein IC_{50}-HepG2 = 1.1 ± 0.7 μM, IC_{50}KB = 0.56 ± 0.17 μM).[101,102].

Several aza-flavonoid analogues, instead, showed potential inhibitory activity against cyclin-CDKs complexes, key targets overexpressed/upregulated in cancer cells, which positively regulate cell cycle progression (Figure 16).[103,104]

For example, Ling and co-workers, inspired by the promising anticancer activity of vitexin (a glycosylated flavonoid) against acute lymphoblastic leukemia cells, developed a series of aza-analogues. The 4-quinolone compound **16** (Figure 16), with an N1-sulfonamide moiety, demonstrated better antiproliferative activity than vitexin, with IC_{50} values in the low micromolar range (0.3–10 μM) towards specific ALL cellular models (UOCB1, SEM, KOPN8), and low toxicity in normal cells. An in-depth analysis of the mechanism of action showed that quinolone **16** was capable to induce G0/G1 cell cycle arrest and oxidative stress, consistent with a possible inhibition of cell cycle regulatory proteins. The biological results were further validated by docking studies, which confirmed the capability of compound **16** to tightly bind the hinge region of the CDK9 ATP binding site.[103].

In the discovery of new CDK inhibitors, the aza-flavanone derivative **17** (Figure 16) showed highly potent antiproliferative effects against the aggressive androgen-independent prostate cancer cell line DU145, with an IC_{50} of 0.4 μM. Insight into the mechanism of action based on molecular docking studies, highlighted its capability to bind strongly to the ATP-binding pocket of CDK2, with a better interaction pattern than the reference flavone flavopiridol, and thus block the activation of the CDK2-Cyclin E1 complex through a competitive mechanism. The subsequent biological in vitro/vivo assays confirmed and further enriched the computational evidence: arrest of the cell cycle in G0/G1 and S phase as a consequence of inactivation of the cyclin-CDKs complexes; ROS production and promotion of mitochondrial dysfunction and then apoptosis; suppression of metastatic phenotype of DU145, with down-regulation of the levels of several matrix metalloproteases, involved in cell migration and invasion; capability to suppress the xenograft tumor in mice models by more than 60 % (dose of 5 mg/kg administered via i.p.).[104].

The tetracyclic structure of the isoflavonoid wrightiadione, an inhibitor of the Tropomyosin Receptor kinase A (TrkA), was even bioisosterically substituted to obtain more effective aza-analogues. The switching from the enolone to the enaminone scaffold, which was also provided with different substituents, led to compound **18** (Figure 17), which is in 8-fold more potent as a TrkA inhibitor than the parent compound (for **18**, IC_{50} = 6.6 μM; for wrightiadione IC_{50} = 55.8 μM).[105]

Even though the substitution of oxygen by a sulfur atom occurs less frequently, attempts have been tried to enhance the anticancer effect of flavonoids and remarkable results have been obtained. For example, Kataoka et al. synthetized a series of thio-flavone analogs as part of a lead structure optimization process of the kinase inhibitor flavone **19** (Figure 18). In particular, compound **20** proved to be the most

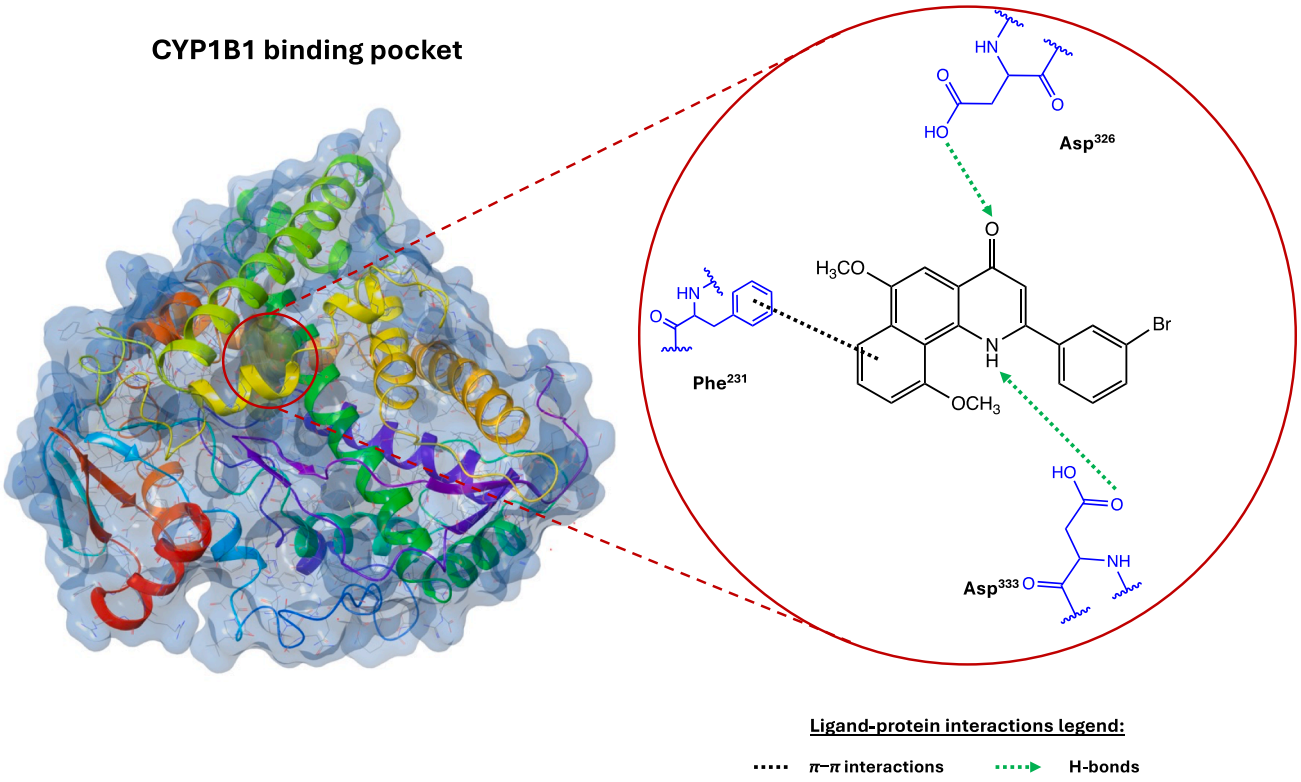


Figure 14. 3D crystal structure of CYP1B1 (PDB id: 3PM0) and representation of the 2D interaction diagram of the complex between **13a** and CYP1B1 binding site, with the crucial interacting amino acids highlighted.

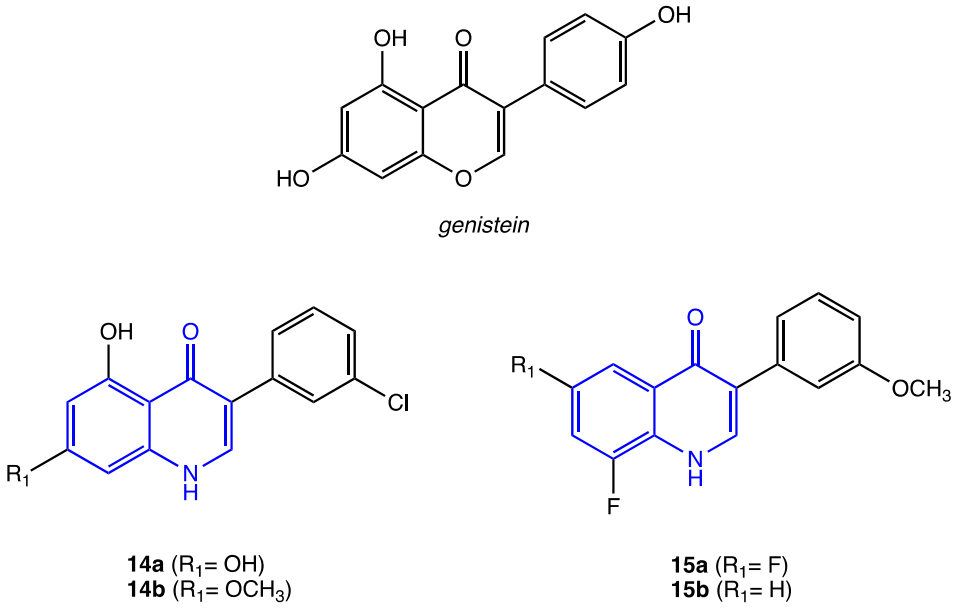


Figure 15. Chemical structures of the isoflavonoid genistein and the corresponding aza-analogues **14a,b**, **15a,b** with EGFR selective inhibitory activity.

interesting, as it was more potent than **19** to inhibit the Raf1-induced activation of the MEK signaling pathway, via an allosteric binding mechanism (IC_{50} of 1.88 μM vs 4.35 μM as determined by the phosphorylation of MEK1, and 2.95 μM vs 13.8 μM as determined by the phosphorylation of ERK1/2). The results of enzymatic inhibition were also confirmed in cell assays, where a more effective antiproliferative activity was measured on cell lines, in which ERK-MAP kinase signaling pathway is constitutively activated (IC_{50} of 8.6 μM vs 29.5 μM for HT1080 cells and IC_{50} of 10.0 μM vs 30.5 μM for WiDr cells).[106].

2.2.4. Other anticancer heterocyclic flavonoid analogues: Miscellanea

In this section several flavonoid heterocyclic analogues are analyzed as promising anticancer drugs, that act on targets other than those already discussed or whose mechanism of action is unclear. Although in some cases the specific target(s) have not yet been identified, the remarkable biological activities deserve a detailed analysis, especially in comparison with the corresponding oxygen derivatives, to guide future investigations.

Several compounds were developed to act specifically on various

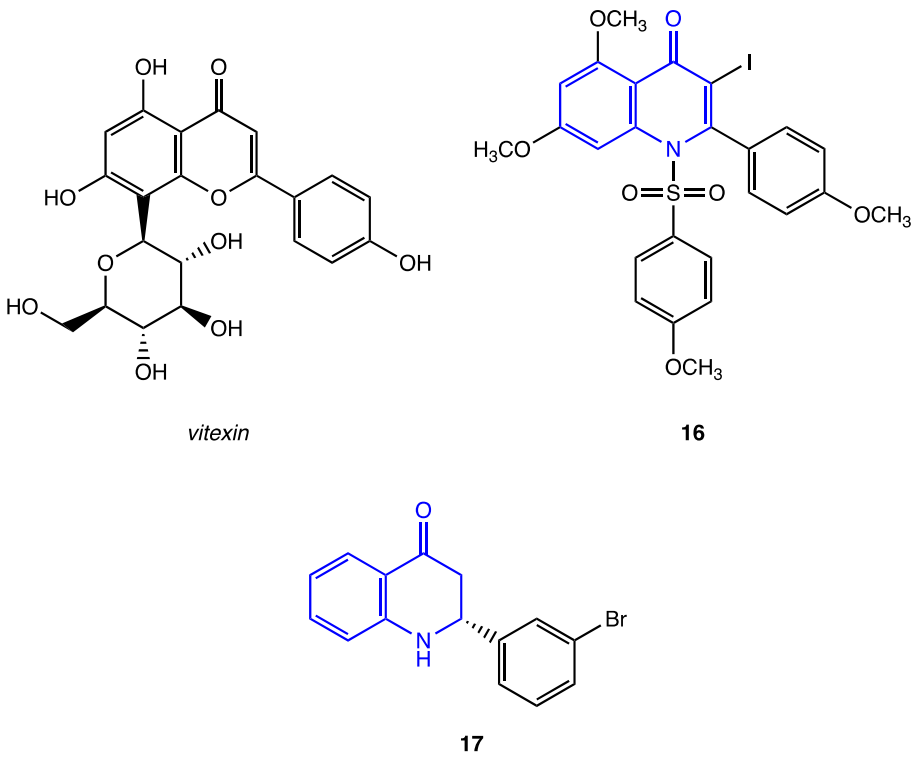


Figure 16. Chemical structures of the flavonoid vitexin and the aza-analogues **16**, potential CDK9 inhibitors (upper part); aza-flavanone compound **17**, a Cyclin E1-Cdk2 complex blocker.

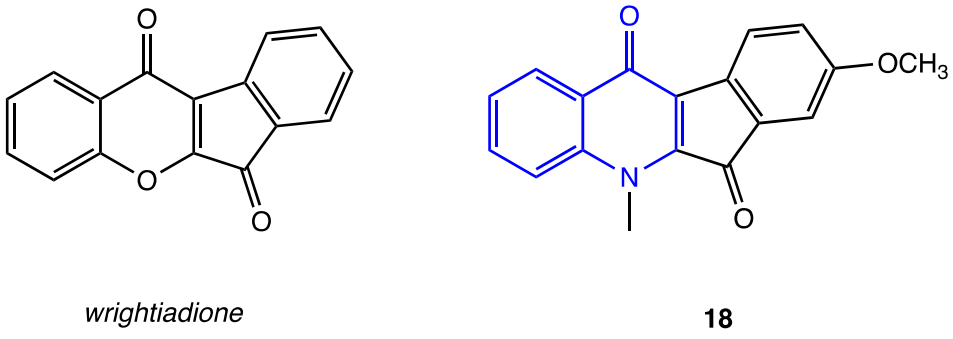


Figure 17. Structures of the tetracyclic isoflavonoid derivative *wrightiadione* and of the aza-analogue **18**, inhibitors of TrkA.

