Synthesis of Anti-angiogenetic Natural-like Acylphloroglucinols

and

Selective ABC Transporter Modulators

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Dedicated

To

My family

路漫漫其修远兮，吾将上下而求索。

The journey is long; I'll search up and down.

屈 原

Qu Yuan

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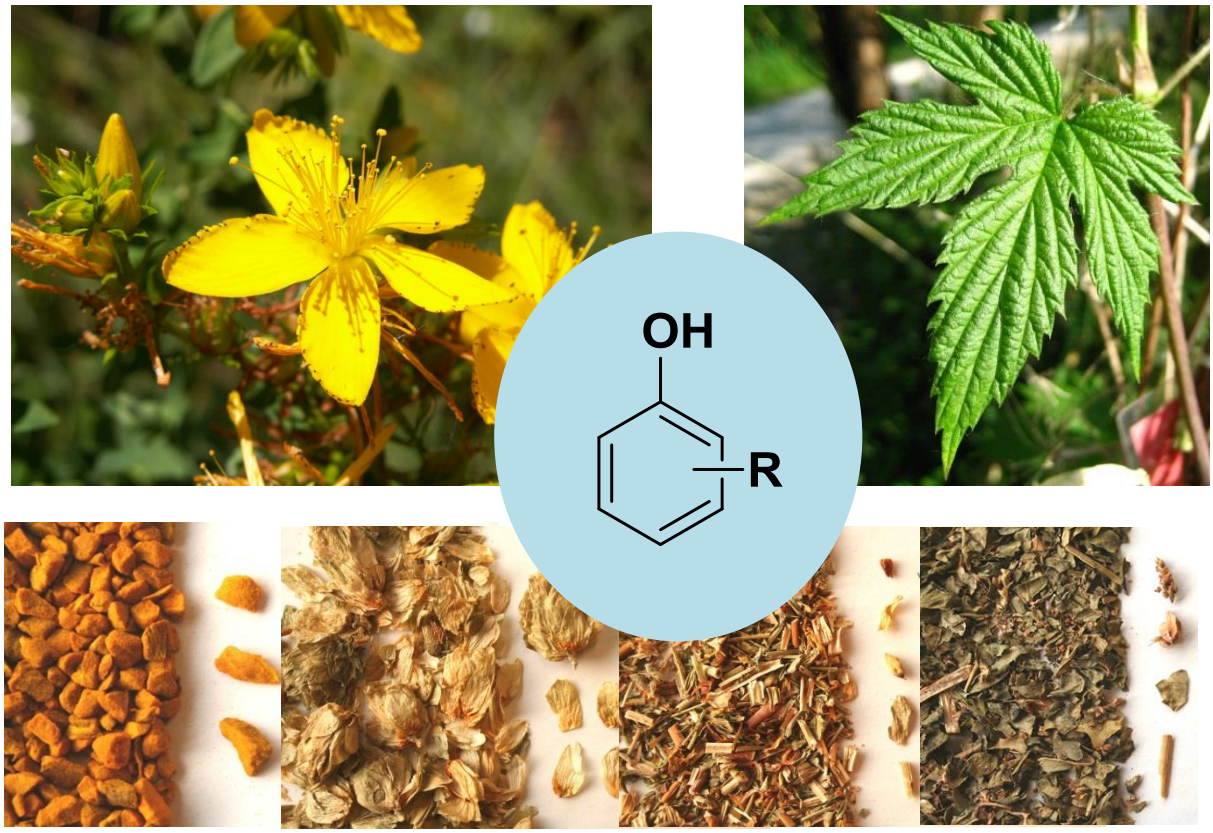
Chapter 1

Natural phenolic metabolites with anti-angiogenic properties – a review from the chemical point of view



Abstract

Within the secondary natural metabolites from plants, phenolic compounds have a special impact on human health as they occur in significant amounts in several fruits, vegetables and medicinal plants. In this review natural phenolic compounds of plant origin with significant anti-angiogenic properties are summarized. Thirteen representatives of eight different natural or natural like

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phenolic subclasses are presented with a particular emphasis on their synthesis and the methods to modify the parent compounds. Whenever available, the consequence of structural variation on the pharmacological activity of the molecules is described.

Keywords

natural phenolic compounds, angiogenesis, synthesis, structure-activity relationship

Introduction

The term “angiogenesis” is commonly used to describe the biological process of blood vessel growth. Nevertheless, it should be more precisely defined as formation of new blood vessels from pre-existing ones. Under physiological conditions angiogenesis is vital for foetal development, tissue regeneration and wound healing. Patho-physiologically, massive vascular growth or abnormal shape formation promotes many diseases including cancer, inflammation, and eye illness. However, inadequate vessel preservation or growth leads to ischemia causing myocardial infarction, stroke, and neurodegenerative or obesity-associated conditions.[1]

The generation of new blood vessels is based on a strictly controlled balance between various soluble and membrane-bound factors showing either anti- or pro-angiogenic activity and thus embedded together with enzymes and signalling molecules (**Table 1**) into a very complex network of signal pathways.[2] In case of cancer development the growing tumor disturbs the angiogenic balance in a tissue and induces the secretion of pro-angiogenic factors either by the tumor cells or by cells of the tumor microenvironment. When the tumor grows to the diameter of 1-2 mm, the tumor cells located far away from blood vessels undergo apoptosis or necrosis resulting from the lack of oxygen and nutrients. At this moment, tumor cells express pro-angiogenic factors including growth factors such as the vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) and enzymes like cyclo-oxygenase 2 (COX-2) and protein kinase A (PKA) as well as signalling molecules like the integrins. The evoked cascades induce the formation of new blood vessels quickly connected with the pre-existing blood vessels providing sufficient supplies for tumor survival.[3] In addition, the new blood vessels allow cancer cells to transfer from a parent location to other new locations causing metastases. Nevertheless, the morphology and pathophysiology of these blood vessels differs significantly from physiological ones as they work less effective and show a lower state of organization and control.[2a] After discovering the mechanism of angiogenesis and its crucial role in the tumor development, different therapies targeting to interfere with this process were investigated.[4] Preferred clinical target are the VEGF receptors leading to the development and approval of monoclonal antibodies against VEGF and VEGF receptor tyrosine kinase inhibitors.[2a] Nevertheless, the existing therapy options with antibodies and VEGF receptor inhibitors showed,

from the clinical point of view, several limitations making the search for further clinically relevant targets and other drugs mandatory to combat tumor related angiogenesis.