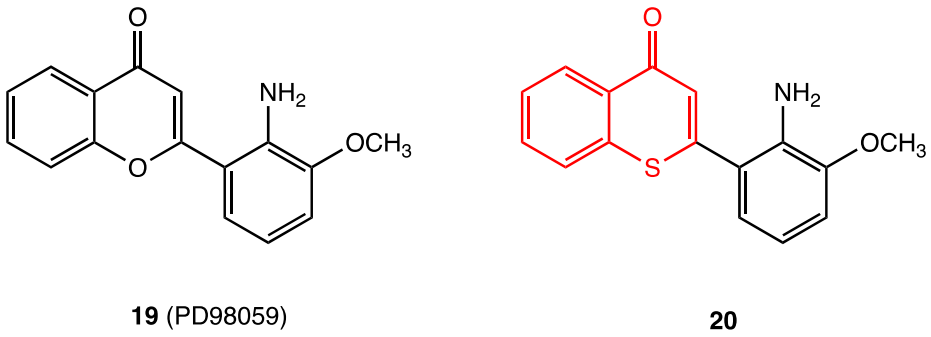


Figure 18. Chemical structure of the semisynthetic flavone derivative **19** (PD98059) and of the corresponding thio-flavone analogue **20** developed as inhibitor of the ERK-MAP kinase pathway.

breast cancer types.

For example, Bharathkumar and co-workers, aiming at improving the phytoestrogen activity of genistein, developed a series of estrogen receptor α ligands with a quinoline scaffold instead of the chromone one. Compound **21** (Figure 19), with a chloro substitution at C7 on the central scaffold and an amino group on the side phenyl ring, was the

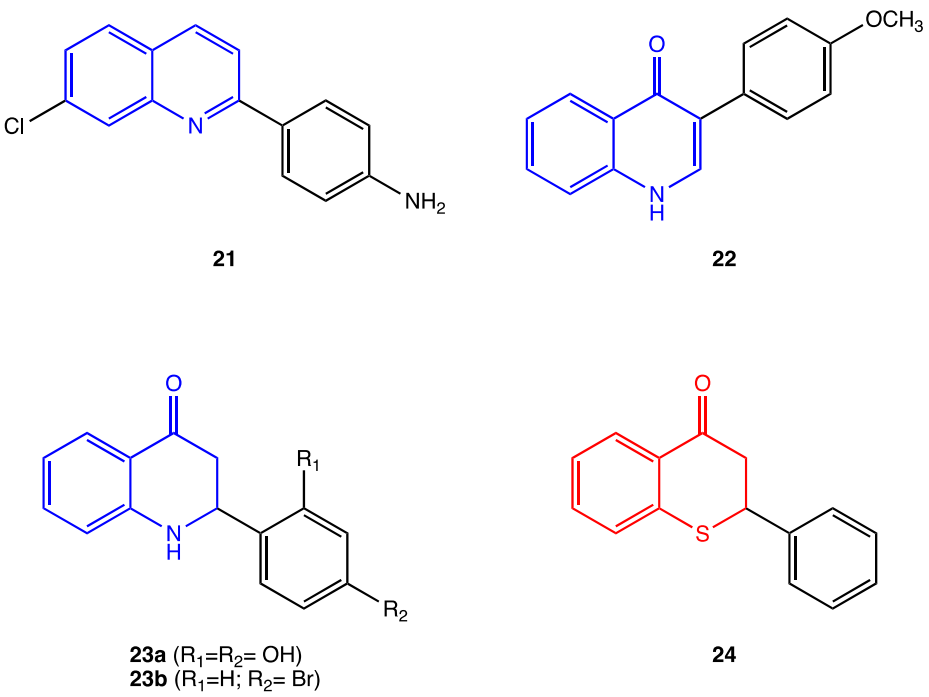


Figure 19. Structures of several targeted aza-flavonoid analogues with anti-breast cancer activity: compound **21**, a quinoline derivative with ERα inhibitory activity; compound **22**, aza-isoflavone compound targeting MTA2/SerRS/VEGFA; compounds **23a,b**, microRNA inhibitors; thioflavanone **24**, a pro-apoptotic cytotoxic agent.

most effective derivative in the antiproliferative assay, with high selectivity against ERα-positive cells, especially from the breast cancer panel (IC_{50} of 6 and 11 μM against MCF-7 and HepG2 cell lines; IC_{50} > 100 μM against the ERα-negative cell lines MDA-MB-231 and BT-549). Docking studies confirmed the tight binding of the selected compound to the ERα binding site, with a mainly hydrophobic interaction-pattern comparable to that of the natural ligand estradiol.[107].

The aza-isoflavone compound **22** (Figure 19) was discovered as potent agent against triple negative breast cancer (TNBC). Its mechanism of action consisted in the downregulation of VEGF-A production and release, which was achieved by acting on the upstream VEGF-A transcriptional repressor system MTA2/SerRS at the transcriptional level. The result of this peculiar effect was a strong anti-angiogenic and antiproliferative activity both in vitro on MDA-MB-231 cells and in vivo on human TNBC xenografts in mice models.[108]

Aza-flavanone compounds **23a,b** (Figure 19) acted at the transcriptional level by inhibiting the expression of the proliferative and anti-apoptotic microRNA miR-4644 in MCF7 breast cancer cells, where they induced cell cycle arrest in G1 phase and a profound and selective cytotoxic effect compared to normal non-cancerous cells.[109]

Even some thio-flavonoid analogues, as type **24** (Figure 19), showed modest anticancer and pro-apoptotic properties on breast cancer cells, with IC_{50} in the range of 62–89 μM against MCF-7, MDA-MB-453, and MDA-MB-231 lines.[110]

Compounds **27** and **28** (Figure 20) with an interesting pyrido[1,2-*a*] pyrimidin-4-one scaffold were developed by scaffold.hopping on the anticancer flavones and isoflavones **25** and **26** with inhibitory activity on human topoisomerase II α (hTopIIα). The diaza substitution was particularly successful and resulted in improved biological activities compared to the reference compounds, such as Topo inhibitor etoposide and the flavonoids **25** and **26**. Derivatives **27** and **28** showed: better hTopIIα inhibitory activity, as revealed by the hTopoIIα-mediated decatenation of kinetoplast DNA assay, with no interaction with TopoI isoform and no DNA-intercalation activity; significant cytotoxicity against various cancer cells (IC_{50} measured in MCF-7, HeLa, A-549, HCT-116 and H-357 in the range 1.8–3.2 μM) with relatively low toxicity against normal cells; induction of apoptosis and inhibition of invasiveness in treated cells.[111]

Many aza-analogues substituted at both C5 and C7 with hydroxy and methoxy groups (a very frequent substitution pattern even in the most active natural active flavonoids) showed significant anticancer properties. The aza-flavone compound **29** (Figure 21), with a 5-hydroxy-7-methoxy-2-phenyl-4-quinolone scaffold, induced significant G2/M cell cycle arrest and antiproliferative effects against the full NCI60 panel (mean IC_{50} = 14.5 μM) and especially on K562 leukemia cells (IC_{50} = 0.8 μM).[112] Compound **30**, which has a regioisomeric aza-isoflavone core compared to **29**, also exhibited great efficacy, reducing cell viability by 85.9–99.0 % in the tested cell lines at a concentration of 25 μM (H460, DU145, A-431, HT-29).[113] In both cases, the presence of a halogen in the *meta*-position of the 2-phenyl ring was decisive for the desired biological activity.

Compound **31** (Figure 21), bearing a dihydroquinazolin-4-one core,

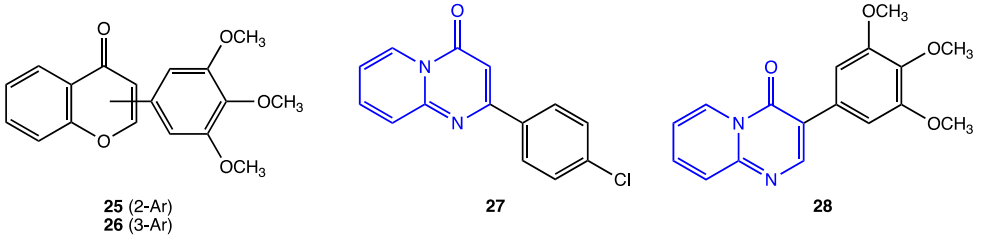


Figure 20. Structures of flavonoid hTopIIα inhibitors **25** and **26** and the related diaza analogues **27** and **28**.

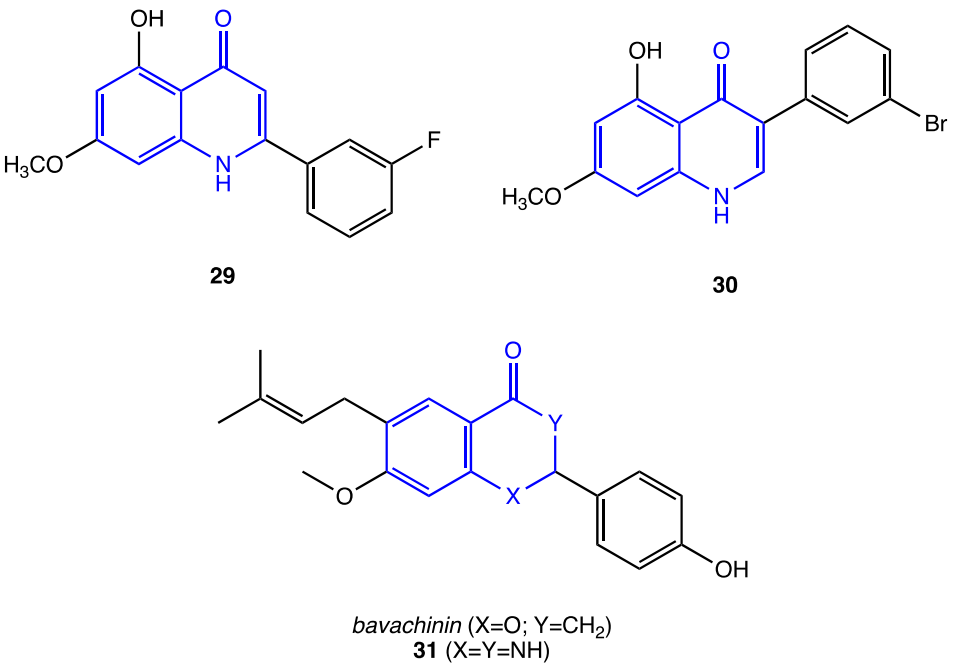


Figure 21. Chemical structure of compounds **29**–**31**, cytotoxic aza-flavonoid analogues with unclear mechanism of action.

is an example of diaza-flavonoid analogue of bavachinin, a natural flavanone well-known for its potent anticancer properties. In biological assays, **31** showed potent antiproliferative activity in vitro against acute myeloid leukemia (AML) cell lines HL-60 and MV4-11, with IC_{50} values of 0.25 and 0.5 μM at 48 h, which were lower than those of the lead flavonoid compound. In addition, it was more active and better tolerated than retinoic acid, a clinical drug for AML therapy, in suppressing the growth of HL-60 cells in vivo in xenograft mouse model. Further tests clarified the putative mechanism of action, which may involve the induction of autophagy and apoptosis as well as the impairment of cell cycle progression.[114]

2.3. Antiviral activity

Many aza-flavonoid analogues have been developed to improve both the potency and selectivity of natural antiviral flavonoids, and some of

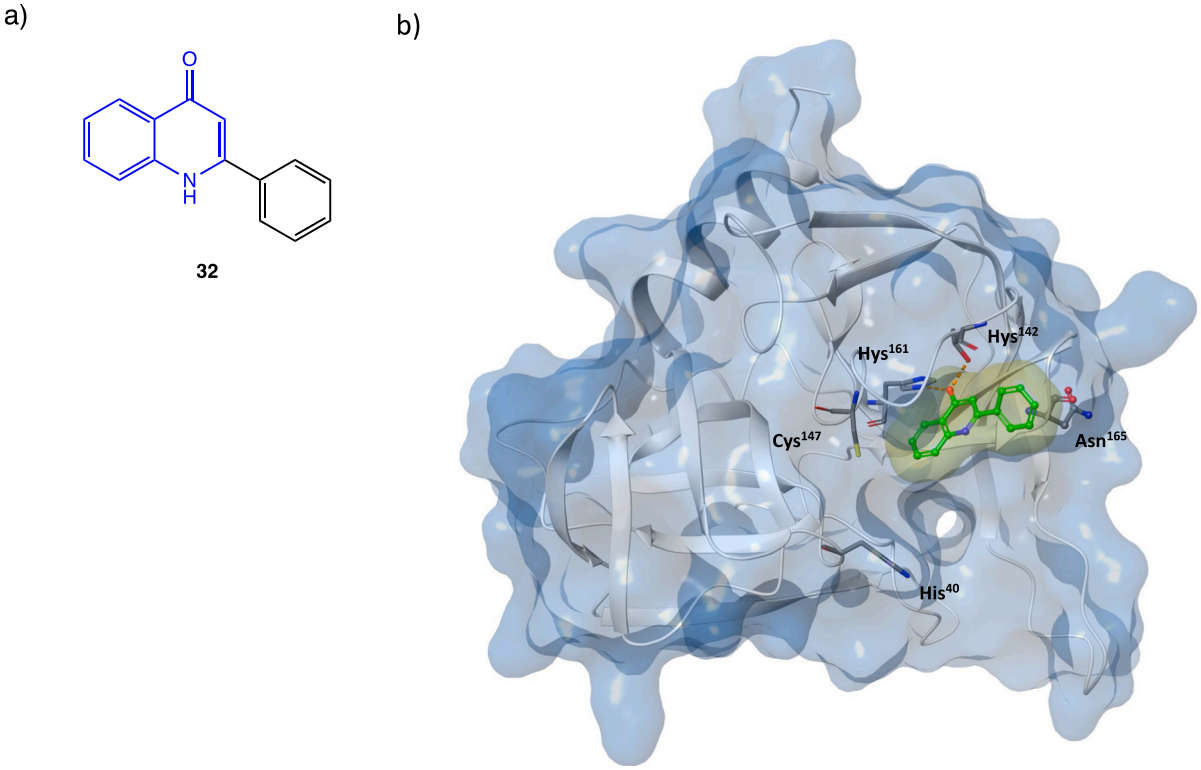


Figure 22. a) Chemical structure of the antiviral aza-flavonoid compound **32**; b) 3D X-ray structure of the complex between **32** and the 3C protease of rhinovirus (PDB id: 2XYA).

them showed a very selective inhibitory activity on specific enzymes involved in crucial steps of viral lifecycle (e.g. proteases, helicases, endonucleases, neuroamidinases, key enzymes for viral replication and assembly processes).[115–117]

For example, thanks to a high-throughput in vitro biochemical screen focused on the 3C protease, a key enzyme of the human rhinovirus (HRV) family, Baxter and co-workers, identified the aza-flavone nucleus **32** (Figure 22a), which shows promising inhibitory activity (3CP pK_d = 3.7; ligand efficiency = 0.30 kcal/mol per HA), while the flavonoid counterpart (3CP $pK_d \sim 3$) has a lower inhibition activity. The X-ray structure of the complex between the catalytic site of 3CP and **32** revealed the binding mode (PDB code 2XYA, Figure 22b) and the key ligand–protein interactions: the quinolone ring interacted through H-bonds, π-π and hydrophobic interactions with the crucial residues His^{40}, His^{142}, Cys^{147}, His^{160}, while the 2-phenyl ring was localized in S1 and interacted with Asn^{165}. This study provided a starting platform for the development of more potent non-covalent 3C-PR inhibitors based on an aza-flavonoid scaffold.[118]

With the aim of discovering compounds with anti-influenza activity, Zima et al. developed a high-throughput in vitro screening assay to evaluate the inhibitory potency of a library of natural derived compounds against the *N*-terminal PA domain (PA-Nter) of influenza RNA-dependent RNA polymerase (endonuclease domain). Quercetin and luteolin, two flavonoid compounds, resulted among the most potent derivatives, and interestingly comparable/increased endonuclease inhibitory activity was observed for the corresponding aza-analogues, aza-quercetin and aza-luteolin (IC_{50} of 0.67 ± 0.02, 0.072 ± 0.002, 0.068 ± 0.002, and 0.2 ± 0.02 μM, respectively) (Figure 23).[119]

X-ray crystal structures and molecular docking studies elucidated the most important pharmacophoric moieties: the lateral bidentate catechol portion, which plays a key role in the coordination of the catalytic divalent cations Mn^{2+} and Mg^{2+}; the central bicyclic core, which is involved in a wide pattern of both hydrophobic interaction and strong H-bonds via the OH groups at the C5 and C7 positions. Even if weak anti-influenza activity was observed in in vitro tests, these results represent a milestone in the development of new PA endonuclease inhibitors with an aza-flavonoid scaffold.[119]

In the same field of anti-influenza derivatives, the aza-flavone **33** (Figure 23) was designed by pursuing a scaffold-hopping on quercetin. It showed interesting antiviral activity with non-competitive inhibition of the neuraminidase (NA), a key surface glycoprotein involved in influenza A/H1N1pdm09 virus replication cycle. In both in silico and in vitro studies, compound **33** exhibited remarkable results, better than the approved reference NA inhibitors quercetin and oseltamivir: more potent antiviral effect with an EC_{50} in the nanomolar range (4.96 nM vs 0.56 μM and 12.7 nM for quercetin and oseltamivir, respectively); improved NA inhibitory activity, with an IC_{50} of 1.3 μM in the enzymatic inhibition assay (IC_{50} values of 8.7 μM and 1.9 μM for quercetin and oseltamivir, respectively); no significant cytotoxicity ($CC_{50} > 200$ μM). Moreover, both kinetics and in silico induced fit docking (IFD) studies, confirmed the non-competitive inhibition of NA by **33**. Indeed, it bound to the allosteric NA-430-cavity and formed stronger stabilizing interactions than quercetin, thanks to the 4-quinolone core and the 2-methoxyphenyl side portion, as evidenced by the higher IFD score.[120]

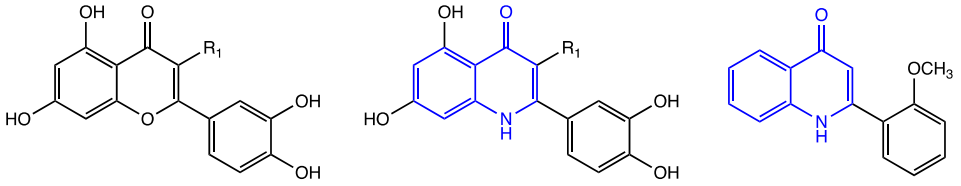
Recently, the significant anti-SARS-CoV-2 properties of several natural flavonoids (e.g. myricetin),[121] have prompted the scientific community to investigate the effect of bioisosteric substitution in this area as well.

For example, the flavone baicalein (Figure 24) was one of the first non-peptidomimetic non-covalent inhibitors of SARS-CoV-2 M^{pro}. Analysis of the X-ray structure of the complex with M^{pro} showed that the C4 carbonyl and the three phenolic groups were key pharmacophoric components, as they were able to form many H-bonds with crucial residues in the active site of the enzyme (Ser^{144}, His^{163}, Leu^{141}, Gly^{143}).[122]

Taking these findings into account, Zhang et al. pursued a scaffold-hopping design-approach, in which they converted the chromen-4-one core of baicalein into aza-analogues quinolon-4-one/quinazolin-4-one privileged scaffolds, to increase both potency and PK properties. While the quinoline analogues showed no inhibitory activity, the quinazolinone analogue **34a** (Figure 24) was only slightly less potent than the parent compound (IC_{50} = 1.372 ± 0.047 μM and 0.966 ± 0.065 μM, respectively), and was therefore selected as the lead compound for further SAR studies at the *C*2 and N3 positions. Compounds **34b** and **34c** (Figure 24) resulted the most interesting compounds, with improved inhibitory activity and IC_{50} = 0.085 ± 0.006 μM and 0.100 ± 0.012 μM, respectively. In addition, compared to the parent flavonoid compound, **34b** also showed: higher selectivity and specificity (no apparent inhibitory activity against host proteases); as well as improved physicochemical and PK properties (better solubility, moderate plasma protein binding, improved human liver microsome stability); 5-fold increase in the antiviral effect against SARS-CoV-2-infected Vero E6 cells (EC_{50} = 1.10 ± 0.12 μM vs 5.15 ± 1.64 μM), with low cytotoxicity ($CC_{50} > 50$ μM). The X-ray crystal structure of compound **34c** in complex with SARS-CoV-2 M^{pro} could be an important aid in the development of new, more effective drugs, as it shows the binding mode and the most relevant interactions (PDB id 8I4S, Figure 25): the trihydroxy-phenyl moiety was confirmed as an essential structural fragment, displaying similar orientation and interaction pattern as the parent compound (H-bonds with the crucial amino acids Asn^{142}, Gly^{143}, Ser^{144}, Cys^{145}, Glu^{166}); the side 3′-methyl-4′-fluorophenyl was deeply inserted into the hydrophobic S2 pocket and forms hydrophobic interactions with Gln^{189} and Met^{49}; the *sec*-butyl at the C2 position is projected into the newly formed cleft S2c, which was formed after the ligand was incorporated into the binding site, where it interacts with Cys^{44}, Thr^{45} and Ser^{46}.[123].

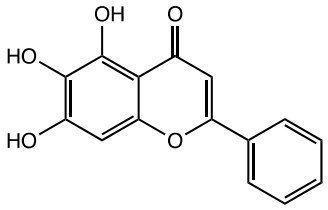
Furthermore, even some thio-flavonoid analogues showed interesting antiviral properties, although the specific target has not yet been identified. For example, compound **35**, a thio-flavone derivative bearing two acetoxy groups (Figure 26), displayed selective anti-HIV activity against HIV in acutely infected H9 lymphocytes, with and an ED_{50} of 0.65 μg/mL.[124]

Thioflavones **36** (Figure 26), instead, were proposed as new lead compounds to develop more effective agents against enterovirus EV71 and the coxsackievirus B3 (CVB3) and B6 (CVB6), due to their promising antiviral activities in the micromolar range measured in cellular assay on Vero cells.[125]

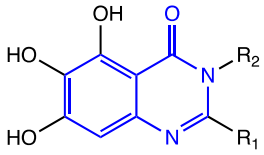


quercetin (R_1=OH) *luteolin* (R_1=H) *azaquercetin* (R_1=OH) *azaluteolin* (R_1=H) **33**

Figure 23. Chemical structures of several anti-influenza virus flavonoid/azaflavonoid compounds.



baicalein



34a-c

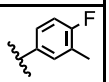
CPD	R_1	R_2
a		H
b		
c		

Figure 24. Chemical structures of baicalein and compounds **34a-c**, aza-flavonoid analogues with anti-SARS-CoV-2 activity.

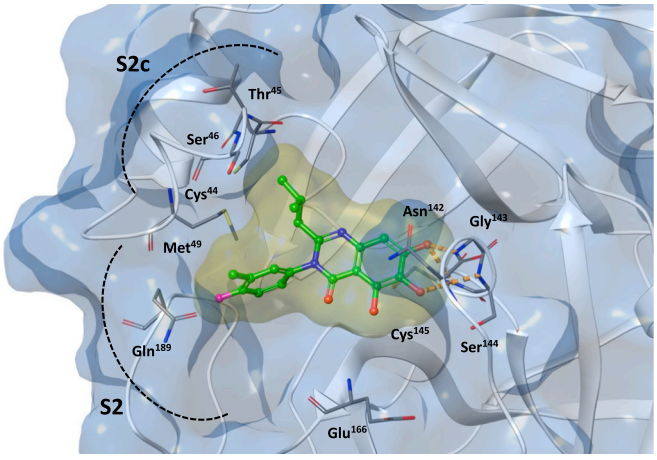
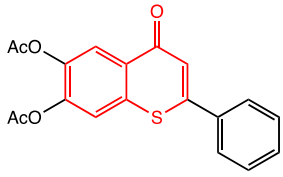
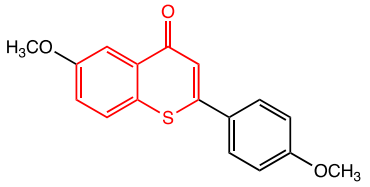


Figure 25. X-ray crystal structure of **34c** in complex with SARS-CoV-2 M^{pro} (detailed view of PDB id: 8I4S).

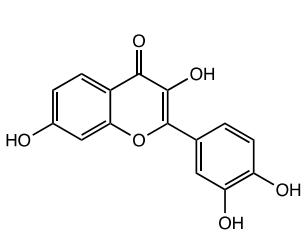


35

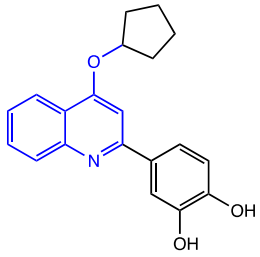


36

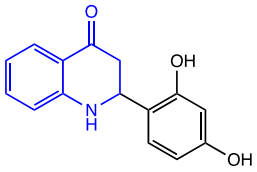
Figure 26. Chemical structures of the antiviral thio-flavonoid compounds **35, 36**.



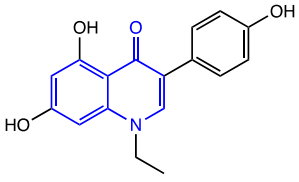
fisetin



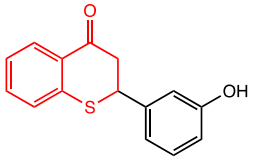
CMS-121



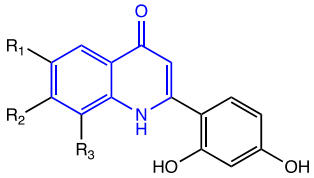
37



38



39



40a (R_1= OH; R_2=R_3= H)
40b (R_2= OH; R_1=R_3= H)
40c (R_3= OH; R_1=R_2= H)

Figure 27. Chemical structures of aza/thio-flavonoid compounds with neuroprotective and antioxidant activity.

2.4. Antioxidant, anti-inflammatory and neuroprotective activity

Several flavonoids with potential multi-target activity have been discovered to counter oxidative stress and inflammation and to provide beneficial effects on the nervous system health, particularly in neurodegenerative diseases.

Fisetin, for example, a natural flavonol (Figure 27), has demonstrated multiple valuable biological effects in vitro, including antioxidant, anti-inflammatory and neurotrophic properties, which are important for maintaining CNS health and function. These effects may be particularly beneficial in stroke or other neurological disorders (e.g.: Alzheimer's disease, AD). However, the low activity in cell-based assays, poor bioavailability and suboptimal physicochemical and PK parameters required for the drug to cross the blood–brain barrier (BBB; too low lipophilicity, high TPSA, high number of hydrogen bond donors) prompted the researchers to perform a scaffold-hopping strategy and synthetize new heterocyclic analogues of fisetin.[126,127]

The quinoline analogue CMS-121 (Figure 27), with a retained dihydroxyphenyl side moiety of the lead compound and a 4-O-cyclopentil ring, showed a > 400-fold increase in neuroprotective activity compared to fisetin (EC_{50} of 0.007 μM vs 3 μM in vitro in ischemia models), in addition to GSH-maintaining activity (antioxidant effect); anti-inflammatory activity (inhibition of bacterial lipopolysaccharide induced microglial activation); neurotrophic effect (induction of PC12 cells differentiation). In addition, CMS-121 has improved physicochemical and PK properties for brain penetration, such as optimal TPSA and cLogP values (increased lipophilia), better metabolic stability and bioavailability (absence of hydroxyl groups on ring A, which are subject to deactivating metabolism).[126,127] In view of these encouraging preliminary results, CMS-121 has been extensively studied in in vivo animal models, confirming its beneficial properties even in complex aging systems: preservation of brain mitochondrial homeostasis in SAMP8 mice, as models of aging and dementia, by maintaining high levels of acetyl-CoA and acetylated histone H3K9, which have neuroprotective effects (prevention of decline in cognitive functions, memory enhancement);[128,129] reduction of AD-related cognitive decline, modulation of lipid peroxidation and reduction of neuroinflammation/degeneration in

the brains of APPswe/PS1ΔE9 transgenic AD mice by modulating the fatty acid synthetase and the oxytosis/ferroptosis cell death process;[130] protective effect against age-related hearing impairment (ARHI) in SAMP8 mice;[131] alleviation of diabetes, inflammation and renal damage in db/db mice with leptin receptor deficiency;[132] slowing of motor disfunction and increase in median life span of R6/2 and YAC128 mouse models of Huntington's disease.[133]