Table 1. Condensed overview on endogenous modulators involved in angiogenic processes

Group name	Modulators (abbreviation)
Growth factors	Vascular endothelial growth factor (VEGF)
	Fibroblast growth factor (FGF)
	Platelet-derived growth factor (PDGF)
	Transforming growth factor β1 (TGF-β1)
	Angiopoetins (Ang)
Adhesion molecules	Integrins
	Cadherins
Proteinases	Matrix metalloproteinases (MMPs)
	Urokinase plasminogen activator (uPA)
Extracellular matrix proteins	Fibronectin
	Collagens
Transcription factors	Nuclear factor (erythroid-derived 2)-related factor (Nrf2)
	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB)
	Activator protein (AP-1)
	Hypoxia inducible factor (HIF)
Other signalling molecules	Mammalian target of rapamycin (mTOR)
Other enzymes	Mitogen-activated-protein-kinases (MAP-kinases)
	Proteinkinases A and C (PKA and PKC)
	Proteinkinase B/Akt
	Cyclo-oxygenase 2 (COX-2)
	Nitric oxide synthase (NOS)

Accumulating research also showed that secondary natural metabolites are attractive candidates for the therapy of pathologically induced angiogenesis.[5] Among such natural products phenolic or polyphenolic compounds with anti-angiogenic properties have been investigated and the opinion on their pharmacological impact has changed over the last years. While the pharmacological activity of polyphenols was previously considered as unspecific, more recently observations of a specific interference with biological mechanisms at the molecular level are exponentially growing. Especially in the field of anti-inflammatory activity, chemoprevention and cytoprotection natural phenolic metabolites like flavonoids, caffeic acid derivatives and diarylheptanoids showed pleiotropic influence on cellular signalling e. g. by the inhibition of transcription factors like NF-κB or Nrf_2,[6] or anti-oxidative effects.[6b, 7] Furthermore, polyphenols are abundant in many plants used as fruits and vegetables in high concentrations, resulting in a continuous and long-term intake of such plant phenols. Consequently, their beneficial and protective impact on unbalanced angiogenic processes has been intensively discussed.[5]

In the last decade, many excellent review articles summarized the biological and pharmacological aspects of anti-angiogenic compounds including natural compounds with a phenolic substructure.[8] Complementary to the previously discussed pharmacological point of view this review focuses on recent reports of anti-angiogenic natural phenolic compounds specifically addressing their chemistry, synthesis and possible structure modifications. Nevertheless, it should be mentioned that the selection of compounds for this review is based on the reports on their pharmacological activity. As the term “anti-angiogenic compound” is not unequivocally defined and somewhat inflationarily used, appropriate inclusion criteria for the review had to be defined. Compounds included in our survey have shown anti-angiogenic activity not only in convenient (and often descriptive) cellular *in vitro* assays (**Table 2**), but also in molecular *in vitro* test systems related to the signalling cascades of pathological angiogenesis. Further inclusion criteria were the existence of anti-agiogenic activity obtained in *ex vivo* and *in vivo* assays (**Table 2**). Additionally, the observed *in vitro* activity should be in the lower µM range (or better) and thus high enough to realistically speculate on an anti-angiogenic activity *in vivo*. As endothelial cells (ECs) have an extraordinary significance in angiogenesis, results from cellular assays using primary or immortalized ECs like human umbilical vein endothelial cells (HUCEC) or human microvascular endothelial cells (HMEC-1) have been given special attention.

In contrast, compounds showing *in vitro* anti-angiogenic activity, but also most likely signs of strong unspecific cytotoxic effects *in vitro* have been excluded. The discussed secondary metabolites include six flavonoids from different subclasses namely quercetin, fisetin, epigallocatechin-3-O-gallate, xanthohumol, (2S)-7,2',4'-triihydroxy-5-methoxy-8-dimethylallyl flavanone and genistein. Other compounds belong to the groups of simple phenols (4-hydroxybenzyl alcohol), hydrolysable tannins (ellagic acid), stilbenoids (resveratrol) and diarylheptanoids (curcumin). In addition, acylphloroglucinols, quinoline substituted phenols and 4-amino-2-sulfanylphenol derivatives were discussed. Some important aspects of the described pharmacological activities of the compounds are summarized in **Table 3**.

Table 2. *In vitro*, *ex vivo* and *in vivo* assays to characterize anti-angiogenic activity*

***In vitro* assays**	**Assay principles / detection, read out**
Endothelial cell proliferation assays	Cell counting / Increase of cell number Crystal violet / Increase of cell number MTT / Activity of dehydrogenase activity (positively correlated to cell number) Incorporation of [^{3}H]thymidine, 5-bromodeoxyuridine into DNA / DNA synthesis (positively correlated to cell number)
Endothelial cell migration assays	Scratch assay / Migration into a denuded area (wound healing)
Endothelial cell differentiation assays	Tube formation e.g. in Matrigel/ Formation of capillary like tubules
Endothelial-Mural cell co-culture assays	Interaction between two cell types (endothelial/mural) / Influence on cell differentiation and proliferation
***Ex vivo* assays**	
Aortic ring assay	Aorta of rodents cultured in biological matrices / Outgrowth of branching microvessels
***In vivo* assays**	

-**Table 2** continued-

Chick chorioallantoic membrane assay (CAM)	Extra-embryonic membrane (in ovo, ex ovo) / growth and branching of blood vessels
Hen's egg test on chorioallantoic membrane (HETCAM)	CAM modification / Growth and branching of blood vessels
Zebrafish	Zebrafish embryos or transgenic zebrafish embryos / Visualization of vascularisation (e.g. with confocal microscopy)
Corneal angiogenesis assay	Corneal injury or implantation of pellets / Vascular response of the cornea
Dorsal air sac model	Ring (filled with tumor cell suspension) implantation (dorsal skin) / Tumor induced angiogenesis
Mouse models	Genetic engineered mouse models; xenografts

*****Molecular or enzyme assays not included

Table 3. Natural phenolic compounds with anti-angiogenic activity and their evaluated molecular mechanisms of anti-angiogenesis

Compound name	**Mechanisms of anti-angiogenic action**
4-Hydroxybenzyl alcohol	Down-regulation of VEGF and MMP9 protein expression
Curcumin	Reduction of VEGF expression, inhibition of transcription factors, mTOR pathway and MMP9 protein expression
Ellagic acid	Inhibition of VEGF and PDGF receptor phosphorylation
Resveratrol	Abrogation of VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin, inhibition of VEGF-induced and FGF-2 neovascularization
Quinoline substituted phenols	Inhibition of VEGF and transforming growth factor-β1 (TGF-β1) expression

-**Table 3** continued-

4-Amino-2-sulfanylphenol derivatives	Inhibition of protein kinase B/Akt and ABL tyrosine kinase
Nature-like acylphloroglucinol derivatives	Under investigation
(-)-Epigallocatechin gallate (EGCG)	Inhibition of estrogen-stimulated VEGF expression, HIF-1α and Nf-κB, inhibition of MMP-2 and MMP-9, inhibition of urokinase plasminogen activator
Xanthohumol	Inhibition of Nf-κB and Akt pathways
Genistein	Inhibition of VEGF and HIF-1*α* protein expression
Fisetin	Down-regulation of VEGF and eNOS expression, inhibition of MMPs activities
Quercetin	Inhibition of the expression of VEGF-2, inhibition of COX-2 and arachidonate 5-lipoxygenase (LOX-5), inhibition of Nf-κB, In some cell types it activates angiogenesis.
(2S)-7,2',4'-Triihydroxy-5-methoxy-8-dimethylallyl flavanone	Down-regulation of reactive oxygen speics (ROS) levels and VEGF expression