Compound **37**, instead, with an aza-flavanone scaffold (Figure 27), showed promising antioxidant and neuroprotective effects in Parkinson's disease (PD) models of transgenic *Drosophila melanogaster* parkin mutant flies, a system very similar to humans. In detail, it was capable to improve the neuromotor coordination system (reduction of motor deficit), increase the lifespan and improve the learning capabilities of treated flies, in comparison to untreated ones. These anti-PD effects were confirmed by more detailed in silico and in vitro analyses, in order to better clarify the possible mechanism of action. Molecular docking studies underlined the capability of **37** to bind strongly to the dopamine binding site of D2 dopamine receptor and to form the same pattern of interactions of the natural neurotransmitter (Asp^{114}, which binds the amino group of dopamine, is coordinated by the resorcinol group; Ser^{193} interacts with the NH of the central aza-flavone scaffold), suggesting a potential agonist activity. Furthermore, it was capable to increase in vitro the expression of the tyrosine hydroxylase (enzyme involved in dopamine biosynthesis) and to maintain mitochondrial health, indicating a betterment of dopaminergic neurons and a delay in their loss.[134]

Several other compounds showed antioxidant neuroprotective activity by displaying different modes of action. For example, the *N*-ethyltrihydroxy aza-isoflavone **38** (Figure 27) blocked in a dose-dependent manner the production/release of the proinflammatory mediator nitric oxide (NO) in LPS activated BV-2 microglia cells (IC_{50} = 7.83 μM), which are involved in pathological inflammatory events in CNS.[135] Similarly, 3′-hydroxythioflavanone **39** (Figure 21) showed potent antioxidant activity and capability to inhibit NO production in LPS-stimulated macrophage cells, with an IC_{50} of 10.55 μM.[136]

Better antioxidant and neuroprotective activity was also found for aza-flavone compounds **40a-c** (Figure 27) in comparison to the corresponding flavonoid compounds, as evidenced by the analysis of the peroxyl and hydroxyl radical scavenging and ferric ion reducing activities.[137]

In general, from a structural analysis of the flavonoid analogues in Figure 27, the presence of at least one hydroxy group attached to an aromatic system is an essential prerequisite to achieve the desired improved antioxidant activity, and, noteworthy, even in this case the bioisosteric substitution affected positively the desired antioxidant activity, leading to compounds with improved properties compared to the parent flavonoid compounds.

Furthermore, many flavonoid compounds, due to their capability to interfere with the prostanoid production, were also considered for their beneficial effects in the treatment of inflammatory and autoimmune diseases. In this light, several noteworthy heterocyclic aza-analogues have been investigated and reported in the literature.

For example, compounds **41a,b** (Figure 28), 2-arylquinoline analogues of the flavonoid baicalein (Figure 24), were rationally designed as the first selective inhibitors of 12R-lipoxygenase (12R-LOX), a metalloenzyme which catalyzes the conversion of arachidonic acid in its key pro-inflammatory metabolites and involved in many skin related inflammatory diseases, such as psoriasis. Indeed, when screened in vitro, they were capable to inhibit 12R-LOX activity by 65 and 81 % at 100 μM with an IC_{50} of 28.25 μM and 12.48 μM, respectively.[138]

Even if the flavonoid reference compound showed a much lower IC_{50} (6.5 μM), **41a,b** have a higher selectivity towards 12R-hLOX than towards the other lipoxygenase 12S-hLOX, 15-hLOX and 15-hLOXB than baicalein, which, in contrast, at a concentration of 100 μM, almost completely inhibited the activity of all of them (% of inhibition in the range > 80). In addition, in colony formation assay they reduced the hyperproliferative state of psoriatic keratinocytes. These results were further corroborated by in silico studies of molecular docking and dynamics simulations, which underscored the capability of both compounds to interact, among all, with the crucial amino acid Val^{631}, which is specific of the 12R-hLOX binding site, and to form stable complexes along time with the only 12R-hLOX, explaining the evidences of target selectivity.[138]

Similarly, the azaisoflavone compound **42** (Figure 28) was rationally designed in-silico as potential dual inhibitor of both thromboxane prostanoid receptor (TP) and cyclooxygenase-2 (COX-2).[139]

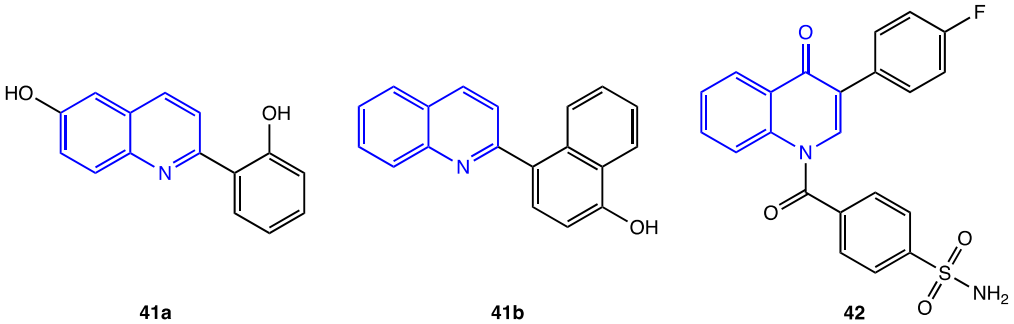
2.5. Estrogen modulatory activity

Several flavonoids and polyphenolic compounds, thanks to their phytoestrogen activity, exhibited capability to bind and interfere with the estrogen receptor, thus with potential application in estrogen-related endocrine disfunctions.[140,141]

Genistein (Figure 15), for example, was discovered to be a selective modulator of estrogen-mediated endocrine signaling, due to its capability to bind effectively and selectively to ERβ with modest affinity for ERα. Considering the implication of the β isoform in various diseases such as breast cancer, genistein has been widely used as a template to design new even more selective ER β modulators, and, even in this case the scaffold-hopping strategy was successful.

In this context, Vu and co-workers reported a series of heterocyclic flavonoid analogues as estrogen receptor beta-modulators (Figure 29), designed by replacing the isoflavone core of genistein with a 2-phenylquinoline one and maintaining two of the hydroxyl groups at the 6 and 4′ positions of the parent natural lead compound. Among all, the dihalogenated compound **43** (Figure 29) resulted the most potent and selective compound in vitro, with ERβ IC_{50}: 3.4 ± 1.5 nM, ERα IC_{50}: 283 ± 113 nM, an outstanding subtype selectivity (index ERα/ERβ: 83, >2-fold higher than genistein).[142]

Consistent with the mechanism of action, cell-based transcriptional assay performed on in vivo models showed that compound **43** acts as a selective partial agonist for ERβ, with no appreciable ability to stimulate and activate ERα. Analysis of molecular docking data conducted on both



41a **41b** **42**

Figure 28. Example of aza-flavonoid compound with anti-inflammatory activity.

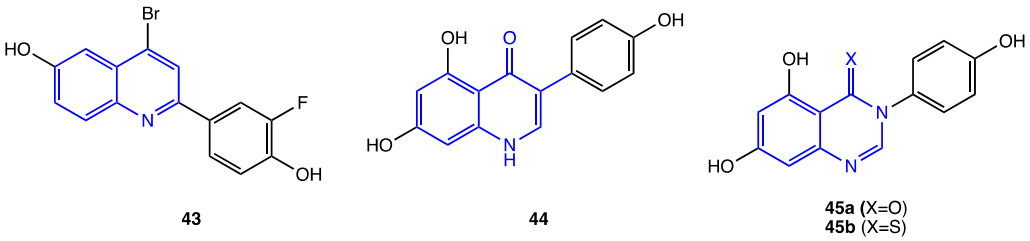


Figure 29. 2D structures of the most representative heterocyclic analogues of genistein with estrogen receptor modulatory activity.

the ERα and ERβ binding pockets, emphasized the two electronegative halogen substituents are essential for ERβ selectivity, as they were able to form strong van der Waals interactions with specific hydrophobic residues (e.g.: Ile^{373}) within the single ERβ catalytic site; in silico findings even explained the higher affinity for β isoform at microscopic level.[142]

With the same aim, Gim et al. synthetized the aza-isoflavone analogue of genistein **44** (Figure 29), which exhibited remarkable ERβ and relatively low ERα binding affinity (IC_{50} of 4.2 μM and 74.5 μM, respectively), as well as a biphasic effect on MCF-7 cell growth (stimulation of proliferation at low concertation and inhibition at higher concentration).[143]

A bioisosteric replacement with the quinazolinone scaffold was also carried out, with remarkable results in terms isoform selectivity enhancement. Among all, 5,7-dihydroxy-3-(4-hydroxyphenyl)-4(3H)-quinazolinone **45a** (Figure 29) showed agonist activity on both ER subtypes, but with a preferential selectivity for ERβ isoform, (compared to genistein: 62-fold higher binding affinity, with an IC_{50} of 179 nM and an EC_{50} of 76 nM in the transcription assay). Noteworthy, the switch from the C═O moiety into a C═S group, yielded the 5,7-dihydroxy-3-(4-hydroxyphenyl)-4(3H)-quinazoline-thione **45b** (Figure 29), which had a 4-fold improved binding affinity and selectivity for ERβ over ERα, in comparison with **45a** (IC_{50} value of 47 nM, and EC_{50} value of 13 nM).[144]

In general, for the entire class of heterocyclic derivatives, it can be observed that the hydroxyl groups of the lead genistein needs to be retained to maintain the desired activity and β-selectivity, as they form a strong pattern of interactions (H-bonds) with key residues within ERβ binding site, such as Glu^{353}, Arg^{394} and His^{524} of ERα/Glu^{305}, Arg^{346} and His^{47}. Any modification of to the hydrogen-bonding network, whether by removing, shifting, or protecting the hydroxyl groups could led to significant loss in ERβ binding.

2.6. Miscellanea

In this section aza-flavonoids with different biological activity are briefly discussed due to the small number of studies in these areas.

Flavonoid compounds showed interesting beneficial effects on metabolic diseases, such as dyslipidemia and diabetes. For example compound **46** (Figure 30), an scaffold-hopping aza-analogue of the flavanone bavachinin (Figure 15), was identified as an agonist of pan-peroxisome proliferator activated receptors (PPARs, involved in the regulation of glucose, lipid and cholesterol metabolism) with comparable/higher activity than the natural reference compound.[145]

Similarly, aza-flavanone compound **47** (Figure 30) was identified as a potent inhibitor of α-glucosidase, an enzyme involved in carbohydrate metabolism and absorption and thus a potential target for antidiabetic therapy. Specifically, it displayed in vitro inhibitory activity 6–8 fold higher than that of the reference standards acarbose, voglibose and miglitol (IC_{50} of 53 μM vs 324–462 μM) and also a favorable pose and interactions, as demonstrated by docking analysis.[146]

Even some diaza flavonoid analogues with a bioisosteric 2-phenyl-pyrido[1,2-*a*]pyrimidin-4-one scaffold exhibited extremely potent and selective antidiabetic activity. Compound **48** (Figure 30), with a crucial catechol fragment was the most interesting compound as it led to the following results: strong inhibitory activity on aldose reductase 2 (ALR2), an enzyme involved in the pathogenesis of diabetes, with a 10-fold increase compared to the lead compound quercetin (IC_{50} of 0.10 and 7.81 μM, respectively); potent antioxidant effect, with ROS scavenging activity. As highlighted in molecular modelling studies, the pyrido-pyrimidinone nucleus as well as the polyphenolic decoration were essential for both the enzyme inhibition and the antioxidant effect, confirming the importance of scaffold-hopping as efficient lead-optimization strategy.[147]

In addition to flavonoid analogues with anticancer activity (see Table 3), the pyrrolo-azaflavone scaffold also yielded compounds with remarkable platelet aggregation inhibitory activity, such as compound **49**. With a 3-phenylpyrroloquinazolinone core (Figure 31), it induced complete inhibition of collagen- and thrombin-induced platelet aggregation in the micromolar range, better than the corresponding quinolinone derivatives.[148]

For the therapeutic treatment of cardiovascular disorsers (e.g. restenosis, atherosclerosis, thrombosis, ischemia) a series of diaza-flavonoid analogues with 2,3-diphenyl-4H-pyrido[1,2-*a*]pyrimidin-4-one core was designed by following a scaffold-hopping approach on the structures of quercetin and apigenin (Figure 23 and 32), which are also known for their cardioprotective effects. Among all, compound **DB103** (Figure 32) showed much more improved properties in regulating inflammation and endothelial cell dysfunction compared to the two flavonoid parent compounds, such as: no reduction of cell viability on human umbilical vein endothelial cells (HUVECs) cells in the range 10–50 μM with improved vasoprotective and antiapoptotic effects (toxicity was observed for quercetin and apigenin, at the same concentrations); selective inhibition of tumor necrosis factor α (TNF-α) and matrix metalloprotease-9 (MMP-9) production; reduction of chronic

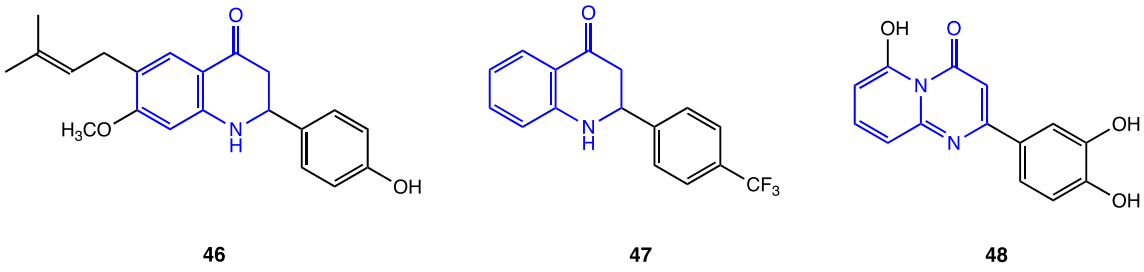
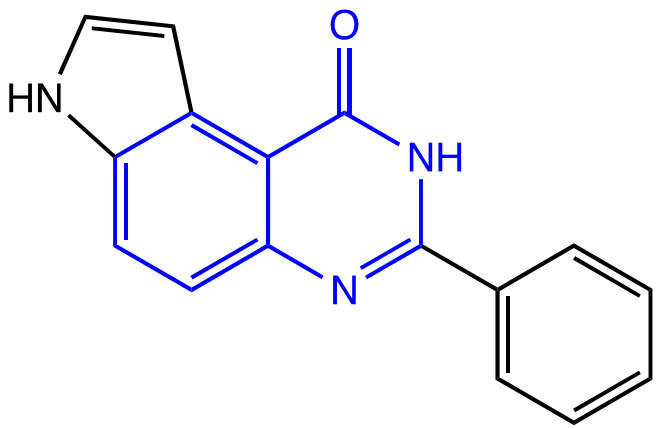


Figure 30. Chemical structures of compounds **46**–**48**, two aza-flavanone analogues with antilipidemic and antidiabetic activity.



49

Figure 31. Chemical structure of 3-phenylpyrroloquinazolinone derivative **49**, an aza-flavonoid analogue with platelet aggregation inhibitory activity.

inflammation, angiogenesis and smooth muscle cell proliferation and migration; better inhibition of collagen-induced platelet activation and aggregation in a concentration-dependent manner when evaluated in ex vivo studies on platelet-rich plasma (IC_{50} of 27, 70 and 55 μM for DB103, quercetin and apigenin, respectively); inhibition of activity and expression of several factors involved in platelet aggregation and platelet-endothelial adhesion, e.g. P-selectin. [149-151]

Since compound **DB103**, unlike the natural flavonoids quercetin and apigenin, exhibits these beneficial effects at concentrations fully consistent with its in vivo administration, it might be used both as a therapeutic agent,[152] and as a dietary supplement/additive in functional foods as a substitute for of natural flavonoids in the prevention and treatment of cardiovascular diseases.

Moreover, considering that several flavonoid analogues have shown interesting agonist activity at the allosteric benzodiazepine site of GABAa receptor, the effects of aza-bioisoster substitution have also been investigated in this field. As an example, in Figure 33 the chemical structure of **50**, one of the most representative aza-flavone ligands of GABAa receptor, is reported; in particular, preliminary studies evidenced its potent affinity against the target receptor, with a k_i in the nanomolar range and higher than the corresponding flavonoid compound.[153]

3. Conclusions and future perspectives

Natural products still represent nowadays one of the most fruitful sources of new molecular entities with high structural diversity and unexplored scaffolds endowed with various and interesting bioactivities.[1]

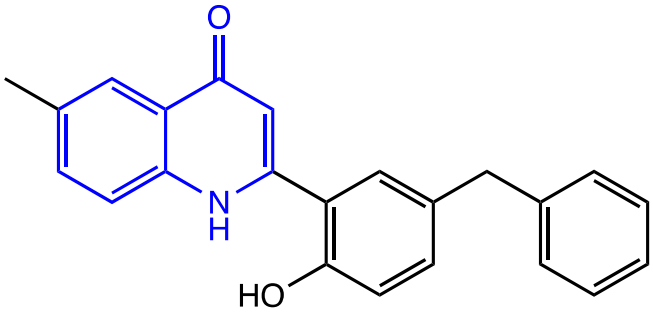
In this context, flavonoids, a class of natural polyphenolic compounds, stand among the most interesting and investigated families, attracting continuously increasing attention in drug discovery campains.[3–5,154] However, despite these enormous potentials, their modest physicochemical, pharmacokinetic, and drug-like properties (suboptimal solubility, low cell permeability and bioavailability, chemical instability) as well as their often promiscuous pharmacodynamics (high target promiscuity and off-target interactions/limited selectivity of action), have slowed or even halted the progress of these compounds in the most advanced phases of drug development.[1,2,155]

Many efforts have been made, both in the field of drug delivery (particular carrier systems to increase stability and improve bioavailability) and in medicinal chemistry (decoration of the central phenylchromen-4-one core) to overcome these issues.[5,6,10–18].

However, such an interesting lead optimization strategy is represented by the scaffold-hopping approach, standing as innovative and successful tool to design new druggable molecular spaces, bioactive agents, and more potent compounds, i.e. with improved P3 properties.[20–22] In this regard, to the best of our knowledge there has been no critical analysis on the impact of such structural modification on flavonoid compounds.

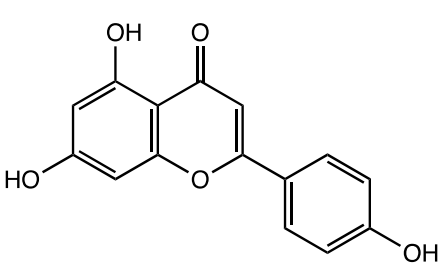
In this light, in the present study we reported and analyzed in detail the example of scaffold-hopping in this area, evidencing the benefit of substituting the chromon-4-one flavonoid core with other bioisosteric heterocycles (e.g. quinolones, quinazolinones, quinazolones, thioflavones, thioflavanones, pyridopimidinones etc…), whose importance in the design of bioactive small molecules is widely documented in the literature.

In general, from the data herein discussed the newly designed heterocyclic flavonoid analogues showed improved pharmacokinetics and dynamics properties in comparison to the parent compounds:

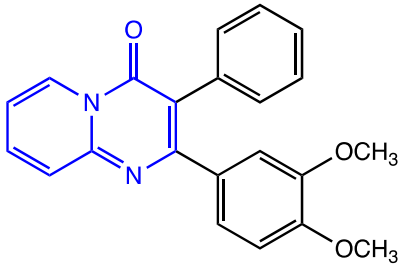


50

Figure 33. Chemical structure of aza-flavone analogue **50**, as ligand of the benzodiazepine binding site of brain GABAa receptor.



apigenin



DB103

Figure 32. Chemical structure of apigenin and the diaza derivative DB103.

- Lower target promiscuity and off-target interactions: appropriate decoration of the scaffold-hopped derivatives led to new chemical entities with better selectivity/specificity of action in comparison to the multi-targeted lead flavonoid (e.g. compounds **14**, **15**, **21**, **43**–**45** derived from genistein; compounds **33**, **48**, **DB103** derived from quercetin; compounds **33** and **46** derived from bavachinin; compounds **34** and **41** derived from baicalein).
- Improved chemical stability, solubility, and bioavailability: nitrogen heterocycles are often more stable and with a better solubility/lipophilicity ratio in comparison to O one.
- Stronger interactions with the target proteins, as shown both by in silico and in vitro enzyme inhibition studies: in most cases, the switch from the H-bond acceptor (O) to the H-bond donor and basic group (NH) allowed for a higher number of interactions (H-bonds) within the protein catalytic site.

From a structural point of view, the scaffold-hopping with nitrogen heterocycle has been more frequent than the sulphur one, which instead was less practiced. In this regard, a special attention should be paid also to the easier synthetic accessibility, and consequently, better versatility in the derivatization and more chances for SAR explorations particularly of the nitrogen cores described. Indeed, chemical synthesis of these scaffolds is highly studied and well documented in the literature, and moreover, they are more suitable skeletons for relatively simple chemical modifications, in comparison to O ones, whose synthetic routes are scarce and often challenging.[156–158] Of note, the possibility of *N*-derivatization (*N*-alkylation/acylation/sulfonation/arylation), which is not suitable for S and O, allowed, in many cases, to introduce crucial fragments, responsible for increased biological activity (e.g. compounds **4a-c**, **12**, **16**, **18**, **38**). In addition, scaffold-hopping with diaza heterocycles, where a second endocyclic heteroatom was added, was interestingly confirmed as a fruitful path in the lead optimization of natural flavonoids, as witnessed by dyhydroquinazolin-4-one (e.g. compound **31, 45a,b**, **49**), quinazolin-4-one (e.g. compound **34a-c)** pyridopyrimidones (e.g. compounds **27**, **28**, **48**, DB103), naphthyridin-4-one (e.g. CSC-3436) derivatives above described.

From another point of view, several interesting SAR considerations can be extrapolated by focusing on the major therapeutic classes analyzed, aiming at guiding future lead optimization strategy of flavonoids through scaffold-hopping approach:

- Antibiotic activity: in this field a wide series of quinol-4-one and quinoline derivatives with anti-AMR activity have been reported. In particular, a strong correlation was found between the position of the side phenyl group and the potential target/therapeutic indication: on one hand, an azaflavone core was required for NorA inhibitory activity, i.e. for treatment of MRSA infections; on the other hand, the azaisoflavone core, with the phenyl ring attached in the C3 position, was required for MAV/MAC efflux pump, which is specific in NTM bacteria. Furthermore, other crucial structural fragments were shared between both subgroups, constituting a leitmotif for the whole class: presence of alkoxy groups (OCH_3, OiPr) and of alkylamine side chains to modulate the solubility and the cell permeability.
- Anticancer activity: this was the most heterogenous class, with a lot of bioactive heterocyclic analogues with various mechanism of actions, and also the most explored from a structural point of view:
  - o Antitubulin and antimitotic compounds were characterized by an azaflavone core with various methoxy groups attached or by an unusual tryciclic pyrrolo-azaflavone core;
  - o Aza-isoflavonoid core was reported in many analogues with EGFR inhibitory activity;
  - o Scaffold-hopping with diaza cores, such as pyrido-pyrimidone scaffold, led to potent inhibitors of Topo II;
- Antiviral activity: aza-flavone scaffold decorated with both OH and OCH_3 resulted privileged for anti-influenza activity. On the other hand, a second aromatic ring attached at C3 or C4 of the central aza-flavonoid core seems required in anti-SARS-CoV-2 analogues.
- Antioxidant, anti-inflammatory and neuroprotective activity: multiple phenolic groups, which are responsible for high water solubility, resulted essential for the desired radical scavenging effect. However, the presence of a nitrogen heterocycle, instead of an O one, provided compounds with a better balance between hydrophilicity and lipophilicity, i.e. acceptable water solubility and, meanwhile, lipophilicity compatible with cell and membrane permeation (higher absorption, enhanced BBB penetration). As an example, CMS-121, thanks to its quinoline nucleus, exhibited an improved antioxidant and neuroprotective properties in vivo than the reference flavone fisetin.
- Estrogen modulator activity: the aza-isoflavonoid core as well as the presence of OH groups characterized the most interesting compounds of this class.

In conclusion, in the present Perspective, we provided a detailed and critical analysis of the examples of scaffold-hopping approaches on natural flavonoids published in the literature in the last decades, focusing on the potential benefits of this lead-optimization strategy, as well as potential future directions and challenges, in the hope that this will be useful for the whole medicinal chemistry community to better exploit the enormous potential of these natural products.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

CRediT authorship contribution statement

Gabriele La Monica: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alessia Bono:** Writing – original draft, Visualization, Data curation. **Federica Alamia:** Writing – original draft, Visualization, Formal analysis, Data curation. **Antonino Lauria:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Funding acquisition. **Annamaria Martorana:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by SiciliAn MicronanOTecH Research And Innovation Center "SAMOTHRACE" (MUR, PNRR-M4C2, ECS_00000022), spoke 3 -Università degli Studi di Palermo "S2-COMMs - Micro and Nanotechnologies for Smart & Sustainable Communities and National Biodiversity Future Center (NBFC) - SPOKE 6 - B73C22000790001.

References

1. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT, Taskforce INPS. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov*. 2021; 20:200–216. https://doi.org/10.1038/s41573-020-00114-z.
2. Dzobo K. The role of natural products as sources of therapeutic agents for innovative drug discovery. *Comprehensive Pharmacology*. 2022;2:408–422.