4-Hydroxybenzyl alcohol

4-Hydroxybenzyl alcohol (HBA, **1**) (**Figure 1**) is a well-known phenolic compound from plants and has been for example found in flowers of carrot (*Daucus carrota* L., Apiaceae). In 2007, Park and co-workers [9] found in the chick chorioallantoic membrane (CAM) assay no change of the vascular density in the presence of HBA, indicating that HBA has no influence on the growth of blood vessels. In contrast, the branching pattern of blood vessels was reduced dose-dependently in the same assay, making an inhibition of angiogenesis likely. Later, Laschke et al. (2011) [10] performed experiments *in vitro* with an aortic ring assay and *in vivo* in an

endometriosis model as well as systematic analysis of the mechanism underlying the anti-angiogenic activity of HBA. They found that HBA is capable of inhibiting several steps of the angiogenic mechanism. Western blot analysis showed the down-regulation of VEGF and MMP9 protein expression. The effect of HBA was confirmed [11] by mouse dorsal skinfold chamber experiments. Incubation of CT26.WT colon carcinoma cells with HBA showed a dose dependent decrease of their viability and integrity. In addition, the cells expression of the apoptosis marker cleaved caspase-3 increased significantly and the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9 decreased compared to controls. No influence on the normal behaviour of the animals was observed. In general, HBA represents an interesting anti-angiogenic agent for the treatment of angiogenic diseases.

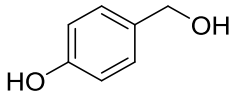

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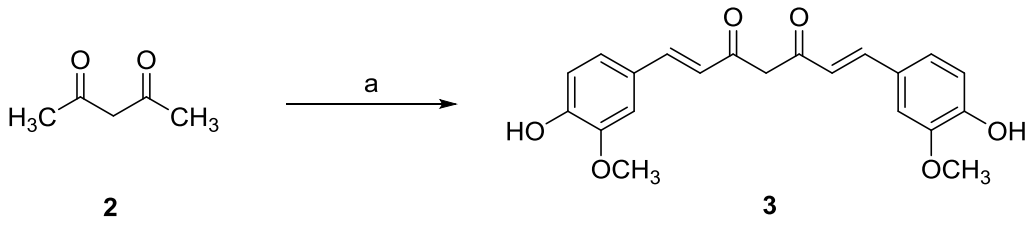
Figure 1. Structure of 4-hydroxybenzyl alcohol (HBA).

Curcumin

Curcumin (**3**) is a natural product isolated from different *Curcuma* species (Zingiberaceae) some of them used as raw material of the spice turmeric. It has been evaluated as a chemopreventive agent since the early nineties and in 1998, Arbiser and his co-workers found that the compound showed also anti-angiogenic properties *in vitro* and *in vivo.*[12] In the following years, many studies on the anti-angiogenic properties in different tumor cell lines or in animal models were reported.[13] They include interactions with the transcription factor Nf-κB, mTOR pathway, and reduction of VEGFA and MMP9 expression. Despite of its promising pharmacological properties, curcumin suffers from a low *in vivo* bioavailability as a consequence of its low aqueous solubility, poor chemical stability and low adsorption. Therefore many analogues (**Figure 2**) were synthesised in order to overcome these drawbacks and enhance the activity. In addition, their structure-activity relationships were studied to gain better insight into the mode of action. The general synthesis of curcumin itself (**Scheme 1**) requires masking of the reactive

methylene group of acetylacetone by formation of a complex with boric oxide, followed by reaction with vanillin. Instead of boric oxide, alkyl borate esters and boric acid can be used.[14] The first attempt of modification was to truncate the general structure to either a single enone or dienone system. The latter group showed a diarylpentanoid instead of the natural diarylheptanoid backbone, in some cases amended by a central ring system, and was labelled as monocarbonyl analogues of curcumin (MACs, **Figure 3**). Bowen et al.(2003) [15] used Claisen–Schmidt reaction for synthesis of these analogues. The C_7- chain between the two aromatic rings was shortened and a series of compounds (**Scheme 2)** with different substitutions on the aromatic rings was synthesised to explore stereoelectronic effects. It was demonstrated that those analogues of curcumin were excellent anti-angiogenic compounds, having inhibition patterns equivalent or better than the parent natural product. This work was continued by more comprehensive bioactive studies on aromatic enones utilizing the substituted chalcone backbone.[16] The study showed that the presence of the enone moiety played an important role in maintaining the activity in the curcumin analogues. Ahn et al. (2005) [17] left the enone part unchanged to the previous principle and prepared various curcumin mimics with asymmetric units with bearing alkyl amide, chloro-substituted benzamide, or heteroaromatic amide moieties. Those analogues showed stronger anti-angiogenic activity than curcumin against HUVEC. Up to now the number of synthesised single enones and MACs clearly broke the 1000 mark.



2 **3**

Scheme 1: Synthesis of Curcumin **3**. Reagents and conditions: (a) vanillin, 1,2,3,4-tetrahydroquinolin, HOAc, H_3BO_3, DMF, Δ 4 h.

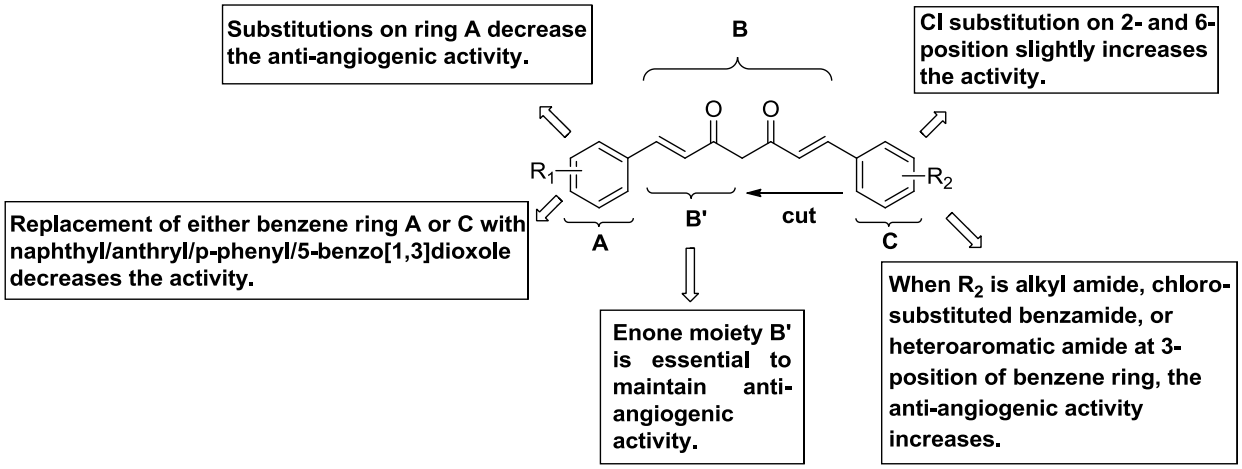
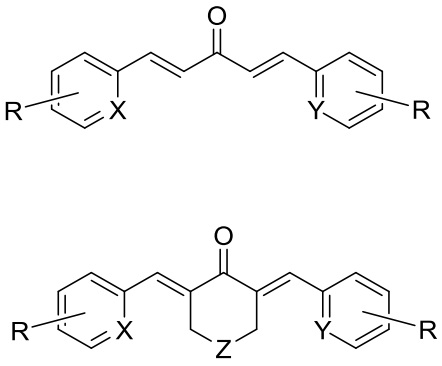


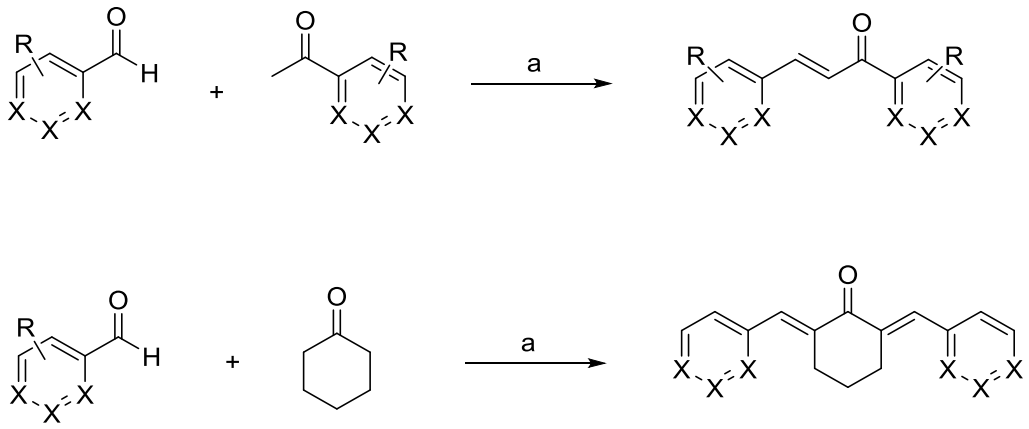
Figure 2. Structure-activity relationship of curcumin analogues.



X, Y = C, N

Z = $C_{subst.}$, $N_{subst.}$, O, $S_{subst.}$

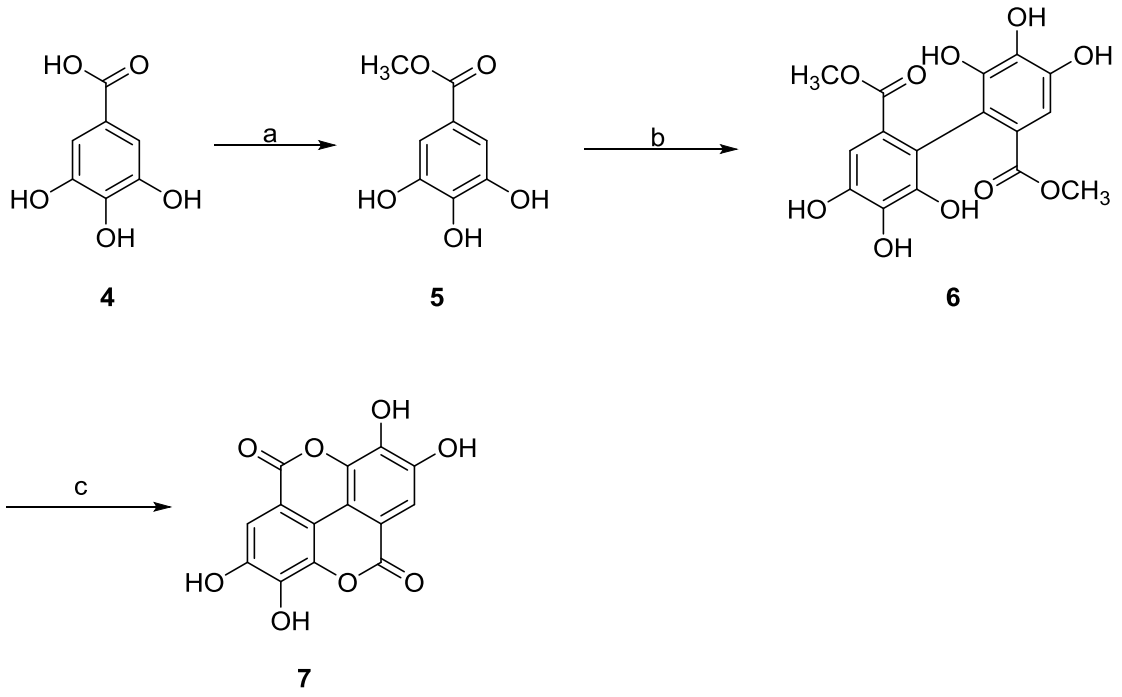
Figure 3. Backbone and substitution of monocarbonyl analogues of curcumin (MACs) showing their structural diversity.



Scheme 2. Exemplary synthesis of monocarbonyl analogues of curcumin (MACs). Reagents and conditions: (a) 40% KOH, EtOH, 5 ℃, stir 10 h, rt. X=C, N. R=OH, OMe, Cl, F.

Ellagic acid

Ellagic acid (**7**) is a naturally existing phenol antioxidant widely found in numerous fruits and vegetables like raspberries (*Rubus idaeus* L., Rosaceae), strawberries (*Fragaria* spec. L., Rosaceae) and pomegranates (*Punica granatum* L., Lythraceae). It shows potent antioxidant effects by radical scavenging and the inhibition of lipid peroxidation.[18] Ellagic acid is also capable of interfering with some angiogenesis-dependent pathways. It possesses anti-carcinogenic activity through inhibiting tumor cell proliferation, migration and induction of apoptosis. In addition, it is a dual inhibitor of the phosphorylation of VEGF and PDGF receptors, intercepting the angiogenesis processes required for tumor growth.[19] Recently, it was reported that its anti-angiogenesis mechanism affects the VEGFR-2 signalling pathway by forming hydrogen bonds and aromatic interactions within the ATP-binding region of the VEGFR-2 kinase unit.[20] Shankar and Srivastava et al. (2013) [21] treated PANC-1 xenografted mice with the ellagic acid and measured the expression of Akt, Shh and Notch. The results suggested that ellagic acid effectively inhibited human pancreatic cancer growth by suppressing protein kinase B (Akt), sonic hedgehog (Shh) and Notch pat hways. The preparation of ellagic acid (**7**) can be achieved by oxidative coupling of gallic acid (**Scheme 3**).[22] In the presence of H_2SO_4, gallic acid (**4**) was esterified to methyl gallate (**5**). The gallate (**5**) was oxidized by o-chloranil followed by reduction with $Na_2S_2O_4$ to obtain the hexahydroxy biphenyl (**6**). Subsequent lactonization afforded the final product ellagic acid (**7**) in high yield.