3. Liga S, Paul C, Flavonoids PF. Overview of biosynthesis, biological activity, and current extraction techniques. *Plants (Basel)*. 2023;12. https://doi.org/10.3390/plants12142732.
4. Chen S, Wang X, Cheng Y, Gao H, Chen X. A review of classification, biosynthesis, biological activities and potential applications of flavonoids. *Molecules*. 2023;28. https://doi.org/10.3390/molecules28134982.
5. Singh M, Kaur M, Silakari O. Flavones: an important scaffold for medicinal chemistry. *Eur J Med Chem*. 2014;84:206–239. https://doi.org/10.1016/j.ejmech.2014.07.013.
6. Thilakarathna SH, Rupasinghe HP. Flavonoid bioavailability and attempts for bioavailability enhancement. *Nutrients*. 2013;5:3367–3387. https://doi.org/10.3390/nu5093367.
7. Sajid M, Channakesavula CN, Stone SR, Kaur P. Synthetic biology towards improved flavonoid pharmacokinetics. *Biomolecules*. 2021;11. https://doi.org/10.3390/biom11050754.
8. Amawi H, Ashby CR, Tiwari AK. Cancer chemoprevention through dietary flavonoids: what's limiting? *Chin J Can*. 2017;36:50. https://doi.org/10.1186/s40880-017-0217-4.
9. Tritsch D, Zinglé C, Rohmer M, Grosdemange-Billiard C. Flavonoids: true or promiscuous inhibitors of enzyme? The case of deoxyxylulose phosphate reductoisomerase. *Bioorg Chem*. 2015;59:140–144. https://doi.org/10.1016/j.bioorg.2015.02.008.
10. Halevas EG, Avgoulas DI, Katsipis G, Pantazaki AA. Flavonoid-liposomes formulations: physico-chemical characteristics, biological activities and therapeutic applications. *Eur J Medi Chem Rep*. 2022;5:C7–100059. https://doi.org/10.1016/j.ejmcr.2022.100059 Scopus.
11. Ranjbar S, Emamjomeh A, Sharifi F, et al. Lipid-based delivery systems for flavonoids and flavonolignans: liposomes, nanoemulsions, and solid lipid nanoparticles. *Pharmaceutics*. 2023;15. https://doi.org/10.3390/pharmaceutics15071944.
12. Dewanjee S, Chakraborty P, Bhattacharya H, et al. Recent advances in flavonoid-based nanocarriers as an emerging drug delivery approach for cancer chemotherapy. *Drug Discov Today*. 2023;28, 103409. https://doi.org/10.1016/j.drudis.2022.103409.
13. Zverev YF, Rykunova AY. Modern nanocarriers as a factor in increasing the bioavailability and pharmacological activity of flavonoids. *Appl Biochem Microbiol*. 2022;58:1002–1020. https://doi.org/10.1134/S0003683822090149.
14. Chen L, Cao H, Huang Q, Xiao J, Teng H. Absorption, metabolism and bioavailability of flavonoids: a review. *Crit Rev Food Sci Nutr*. 2022;62:7730–7742. https://doi.org/10.1080/10408398.2021.1917508.
15. Li C, Dai T, Chen J, et al. Modification of flavonoids: methods and influences on biological activities. *Crit Rev Food Sci Nutr*. 2023;63:10637–10658. https://doi.org/10.1080/10408398.2022.2083572.
16. Massi A, Bortolini O, Ragno D, et al. Research progress in the modification of quercetin leading to anticancer agents. *Molecules*. 2017;22. https://doi.org/10.3390/molecules22081270.
17. Alizadeh SR, Ebrahimzadeh MA. Quercetin derivatives: drug design, development, and biological activities, a review. *Eur J Med Chem*. 2022;229, 114068. https://doi.org/10.1016/j.ejmech.2021.114068.
18. Kasprzak MM, Erxleben A, Ochocki J. Properties and applications of flavonoid metal complexes. *RSC Adv*. 2015;5:45853–45877. https://doi.org/10.1039/c5ra05069c Scopus.
19. Harrison C. Patenting natural products just got harder. *Nat Biotechnol*. 2014;32:403–404. https://doi.org/10.1038/nbt0514-403a.
20. Acharya A, Yadav M, Nagpure M, Kumaresan S, Guchhait SK. Molecular medicinal insights into scaffold hopping-based drug discovery success. *Drug Discov Today*. 2023;29, 103845. https://doi.org/10.1016/j.drudis.2023.103845.
21. Hu Y, Stumpfe D, Bajorath J. Recent advances in Scaffold hopping. *J Med Chem*. 2017;60:1238–1246. https://doi.org/10.1021/acs.jmedchem.6b01437.
22. Sun H, Tawa G, Wallqvist A. Classification of scaffold-hopping approaches. *Drug Discov Today*. 2012;17:310–324. https://doi.org/10.1016/j.drudis.2011.10.024.
23. Gupta T, Kataria R, Sardana S. A comprehensive review on current perspectives of flavonoids as antimicrobial agent. *Curr Top Med Chem*. 2022;22:425–434. https://doi.org/10.2174/1568026622666220117104709.
24. González A, Casado J, Lanas Á. Fighting the antibiotic crisis: flavonoids as promising antibacterial drugs against *Helicobacter pylori* infection. *Front Cell Infect Microbiol*. 2021;11, 709749. https://doi.org/10.3389/fcimb.2021.709749.
25. Kumar G, Kiran Tudu A. Tackling multidrug-resistant Staphylococcus aureus by natural products and their analogues acting as NorA efflux pump inhibitors. *Bioorg Med Chem*. 2023;80, 117187. https://doi.org/10.1016/j.bmc.2023.117187.
26. Waditzer M, Bucar F. Flavonoids as inhibitors of bacterial efflux pumps. *Molecules*. 2021;26. https://doi.org/10.3390/molecules26226904.
27. Rindi L. Efflux pump inhibitors against nontuberculous mycobacteria. *Int J Mol Sci*. 2020;21. https://doi.org/10.3390/ijms21124191.
28. Gibbons S. Anti-staphylococcal plant natural products. *Nat Prod Rep*. 2004;21:263–277. https://doi.org/10.1039/b212695h.
29. Gao C, Fan YL, Zhao F, et al. Quinolone derivatives and their activities against methicillin-resistant Staphylococcus aureus (MRSA). *Eur J Med Chem*. 2018;157:1081–1095. https://doi.org/10.1016/j.ejmech.2018.08.061.
30. Dorababu A. Recent update on antibacterial and antifungal activity of quinoline scaffolds. *Arch Pharm (weinheim)*. 2021;354:e2000232.
31. Pathania S, Narang RK, Rawal RK. Role of sulphur-heterocycles in medicinal chemistry: an update. *Eur J Med Chem*. 2019;180:486–508. https://doi.org/10.1016/j.ejmech.2019.07.043.
32. Guz NR, Stermitz FR, Johnson JB, et al. Flavonolignan and flavone inhibitors of a Staphylococcus aureus multidrug resistance pump: structure-activity relationships. *J Med Chem*. 2001;44:261–268. https://doi.org/10.1021/jm0004190.
33. Sabatini S, Gosetto F, Manfroni G, et al. Evolution from a natural flavones nucleus to obtain 2-(4-Propoxyphenyl)quinoline derivatives as potent inhibitors of the S. aureus NorA efflux pump. *J Med Chem*. 2011;54:5722–5736. https://doi.org/10.1021/jm200370y.
34. Sabatini S, Gosetto F, Iraci N, et al. Re-evolution of the 2-phenylquinolines: ligand-based design, synthesis, and biological evaluation of a potent new class of Staphylococcus aureus NorA efflux pump inhibitors to combat antimicrobial resistance. *J Med Chem*. 2013;56:4975–4989. https://doi.org/10.1021/jm400262a.
35. Felicetti T, Cannalire R, Pietrella D, et al. 2-Phenylquinoline S. aureus NorA Efflux pump inhibitors: evaluation of the importance of methoxy group introduction. *J Med Chem*. 2018;61:7827–7848. https://doi.org/10.1021/acs.jmedchem.8b00791.
36. Felicetti T, Cannalire R, Nizi MG, et al. Studies on 2-phenylquinoline Staphylococcus aureus NorA efflux pump inhibitors: New insights on the C-6 position. *Eur J Med Chem*. 2018;155:428–433. https://doi.org/10.1016/j.ejmech.2018.06.013.
37. Felicetti T, Cedraro N, Astolfi A, et al. New C-6 functionalized quinoline NorA inhibitors strongly synergize with ciprofloxacin against planktonic and biofilm growing resistant Staphylococcus aureus strains. *Eur J Med Chem*. 2022;241, 114656. https://doi.org/10.1016/j.ejmech.2022.114656.
38. Brawley DN, Sauer DB, Li J, et al. Structural basis for inhibition of the drug efflux pump NorA from Staphylococcus aureus. *Nat Chem Biol*. 2022;18:706–712. https://doi.org/10.1038/s41589-022-00994-9.
39. Palazzotti D, Felicetti T, Sabatini S, et al. Fighting antimicrobial resistance: insights on how the Staphylococcus aureus NorA efflux pump recognizes 2-phenylquinoline inhibitors by supervised molecular dynamics (SuMD) and molecular docking simulations. *J Chem Inf Model*. 2023. https://doi.org/10.1021/acs.jcim.3c00516.
40. Machado D, Cannalire R, Santos Costa S, et al. Boosting effect of 2-phenylquinoline efflux inhibitors in combination with macrolides against mycobacterium smegmatis and Mycobacterium avium. *ACS Infect Dis*. 2015;1:593–603. https://doi.org/10.1021/acsinfecdis.5b00052.
41. Lechner D, Gibbons S, Bucar F. Plant phenolic compounds as ethidium bromide efflux inhibitors in Mycobacterium smegmatis. *J Antimicrob Chemother*. 2008;62:345–348. https://doi.org/10.1093/jac/dkn178.
42. Cannalire R, Machado D, Felicetti T, et al. Natural isoflavone biochanin A as a template for the design of new and potent 3-phenylquinolone efflux inhibitors against Mycobacterium avium. *Eur J Med Chem*. 2017;140:321–330. https://doi.org/10.1016/j.ejmech.2017.09.014.
43. Felicetti T, Machado D, Cannalire R, et al. Modifications on C6 and C7 positions of 3-phenylquinolone efflux pump inhibitors led to potent and safe antimycobacterial treatment adjuvants. *ACS Infect Dis*. 2019;5:982–1000. https://doi.org/10.1021/acsinfecdis.9b00041.
44. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. *Nutrients*. 2020;12:2. https://doi.org/10.3390/nu12020457.
45. Rauf A, Imran M, Khan IA, et al. Anticancer potential of quercetin: a comprehensive review. *Phytother Res*. 2018;32:2109–2130. https://doi.org/10.1002/ptr.6155.
46. Beutler JA, Cardellina Ii JH, Lin CM, Hamel E, Cragg GM, Boyd MR. Centaureidin, a cytotoxic flavone from Polymnia fruticosa, inhibits tubulin polymerization. *Bioorg Med Chem Lett*. 1993;3:581–584. https://doi.org/10.1016/S0960-894X(01)81233-6 Scopus.
47. Beutler JA, Hamel E, Vlietinck AJ, et al. Structure-activity requirements for flavone cytotoxicity and binding to tubulin. *J Med Chem*. 1998;41:2333–2338. https://doi.org/10.1021/jm970842h.
48. Lewin G, Maciuk A, Thoret S, Aubert G, Dubois J, Cresteil T. Semisynthesis of natural flavones inhibiting tubulin polymerization, from hesperidin. *J Nat Prod*. 2010;73:702–706. https://doi.org/10.1021/np100065v.
49. Shi Q, Chen K, Li L, et al. Antitumor agents, 154. Cytotoxic and antimitotic flavonols from Polanisia dodecandra. *J Nat Prod*. 1995;58:475–482. https://doi.org/10.1021/np50118a001.
50. Xia Y, Yang ZY, Xia P, et al. Antitumor agents. Part 226: synthesis and cytotoxicity of 2-phenyl-4-quinolone acetic acids and their esters. *Bioorg Med Chem Lett*. 2003;13:2891–2893. https://doi.org/10.1016/s0960-894x(03)00624-3.
51. Chang YH, Hsu MH, Wang SH, et al. Design and synthesis of 2-(3-benzo[b]thienyl)-6,7-methylenedioxyquinolin-4-one analogues as potent antitumor agents that inhibit tubulin assembly. *J Med Chem*. 2009;52:4883–4891. https://doi.org/10.1021/jm900456w.
52. Cheng YY, Liu CY, Tsai MT, et al. Design, synthesis, and mechanism of action of 2-(3-hydroxy-5-methoxyphenyl)-6-pyrrolidinylquinolin-4-one as a potent anticancer lead. *Bioorg Med Chem Lett*. 2013;23:5223–5227. https://doi.org/10.1016/j.bmcl.2013.06.083.
53. Xia Y, Yang ZY, Xia P, et al. Antitumor agents. 181. Synthesis and biological evaluation of 6,7,2',3',4'-substituted-1,2,3,4-tetrahydro-2-phenyl-4-quinolones as a new class of antimitotic antitumor agents. *J Med Chem*. 1998;41:1155–1162. https://doi.org/10.1021/jm9707479.
54. Xia Y, Yang ZY, Hour MJ, et al. Antitumor agents. Part 204: synthesis and biological evaluation of substituted 2-aryl quinazolinones. *Bioorg Med Chem Lett*. 2001;11:1193–1196. https://doi.org/10.1016/s0960-894x(01)00190-1.
55. Zhang SX, Bastow KF, Tachibana Y, et al. Antitumor agents. 196. Substituted 2-thienyl-1,8-naphthyridin-4-ones: their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *J Med Chem*. 1999;42:4081–4087. https://doi.org/10.1021/jm990208z.