Scheme 3. Synthesis of ellagic acid **7.** Reagents and conditions: (a) H_2SO_4, CH_3OH; (b) (1) o-chloranil, Et_2O, -40 ℃; -40 ℃ → r.t, 3 h; (2) $Na_2S_2O_4$, r.t, 30 min; (c) MeOH:H_2O=1:1, reflux.

Resveratrol

Resveratrol (**8**) is a natural polyphenol belonging to the stilbenoids and widely existing in a number of plants. It was primarily extracted from grape (*Vitis vinifera* L., Vitaceae), and mulberry (*Morus* L., Moraceae) (**Figure 4**).[23] It has anti-oxidant effects, anti-estrogenic activities and the ability to reduce the synthesis of hepatic lipids and eicosanoids. It inhibits platelet aggregation and protects vessels from arteriosclerosis.[23-24] In recent years, it has been reported that resveratrol is sufficiently potent to inhibit VEGF-induced and FGF-2 neovascularization *in vivo*.[25] It was also found that resveratrol showed direct inhibition to bovine aorta endothelial cell proliferation, migration and tube formation *in vitro*.[26] Resveratrol has also been found to effectively abrogate VEGF-mediated tyrosine phosphorylation of vascular endothelial (VE)-cadherin and its complex partner, β-catenin.[27] But unfortunately, resveratrol has dual effects on cells depending on the situation and cell type, meaning it can either induce or suppress angiogenic effects.[28] The low oral bioavailability and metabolic stability of resveratrol also limited its application.[29] Therefore, in an attempt to increase its bioavailability and stability,

the structure of resveratrol was modified by methylation of the phenol group [30] and introduction of other groups on the phenyl ring (see compound **9**-**15**).[31] *Trans*-3,4,5,4'-tetramethoxystilbene (DMU-212) has pharmacokinetic properties that are better compared to resveratrol and shows anti-proliferative activities in different cancer cells.[32] The further investigation of its role in angiogenesis by Dai and Zhang et al.(2013) [33] showed that DMU-212, a potential anti-angiogenic agent, inhibits VEGFR2 phosphorylation and thereby acts as a suppressor of signalling pathways mediated by VEGFR2 inducing apoptosis in endothelial cells.

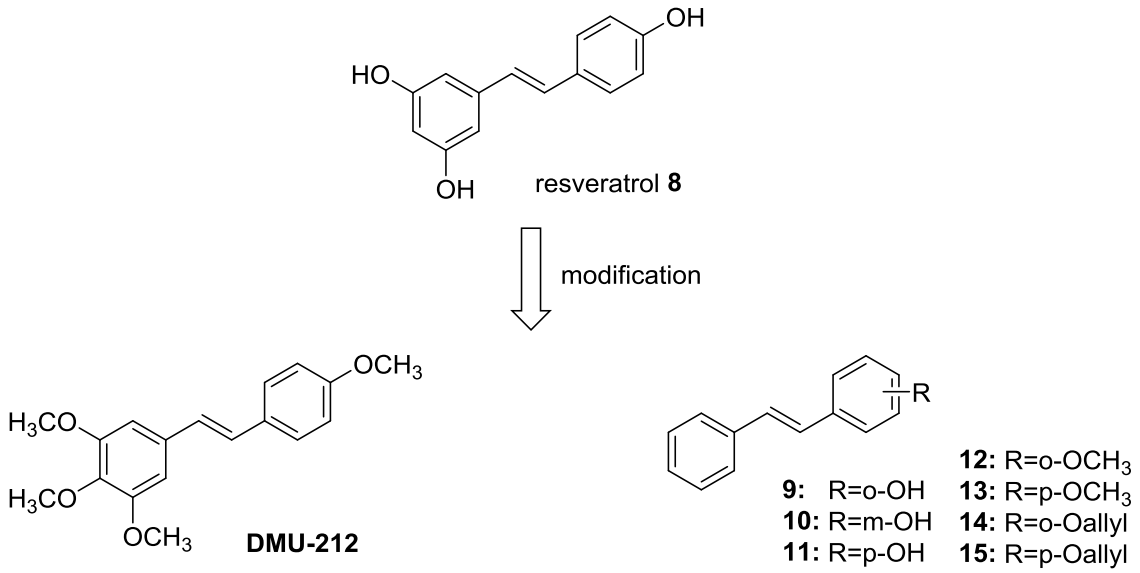
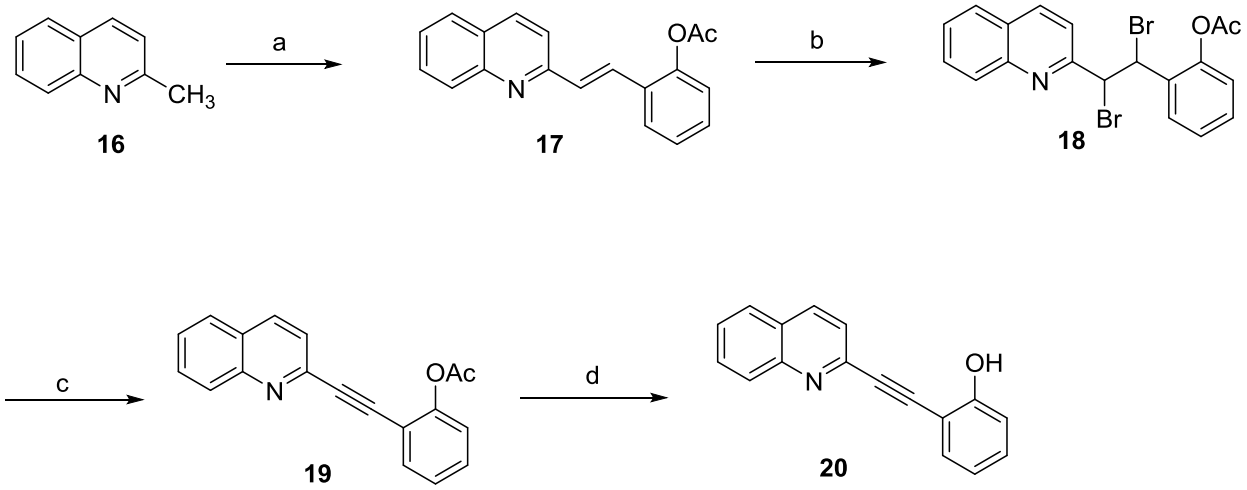


Figure 4. Structure of resveratrol **8** and its analogues.