56. Li L, Wang HK, Kuo SC, Wu TS, Lednicer D, Lin CM, et al. Antitumor agents. 150. 2',3',4',5',5,6,7-substituted 2-phenyl-4-quinolones and related compounds: their synthesis, cytotoxicity, and inhibition of tubulin polymerization. *J Med Chem* **1994**, *37* (8), 1126-1135. DOI: 10.1021/jm00034a010.
57. Chou LC, Chen CT, Lee JC, et al. Synthesis and preclinical evaluations of 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one monosodium phosphate (CHM-1-P-Na) as a potent antitumor agent. *J Med Chem*. 2010;53:1616–1626. https://doi.org/10.1021/jm901292j.
58. Wang SW, Pan SL, Peng CY, et al. CHM-1 inhibits hepatocyte growth factor-induced invasion of SK-Hep-1 human hepatocellular carcinoma cells by suppressing matrix metalloproteinase-9 expression. *Can Lett*. 2007;257:87–96. https://doi.org/10.1016/j.canlet.2007.07.002.
59. Wang SW, Pan SL, Huang YC, et al. CHM-1, a novel synthetic quinolone with potent and selective antimitotic antitumor activity against human hepatocellular carcinoma in vitro and in vivo. *Mol Cancer Ther*. 2008;7:350–360. https://doi.org/10.1158/1535-7163.MCT-07-2000.
60. Liu CW, Lin YC, Hung CM, et al. CHM-1, a novel microtubule-destabilizing agent exhibits antitumor activity via inducing the expression of SIRT2 in human breast cancer cells. *Chem Biol Interact*. 2018;289:98–108. https://doi.org/10.1016/j.cbi.2018.04.007.
61. Chou LC, Yang JS, Huang LJ, et al. The synthesized 2-(2-fluorophenyl)-6,7-methylenedioxyquinolin-4-one (CHM-1) promoted G2/M arrest through inhibition of CDK1 and induced apoptosis through the mitochondrial-dependent pathway in CT-26 murine colorectal adenocarcinoma cells. *J Gastroenterol*. 2009;44:1055–1063. https://doi.org/10.1007/s00535-009-0111-1.
62. Tsai AC, Pan SL, Sun HL, et al. CHM-1, a new vascular targeting agent, induces apoptosis of human umbilical vein endothelial cells via p53-mediated death receptor 5 up-regulation. *J Biol Chem*. 2010;285:5497–5506. https://doi.org/10.1074/jbc.M109.036277.
63. Lai TY, Yang JS, Wu PP, et al. The quinolone derivative CHM-1 inhibits murine WEHI-3 leukemia in BALB/c mice in vivo. *Leuk Lymphoma*. 2010;51:2098–2102. https://doi.org/10.3109/10428194.2010.517279.
64. Lee JC, Chou LC, Huang CH, et al. CHM-1 induces apoptosis via p38-mediated upregulation of DR5 expression in human ovarian cancer SKOV3 cells. *Eur J Pharmacol*. 2011;670:96–104. https://doi.org/10.1016/j.ejphar.2011.08.006.
65. Lin YC, Hou SC, Hung CM, et al. Inhibition of the insulin-like growth factor 1 receptor by CHM-1 blocks proliferation of glioblastoma multiforme cells. *Chem Biol Interact*. 2015;231:119–126. https://doi.org/10.1016/j.cbi.2015.01.016.
66. Chou LC, Tsai MT, Hsu MH, et al. Design, synthesis, and preclinical evaluation of new 5,6- (or 6,7-) disubstituted-2-(fluorophenyl)quinolin-4-one derivatives as potent antitumor agents. *J Med Chem*. 2010;53:8047–8058. https://doi.org/10.1021/jm100780c.
67. Shen L, Chen YL, Huang CC, et al. CVM-1118 (foslinanib), a 2-phenyl-4-quinolone derivative, promotes apoptosis and inhibits vasculogenic mimicry via targeting TRAP1. *Pathol Oncol Res*. 2023;29:1611038. https://doi.org/10.3389/pore.2023.1611038.
68. Study of CVM-1118 for Patients with Advanced Neuroendocrine Tumors - A Phase II, Open-label Study of CVM-1118 Administered Orally to Patients With Advanced Neuroendocrine Tumors. ClinicalTrials.gov Identifier: NCT03600233. https://classic.clinicaltrials.gov/ct2/show/NCT03600233?term=03600233&draw=1&rank=1 (accessed).
69. CVM-1118 and Sorafenib Combination in Subjects With Advanced Hepatocellular Carcinoma - A Phase 2, Open-Label Study With Orally Administered CVM-1118 and Sorafenib in Subjects With Advanced Hepatocellular Carcinoma" ClinicalTrials.gov Identifier: NCT03582618. https://classic.clinicaltrials.gov/ct2/show/NCT03582618?term=03582618&draw=2&rank=1 (accessed).
70. Liu CY, Cheng YY, Chang LC, et al. Design and synthesis of new 2-arylnaphthyridin-4-ones as potent antitumor agents targeting tumorigenic cell lines. *Eur J Med Chem*. 2015;90:775–787. https://doi.org/10.1016/j.ejmech.2014.11.062.
71. Chang LC, Yu YL, Liu CY, et al. The newly synthesized 2-arylnaphthyridin-4-one, CSC-3436, induces apoptosis of non-small cell lung cancer cells by inhibiting tubulin dynamics and activating CDK1. *Cancer Chemother Pharmacol*. 2015;75:1303–1315. https://doi.org/10.1007/s00280-015-2765-0.
72. Wu ST, Sun GH, Cha TL, et al. CSC-3436 switched tamoxifen-induced autophagy to apoptosis through the inhibition of AMPK/mTOR pathway. *J Biomed Sci*. 2016;23:60. https://doi.org/10.1186/s12929-016-0275-y.
73. Huang CC, Cheng YC, Lin YC, et al. CSC-3436 sensitizes triple negative breast cancer cells to TRAIL-induced apoptosis through ROS-mediated p38/CHOP/death receptor 5 signaling pathways. *Environ Toxicol*. 2021;36:2578–2588. https://doi.org/10.1002/tox.23372.
74. Shobeiri N, Rashedi M, Mosaffa F, et al. Synthesis and biological evaluation of quinoline analogues of flavones as potential anticancer agents and tubulin polymerization inhibitors. *Eur J Med Chem*. 2016;114:14–23. https://doi.org/10.1016/j.ejmech.2016.02.069.
75. Řehulka J, Vychodilová K, Krejčí P, et al. Fluorinated derivatives of 2-phenyl-3-hydroxy-4(1H)-quinolinone as tubulin polymerization inhibitors. *Eur J Med Chem*. 2020;192, 112176. https://doi.org/10.1016/j.ejmech.2020.112176.
76. Ferlin MG, Chiarelotto G, Gasparotto V, et al. Synthesis and in vitro and in vivo antitumor activity of 2-phenylpyrroloquinolin-4-ones. *J Med Chem*. 2005;48:3417–3427. https://doi.org/10.1021/jm049387x.
77. Gasparotto V, Castagliuolo I, Chiarelotto G, et al. Synthesis and biological activity of 7-phenyl-6,9-dihydro-3H-pyrrolo[3,2-f]quinolin-9-ones: a new class of antimitotic agents devoid of aromatase activity. *J Med Chem*. 2006;49:1910–1915. https://doi.org/10.1021/jm0510676.
78. Gasparotto V, Castagliuolo I, Ferlin MG. 3-substituted 7-phenyl-pyrroloquinolinones show potent cytotoxic activity in human cancer cell lines. *J Med Chem*. 2007;50:5509–5513. https://doi.org/10.1021/jm070534b.
79. Viola G, Bortolozzi R, Hamel E, et al. MG-2477, a new tubulin inhibitor, induces autophagy through inhibition of the Akt/mTOR pathway and delayed apoptosis in A549 cells. *Biochem Pharmacol*. 2012;83:16–26. https://doi.org/10.1016/j.bcp.2011.09.017.
80. Hagenbuchner J, Lungkofler L, Kiechl-Kohlendorfer U, et al. The tubulin inhibitor MG-2477 induces autophagy-regulated cell death, ROS accumulation and activation of FOXO3 in neuroblastoma. *Oncotarget*. 2017;8:32009–32026. https://doi.org/10.18632/oncotarget.16434.
81. Ferlin MG, Bortolozzi R, Brun P, et al. Synthesis and in vitro evaluation of 3h-pyrrolo[3,2-f]-quinolin-9-one derivatives that show potent and selective anti-leukemic activity. *ChemMedChem*. 2010;5:1373–1385. https://doi.org/10.1002/cmdc.201000180.
82. Carta D, Bortolozzi R, Hamel E, et al. Novel 3-substituted 7-phenylpyrrolo[3,2-f]quinolin-9(6H)-ones as single entities with multitarget antiproliferative activity. *J Med Chem*. 2015;58:7991–8010. https://doi.org/10.1021/acs.jmedchem.5b00805.
83. Carta D, Bortolozzi R, Sturlese M, et al. Synthesis, structure-activity relationships and biological evaluation of 7-phenyl-pyrroloquinolinone 3-amide derivatives as potent antimitotic agents. *Eur J Med Chem*. 2017;127:643–660. https://doi.org/10.1016/j.ejmech.2016.10.026.
84. Bortolozzi R, Mattiuzzo E, Dal Pra M, et al. Targeting tubulin polymerization by novel 7-aryl-pyrroloquinolinones: Synthesis, biological activity and SARs. *Eur J Med Chem*. 2018;143:244–258. https://doi.org/10.1016/j.ejmech.2017.11.038.
85. Bortolozzi R, Carta D, Prà MD, et al. Evaluating the effects of fluorine on biological properties and metabolic stability of some antitubulin 3-substituted 7-phenyl-pyrroloquinolinones. *Eur J Med Chem*. 2019;178:297–314. https://doi.org/10.1016/j.ejmech.2019.05.092.
86. Ferlin MG, Conconi MT, Urbani L, et al. Synthesis, in vitro and in vivo preliminary evaluation of anti-angiogenic properties of some pyrroloazaflavones. *Bioorg Med Chem*. 2011;19:448–457. https://doi.org/10.1016/j.bmc.2010.11.010.
87. Carta D, Ferlin MG. An overview on 2-arylquinolin-4(1H)-ones and related structures as tubulin polymerisation inhibitors. *Curr Top Med Chem*. 2014;14:2322–2345. https://doi.org/10.2174/1568026614666141127120421.
88. Ravelli RB, Gigant B, Curmi PA, et al. Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature*. 2004;428:198–202. https://doi.org/10.1038/nature02393.
89. Ferreira A, Pousinho S, Fortuna A, Falcão A, Alves G. Flavonoid compounds as reversal agents of the P-glycoprotein-mediated multidrug resistance: biology, chemistry and pharmacology. *Phytochem Rev*. 2015;14:233–272. https://doi.org/10.1007/s11101-014-9358-0 Scopus.
90. Hadjeri M, Barbier M, Ronot X, Mariotte AM, Boumendjel A, Boutonnat J. Modulation of P-glycoprotein-mediated multidrug resistance by flavonoid derivatives and analogues. *J Med Chem*. 2003;46:2125–2131. https://doi.org/10.1021/jm021099i.
91. Aboutorabzadeh SM, Mosaffa F, Hadizadeh F, Ghodsi R. Design, synthesis, and biological evaluation of 6-methoxy-2-arylquinolines as potential P-glycoprotein inhibitors. *Iran J Basic Med Sci*. 2018;21:9–18. https://doi.org/10.22038/IJBMS.2017.21892.5616.
92. Feng S, Zhou H, Wu D, et al. Nobiletin and its derivatives overcome multidrug resistance (MDR) in cancer: total synthesis and discovery of potent MDR reversal agents. *Acta Pharm Sin B*. 2020;10:327–343. https://doi.org/10.1016/j.apsb.2019.07.007.
93. Cui J, Meng Q, Zhang X, Cui Q, Zhou W, Li S. Design and synthesis of new α-naphthoflavones as cytochrome P450 (CYP) 1B1 inhibitors to overcome docetaxel-resistance associated with CYP1B1 overexpression. *J Med Chem*. 2015;58:3534–3547. https://doi.org/10.1021/acs.jmedchem.5b00265.
94. Dong J, Wang Z, Cui J, Meng Q, Li S. Synthesis and structure-activity relationship studies of α-naphthoflavone derivatives as CYP1B1 inhibitors. *Eur J Med Chem*. 2020;187, 111938. https://doi.org/10.1016/j.ejmech.2019.111938.
95. Dong J, Wang Z, Meng Q, et al. Development of 2-arylbenzo[h]quinolone analogs as selective CYP1B1 inhibitors. *RSC Adv*. 2018;8:15009–15020. https://doi.org/10.1039/c8ra00465j.
96. Martorana A, La Monica G, Lauria A. Quinoline-based molecules targeting c-met, EGF, and VEGF receptors and the proteins involved in related carcinogenic pathways. *Molecules*. 2020:25 (18). https://doi.org/10.3390/molecules25184279.
97. Lauria A, La Monica G, Bono A, Martorana A. Quinoline anticancer agents active on DNA and DNA-interacting proteins: from classical to emerging therapeutic targets. *Eur J Med Chem*. 2021;220, 113555. https://doi.org/10.1016/j.ejmech.2021.113555.
98. Shagufta; Ahmad, I. An insight into the therapeutic potential of quinazoline derivatives as anticancer agents. *Medchemcomm* **2017**, *8* (5), 871-885. DOI: 10.1039/c7md00097a.
99. Dube PS, Legoabe LJ, Beteck RM. Quinolone: a versatile therapeutic compound class. *Mol Divers*. 2023;27:1501–1526. https://doi.org/10.1007/s11030-022-10581-8.
100. Traxler P, Green J, Mett H, Séquin U, Furet P. Use of a pharmacophore model for the design of EGFR tyrosine kinase inhibitors: isoflavones and 3-phenyl-4(1H)-quinolones. *J Med Chem*. 1999;42:1018–1026. https://doi.org/10.1021/jm980551o.
101. Xiao ZP, Li HQ, Shi L, Lv PC, Song ZC, Zhu HL. Synthesis, antiproliferative activity, and structure-activity relationships of 3-aryl-1H-quinolin-4-ones. *ChemMedChem*. 2008;3:1077–1082. https://doi.org/10.1002/cmdc.200800057.

102. Xiao ZP, Lv PC, Xu SP, Zhu TT, Zhu HL. Synthesis, antiproliferative evaluation, and structure-activity relationships of 3-arylquinolines. *ChemMedChem*. 2008;3: 1516–1519. https://doi.org/10.1002/cmdc.200800160.
103. Ling T, Lang W, Feng X, et al. Novel vitexin-inspired scaffold against leukemia. *Eur J Med Chem*. 2018;146:501–510. https://doi.org/10.1016/j.ejmech.2018.01.004.
104. Raut GK, Sukumar G, Chakrabarti M, et al. Anticancer effect and apoptosis induction by azaflavanone derivative in human prostate cancer cells, 27(11–12), pp. 825–839 *Apoptosis*. 2022. https://doi.org/10.1007/s10495-022-01745-w.
105. Jeong Y, Lim SM, Hong S. Discovery of wrightiadione as a novel template for the TrkA kinase inhibitors. *Bioorg Med Chem Lett*. 2015;25:5186–5189. https://doi.org/10.1016/j.bmcl.2015.09.070.
106. Kataoka T, Watanabe S, Mori E, Kadomoto R, Tanimura S, Kohno M. Synthesis and structure-activity relationships of thioflavone derivatives as specific inhibitors of the ERK-MAP kinase signaling pathway. *Bioorg Med Chem*. 2004;12:2397–2407. https://doi.org/10.1016/j.bmc.2004.02.002.
107. Bharathkumar H, Mohan CD, Ananda H, et al. Microwave-assisted synthesis, characterization and cytotoxic studies of novel estrogen receptor α ligands towards human breast cancer cells. *Bioorg Med Chem Lett*. 2015;25:1804–1807. https://doi.org/10.1016/j.bmcl.2015.01.030.
108. Zhang X, Zou G, Li X, et al. An isoflavone derivative potently inhibits the angiogenesis and progression of triple-negative breast cancer by targeting the MTA2/SerRS/VEGFA pathway. *Cancer Biol Med*. 2020;17:693–706. https://doi.org/10.20892/j.issn.2095-3941.2020.0010.
109. Chandrasekhar S, Pushpavalli SN, Chatla S, et al. aza-Flavanones as potent cross-species microRNA inhibitors that arrest cell cycle. *Bioorg Med Chem Lett*. 2012;22: 645–648. https://doi.org/10.1016/j.bmcl.2011.10.061.
110. Choi EJ, Lee JI, Kim GH. Evaluation of the anticancer activities of thioflavanone and thioflavone in human breast cancer cell lines. *Int J Mol Med*. 2012;29:252–256. https://doi.org/10.3892/ijmm.2011.834.
111. Priyadarshani G, Amrutkar S, Nayak A, Banerjee UC, Kundu CN, Guchhait SK. Scaffold-hopping of bioactive flavonoids: Discovery of aryl-pyridopyrimidinones as potent anticancer agents that inhibit catalytic role of topoisomerase IIα. *Eur J Med Chem*. 2016;122:43–54. https://doi.org/10.1016/j.ejmech.2016.06.024.
112. Hadjeri M, Peiller EL, Beney C, et al. Antimitotic activity of 5-hydroxy-7-methoxy-2-phenyl-4-quinolones. *J Med Chem*. 2004;47:4964–4970. https://doi.org/10.1021/jm049876x.
113. Rajput S, Gardner CR, Failes TW, Arndt GM, Black DS, Kumar N. Synthesis and anticancer evaluation of 3-substituted quinolin-4-ones and 2,3-dihydroquinolin-4-ones. *Bioorg Med Chem*. 2014;22:105–115. https://doi.org/10.1016/j.bmc.2013.11.047.
114. Zhang W, Liu J, Li Y, Guo F. A bavachinin analog, D36, induces cell death by targeting both autophagy and apoptosis pathway in acute myeloid leukemia cells. *Cancer Chemother Pharmacol*. 2022;90:251–265. https://doi.org/10.1007/s00280-022-04462-y.
115. Ninfali P, Antonelli A, Magnani M, Scarpa ES. Antiviral Properties of Flavonoids and Delivery Strategies. *Nutrients*. 2020;12. https://doi.org/10.3390/nu12092534.
116. Badshah SL, Faisal S, Muhammad A, Poulson BG, Emwas AH, Jaremko M. Antiviral activities of flavonoids. *Biomed Pharmacother*. 2021;140, 111596. https://doi.org/10.1016/j.biopha.2021.111596.
117. Zakaryan H, Arabyan E, Oo A, Zandi K. Flavonoids: promising natural compounds against viral infections. *Arch Virol*. 2017;162:2539–2551. https://doi.org/10.1007/s00705-017-3417-y.
118. Baxter A, Chambers M, Edfeldt F, et al. Non-covalent inhibitors of rhinovirus 3C protease. *Bioorg Med Chem Lett*. 2011;21:777–780. https://doi.org/10.1016/j.bmcl.2010.11.110.
119. Zima V, Radilová K, Kožíšek M, et al. Unraveling the anti-influenza effect of flavonoids: Experimental validation of luteolin and its congeners as potent influenza endonuclease inhibitors. *Eur J Med Chem*. 2020;208, 112754. https://doi.org/10.1016/j.ejmech.2020.112754.
120. Malbari K, Saha P, Chawla-Sarkar M, et al. In quest of small-molecules as potent non-competitive inhibitors against influenza. *Bioorg Chem*. 2021;114, 105139. https://doi.org/10.1016/j.bioorg.2021.105139.
121. La Monica G, Bono A, Lauria A, Martorana A. Targeting SARS-CoV-2 main protease for treatment of COVID-19: covalent inhibitors structure-activity relationship insights and evolution perspectives. *J Med Chem*. 2022;65:12500–12534. https://doi.org/10.1021/acs.jmedchem.2c01005.
122. Su HX, Yao S, Zhao WF, et al. Anti-SARS-CoV-2 activities in vitro of Shuanghuanglian preparations and bioactive ingredients. *Acta Pharmacol Sin*. 2020;41:1167–1177. https://doi.org/10.1038/s41401-020-0483-6.
123. Zhang K, Wang T, Li M, et al. Discovery of quinazolin-4-one-based non-covalent inhibitors targeting the severe acute respiratory syndrome coronavirus 2 main protease (SARS-CoV-2 M^{pro}). *Eur J Med Chem*. 2023;257, 115487. https://doi.org/10.1016/j.ejmech.2023.115487.
124. Wang HK, Bastow KF, Cosentino LM, Lee KH. Antitumor agents. 166. Synthesis and biological evaluation of 5,6,7,8-substituted-2-phenylthiochromen-4-ones. *J Med Chem*. 1996;39:1975–1980. https://doi.org/10.1021/jm960008c.
125. Zhang D, Ji X, Gao R, et al. Synthesis and antiviral activities of a novel class of thioflavone and flavonoid analogues. *Acta Pharm Sin B*. 2012;2:575–580. https://doi.org/10.1016/j.apsb.2012.10.005.
126. Chiruta C, Schubert D, Dargusch R, Maher P. Chemical modification of the multitarget neuroprotective compound fisetin. *J Med Chem*. 2012;55:378–389. https://doi.org/10.1021/jm2012563.
127. Schubert DR, Maher PA, Chiruta C. NEUROPROTECTIVE POLYPHENOL ANALOGS (August 2012), PCT/US2012/050324.
128. Currais A, Huang L, Goldberg J, et al. Elevating acetyl-CoA levels reduces aspects of brain aging. *Elife*. 2019:8. https://doi.org/10.7554/eLife.47866.
129. Kepchia D, Currais A, Dargusch R, Finley K, Schubert D, Maher P. Geroprotective effects of Alzheimer's disease drug candidates. *Aging (Albany NY)*. 2021;13: 3269–3289. https://doi.org/10.18632/aging.202631.
130. Ates G, Goldberg J, Currais A, Maher P. CMS121, a fatty acid synthase inhibitor, protects against excess lipid peroxidation and inflammation and alleviates cognitive loss in a transgenic mouse model of Alzheimer's disease. *Redox Biol*. 2020;36, 101648. https://doi.org/10.1016/j.redox.2020.101648.
131. Pham TB, Boussaty EC, Currais A, et al. Attenuation of age-related hearing impairment in senescence-accelerated mouse prone 8 (SAMP8) mice treated with fatty acid synthase inhibitor CMS121. *J Mol Neurosci*. 2023;73:307–315. https://doi.org/10.1007/s12031-023-02119-w.
132. Zahid S, Dafre AL, Currais A, Yu J, Schubert D, Maher P. The geroprotective drug candidate CMS121 alleviates diabetes, liver inflammation, and renal damage in db/db leptin receptor deficient mice. *Int J Mol Sci*. 2023:24 (7). https://doi.org/10.3390/ijms24076828.
133. Ates G, Taguchi T, Maher P. CMS121 partially attenuates disease progression in mouse models of Huntington's disease. *Mol Neurobiol*. 2023. https://doi.org/10.1007/s12035-023-03711-2.
134. Pant C, Chakrabarti M, Mendonza JJ, Ganganna B, Pabbaraja S, Pal Bhadra M. A*za*-Flavanone diminishes parkinsonism in the D*rosophila melanogasterParkin* mutant. *ACS Chem Neurosci*. 2021;12:4380–4392. https://doi.org/10.1021/acschemneuro.1c00285.
135. Jin GH, Ha SK, Park HM, et al. Synthesis of azaisoflavones and their inhibitory activities of NO production in activated microglia. *Bioorg Med Chem Lett*. 2008;18: 4092–4094. https://doi.org/10.1016/j.bmcl.2008.05.106.
136. Lee JI, Lee JH. Antioxidant and inhibitory activities of thioflavanones against nitric oxide production. *Food Sci Biotechnol*. 2014;23:957–963. https://doi.org/10.1007/s10068-014-0129-x Scopus.
137. Greeff J, Joubert J, Malan SF, van Dyk S. Antioxidant properties of 4-quinolones and structurally related flavones. *Bioorg Med Chem*. 2012;20:809–818. https://doi.org/10.1016/j.bmc.2011.11.068.
138. Bhuktar H, Shukla S, Kakularam KR, et al. Design, synthesis and evaluation of 2-aryl quinoline derivatives against 12R-lipoxygenase (12R-LOX): discovery of first inhibitor of 12R-LOX. *Bioorg Chem*. 2023;138, 106606. https://doi.org/10.1016/j.bioorg.2023.106606.
139. Hadianawala M, Mahapatra AD, Yadav JK, Datta B. Molecular docking, molecular modeling, and molecular dynamics studies of azaisoflavone as dual COX-2 inhibitors and TP receptor antagonists. *J Mol Model*. 2018;24:69. https://doi.org/10.1007/s00894-018-3620-0.
140. Kiyama R. Estrogenic flavonoids and their molecular mechanisms of action. *J Nutr Biochem*. 2023;114, 109250. https://doi.org/10.1016/j.jnutbio.2022.109250.
141. Collins-Burow BM, Burow ME, Duong BN, McLachlan JA. Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms. *Nutr Cancer*. 2000;38:229–244. https://doi.org/10.1207/S15327914NC382_13.
142. Vu, A. T.; Cohn, S. T.; Manas, E. S.; Harris, H. A.; Mewshaw, R. E. ERbeta ligands. Part 4: Synthesis and structure-activity relationships of a series of 2-phenylquinoline derivatives. *Bioorg Med Chem Lett* **2005**, *15* (20), 4520-4525. DOI: 10.1016/j.bmcl.2005.07.008.
143. Gim HJ, Li H, Jung SR, et al. Design and synthesis of azaisoflavone analogs as phytoestrogen mimetics. *Eur J Med Chem*. 2014;85:107–118. https://doi.org/10.1016/j.ejmech.2014.07.030.
144. Güngör T, Chen Y, Golla R, et al. Synthesis and characterization of 3-arylquinazolinone and 3-arylquinazolinethione derivatives as selective estrogen receptor beta modulators. *J Med Chem*. 2006;49:2440–2455. https://doi.org/10.1021/jm0509389.
145. Yi J, Du G, Zhao Y, et al. Bavachinin analogues as agonists of pan-peroxisome proliferator-activated receptors. *Med Chem Res*. 2018;27:1851–1862. https://doi.org/10.1007/s00044-018-2197-6 Scopus.
146. Kasturi S, Surarapu S, Bathoju CC, et al. Synthesis, molecular modeling and biological evaluation of aza-flavanones as α-glucosidase inhibitors. *Medchemcomm*. 2017;8:1618–1630. https://doi.org/10.1039/c7md00162b.
147. La Motta C, Sartini S, Mugnaini L, et al. Pyrido[1,2-a]pyrimidin-4-one derivatives as a novel class of selective aldose reductase inhibitors exhibiting antioxidant activity. *J Med Chem*. 2007;50:4917–4927. https://doi.org/10.1021/jm070398a.
148. Ferlin MG, Borgo C, Deana R. Synthesis and evaluation of platelet aggregation inhibitory activity of some 3-phenyl-pyrroloquinazolinones. *Eur J Med Chem*. 2012; 48:275–283. https://doi.org/10.1016/j.ejmech.2011.12.026.
149. Del Turco S, Sartini S, Sentieri C, et al. A novel 2,3-diphenyl-4H-pyrido[1,2-a] pyrimidin-4-one derivative inhibits endothelial cell dysfunction and smooth muscle cell proliferation/activation. *Eur J Med Chem*. 2014;72:102–109. https://doi.org/10.1016/j.ejmech.2013.11.021.
150. Del Turco S, Sartini S, Cigni G, et al. Synthetic analogues of flavonoids with improved activity against platelet activation and aggregation as novel prototypes of food supplements. *Food Chem*. 2015;175:494–499. https://doi.org/10.1016/j.foodchem.2014.12.005.
151. Del Turco S, Quattrini L, Colucci R, Gaggini M, La Motta C, Basta G. A 2,3-diphenylpyrido[1,2-a] pyrimidin-4-one derivative inhibits specific angiogenic factors induced by TNF-α. *Saudi Pharm J*. 2019;27:1174–1181. https://doi.org/10.1016/j.jsps.2019.09.014.
152. Coviello V, Sartini S, Quattrini L, Baraldi C, Gamberini MC, La Motta C. Cyclodextrin-based nanosponges for the targeted delivery of the anti-restenotic agent DB103: A novel opportunity for the local therapy of vessels wall subjected to percutaneous intervention. *Eur J Pharm Biopharm*. 2017;117:276–285. https://doi.org/10.1016/j.ejpb.2017.04.028.

153. Nilsson J, Nielsen E, Liljefors T, Nielsen M, Sterner O. Azaflavones compared to flavones as ligands to the benzodiazepine binding site of brain GABA(A) receptors. *Bioorg Med Chem Lett*. 2008;18:5713–5716. https://doi.org/10.1016/j.bmcl.2008.09.092.
154. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *ScientificWorldJournal*. 2013;2013, 162750. https://doi.org/10.1155/2013/162750.
155. Patridge E, Gareiss P, Kinch MS, Hoyer D. An analysis of FDA-approved drugs: natural products and their derivatives. *Drug Discov Today*. 2016;21:204–207. https://doi.org/10.1016/j.drudis.2015.01.009.
156. In Lee J. A review of the syntheses of (thio)flavones, 4-quinolones, (thio)aurones, and azaaurones from 2′-substituted alkynones. *Bull Kor Chem Soc*. 2021;42:1610–1623. https://doi.org/10.1002/bkcs.12412 Scopus.
157. Pinheiro S, Muri EMF, de Oliveira RPRF, Dias LRS, Greco SJ. Asymmetric catalysis in the synthesis of azaflavanones. *Mini-Rev Org Chem*. 2017;14:400–406. https://doi.org/10.2174/1570193X14666170518120501 Scopus.
158. Nibbs AE, Scheidt KA. Asymmetric methods for the synthesis of flavanones, chromanones, and azaflavanones. *Eur J Org Chem*. 2012;2012:449–462. https://doi.org/10.1002/ejoc.201101228.