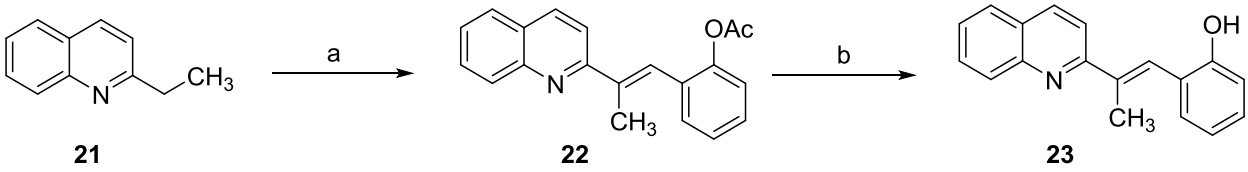
Quinoline substituted phenols

Up to now, quinoline substituted phenols (Qsps) have not been reported as natural secondary metabolites, but the individual substructures quinoline and alkyl phenol are common structural elements of secondary plant products. The quinoline skeleton is present in alkaloids derived from tryptophane, like quinine or camptothecine, whereas alkyl phenols with a varying length of the alkyl side chain are common metabolites from the shikimate pathway. Qsps were reported in a recent patent to be effective for the treatment of angiogenesis-related diseases or disorder. Among other assays a transgenic line of zebrafish that express a fluorescent reporter (EGFP) in vasculature was used in their study to identify anti-angiogenic compounds.[34] They particularly

looked at the integrity of vessels developing in the eyes and in the trunk. Quinoline-substituted phenols were identified being active based on a significant inhibition of the hyaloid vessel formation in the zebrafish model. The synthesis of two representative compounds **20** and **23** of this class are shown in **Scheme 4** and **Scheme 5**.[34]



Scheme 4. Synthesis of quinoline substituted phenol **20**. Reagents and conditions: (a) Ac_2O, 2-hydroxybenzaldehyde, 130 °C; (b) (1) Br_2, AcOH; (2) Ac_2O; (c) (1) DBU, THF; (2) Ac_2O; (d) Na_2CO_3, MeOH/THF.



Scheme 5. Synthesis of quinoline substituted phenol **23.** Reagents and conditions: (a) Ac_2O, 2-hydroxybenzaldehyde; (b) (1) NaOH, EtOH/H_2O, 100 °C; (2) HCl.

After condensation of **16** with an aldehyde, product **17** was subsequently brominated, followed by twofold elimination of HBr forming a triple bond. Finally, ester hydrolysis provides

compound **20**. Compound **23** was obtained from an analogous condensation product **22** hydrolysis under basic condition.

4-Amino-2-sulfanylphenol derivatives

The group of 4-amino-2-sulfanylphenols is obviously an outlier among the reviewed compounds as the 2-sulfanylphenol structure is not a structural element in natural products. The vast majority of thiol groups in secondary metabolites derive from the amino acid from cysteine, producing aliphatic secondary metabolites with thiol functionality instead of phenolic ones. Nevertheless, this group contains thiol-analogues of the naturally occurring o-catechol substructure and thus they have been included to this review. Zhang and Xu et al. (2013) [35] have reported that 4-amino-2-sulfanylphenol compounds display high specific protein kinase and angiogenesis inhibitory activities. Based on their previous findings, the structure of compound **24** was optimized by replacing the naphthalene ring by a phenolic skeleton and a sulfonamide fragment.[36] These compounds show *in vitro* anti-angiogenic activities compared to Pazopanib in both human umbilical vein endothelial cell (HUVEC) tube formation assay and the rat thoracic aorta rings test. They inhibited protein kinase B/Akt and ABL tyrosine kinase in the micro-molar range. The preliminary structure-activity relationship is summarized in **Figure 5**.

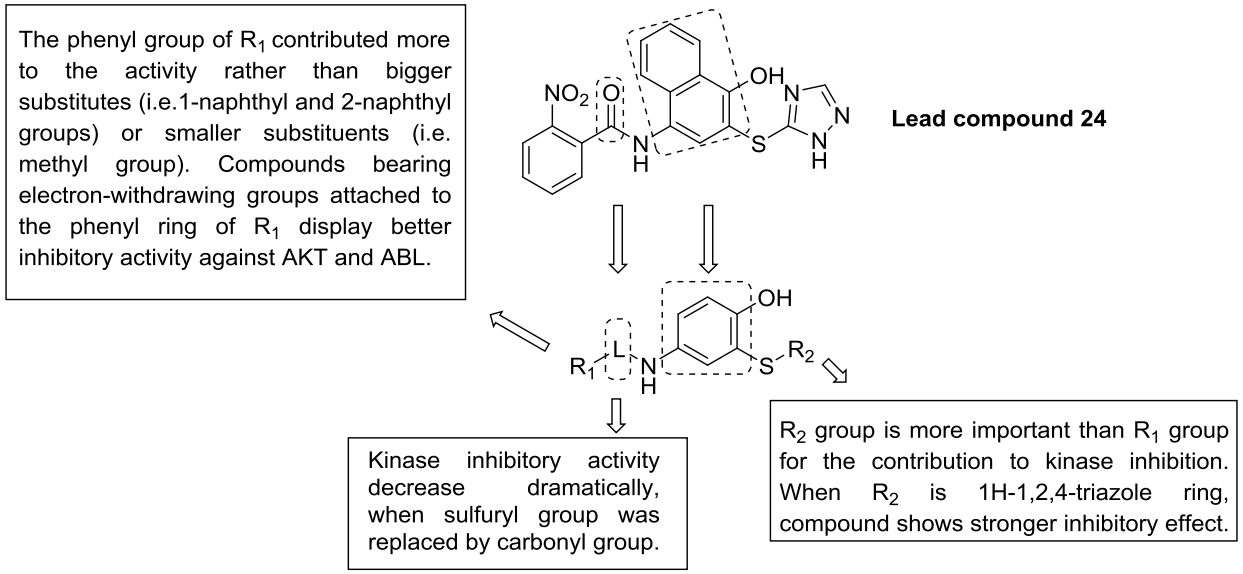
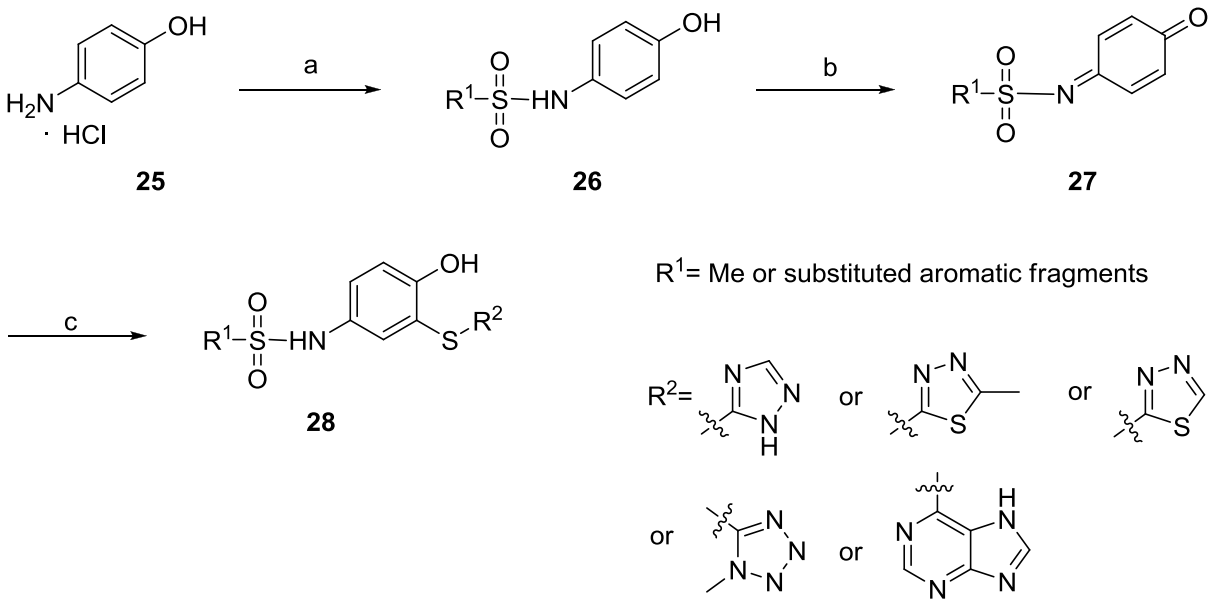


Figure 5. Design of 4-amino-2-sulfanylphenol derivatives and their structure-activity relationship.

The synthesis of 4-amino-2-sulfanylphenol compounds (**Scheme 6**) [37] starts from the 4-aminophenol hydrochloride salt **25**, which was dissolved in pyridine and reacted with various substituted sulfonyl chlorides yielding compounds **26** that differ in the substituent R_1. Oxidation by $NaIO_4/SiO_2$ gives the quinone-type structures **27**, which were reacted without further purification with thiols R_2SH. The addition to the unsaturated system yielded the target compounds **28** with different arylthiol groups under rearomatization.



Scheme 6. Synthesis of 4-amino-2-sulfanylphenol deriatives**.** Reagents and conditions: (a) R^1SO_2Cl, Pyridine, 0 ℃; (b) $NaIO_4/SiO_2$, DCM; (c) DMF, R_2SH.

Acylphloroglucinol derivatives

Acylphloroglucinols are typical secondary metabolites biosynthetically derived from the polyketide pathway and mainly accumulating in Hypericaceae [38] and Clusiaceae. Hyperforin, likely the most prominent acylphloroglucinol derivative and present in higher amounts in St. John's wort (*Hypericum perforatum* L., Hypericaceae), has been recently reported to exhibit strong anti-proliferative effects [39] and strongly inhibited angiogenesis *in vitro* and *in vivo* models. Mechanistically, it interferes with MMP-2 and an urokinase plasminogen activator

(uPA),[40] but due to its instability in aqueous solution, complex structure and limited availability, hyperforin is neither a drug candidate nor a good model compound. The finding that structurally simpler natural acylphloroglucinol derivatives showed also anti-proliferative effects against endothelial cells with inhibitory effects in a tube-formation assay on Matrigel catalysed the search for simple acylphloroglucinols with anti-angiogenic activity.[41] Within this group some geranylated monocyclic and bicyclic acylphloroglucinol derivatives have been found, which are structurally much simpler than hyperforin, but exhibiting potent anti-proliferative activity for a human microvascular endothelial cell line (HMEC-1) at low micromolar concentration.[41] Two series of natural-like acylphloroglucinols were synthesised (**Figure 6**) for the systematic investigations of their anti-angiogenic properties.[42] Compound **47** (R_1=H, R_2=OH, R_3=OH) showed anti-proliferative activity with an IC_{50} of 0.88 ± 0.08 µM *in vitro*. Compound **38** (alkyl = $CH(CH_3)CH_2CH_3$) exhibited moderate anti-proliferative effects (IC_{50} =11.0 ± 1 µM) and inhibited the capillary-like tube formation of HMEC-1 *in vitro*, whereas **47** is inactive. Furthermore some of the compounds showed significant anti-oxidative activity. The most active compound in the ORAC assay was **47**, which exhibited an anti-oxidative effect of 6.6 ± 1.0 TE. However, this compound showed only weak activity during the proliferation assay (IC_{50} = 53.8 ± 0.3 µM) and did not inhibit tube-formation.

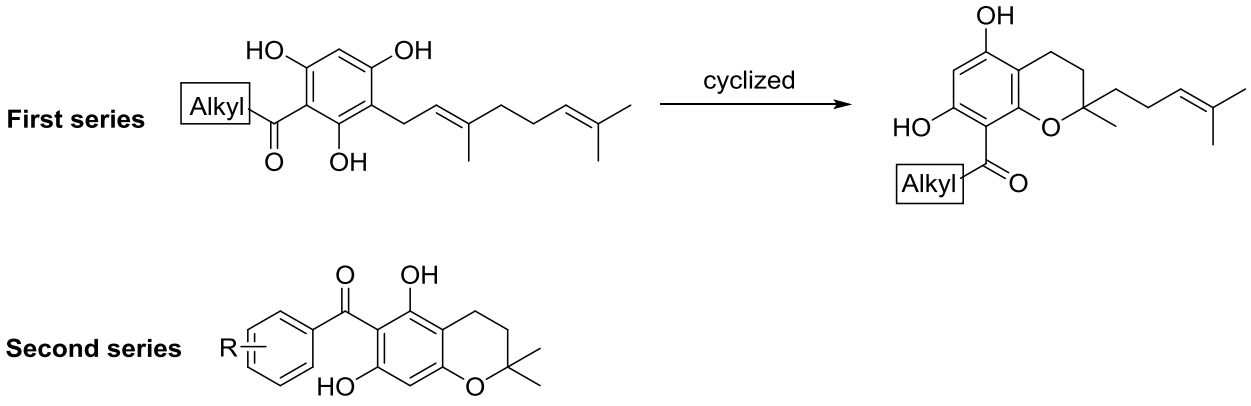
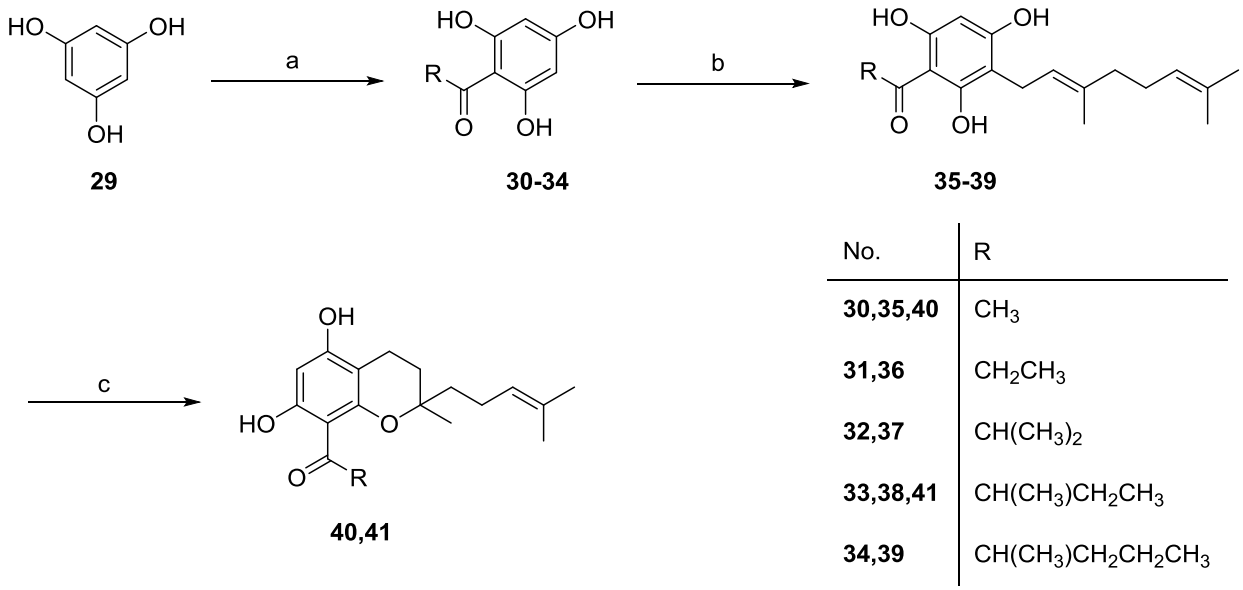


Figure 6. Structures of two series of nature-like acylphloroglucinols.

A basic structure-activity relationship of the aliphatic mono- and bicyclic acylphloroglucinol derivatives with short acyl side chains indicates that the *in vitro* anti-proliferative activity of these acylphloroglucinols in HMEC-1 increases with increasing logP. Increasing the number of carbon atoms in the acyl group provides higher lipophilicity, which allows the compound a better penetration across cellular membranes *in vitro* assay. In contrast, the activity of the derivatives with aromatic acylside chain depends on other properties. The molecular aspects of the observed cellular effects are currently under investigation.

Compounds **30-34** were synthesised via Friedel-Crafts acylation (**Scheme 7**) with 55 to 81% yield. The alkylation of **30-34** and geranyl bromide gave products **35-39** with moderate yields from 55 to 60%. Finally, a para-toluenesulfonic acid (pTSA) catalysed cyclisation afforded the target compounds **40** and **41** in 53 and 65% yield, respectively.

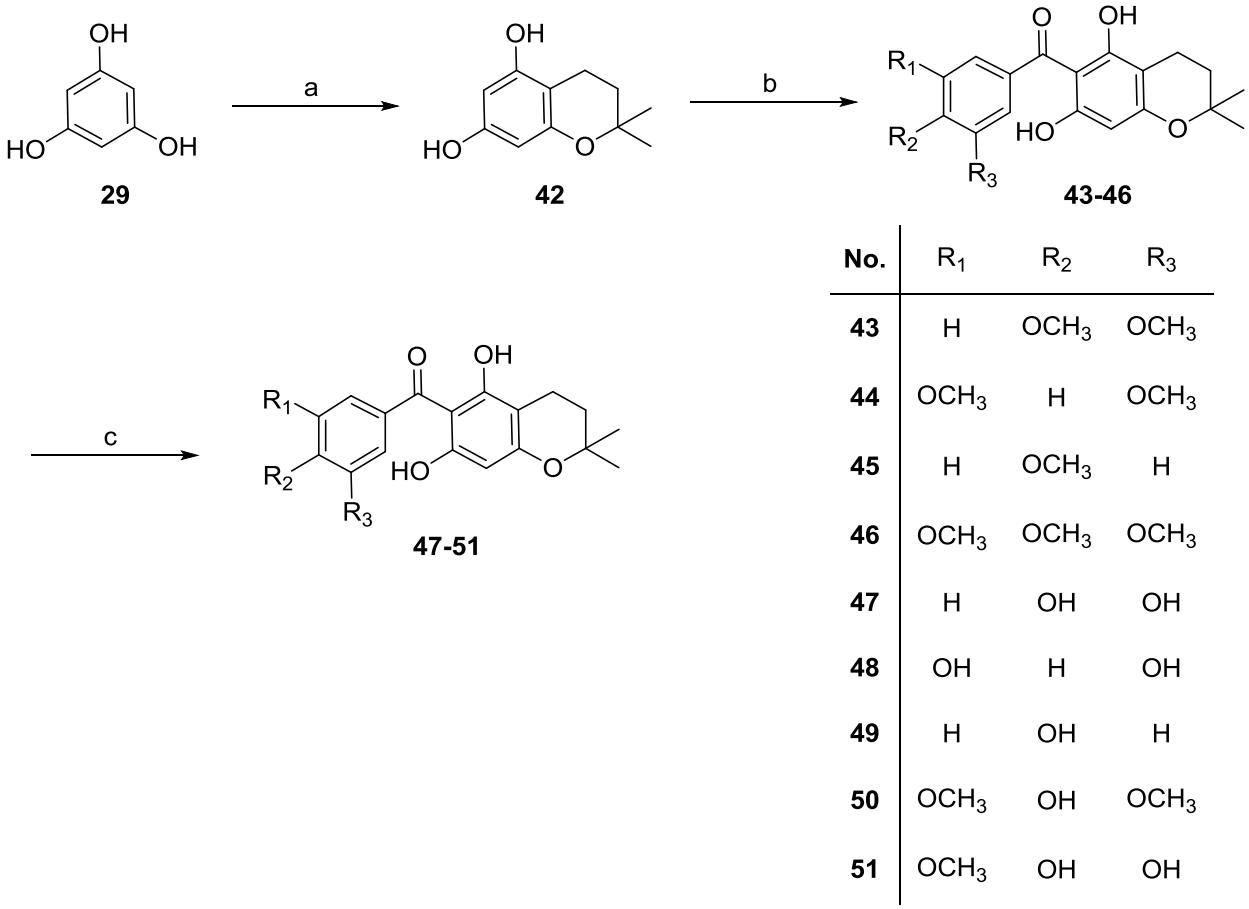


No.	R
30,35,40	CH_3
31,36	CH_2CH_3
32,37	$CH(CH_3)_2$
33,38,41	$CH(CH_3)CH_2CH_3$
34,39	$CH(CH_3)CH_2CH_2CH_3$

Scheme 7. Synthesis of acylphloroglucinol derivatives **35-41**. Reagents and conditions: (**a**) acyl chloride, $AlCl_3$, CS_2-$PhNO_2$, 55 ℃, 2 h; (**b**) geranyl bromide, K_2CO_3, acetone, reflux overnight; (**c**) pTSA, benzene, reflux, 2 h.

Amberlyst 15 efficiently catalysed the condensation of 1,3,5-trihydroxybenzene **29** with isoprene in 53% yield in the synthesis of the second series of compounds (**Scheme 8**). The following

Friedel-Crafts acylation gave intermediates **43-46**, which were subsequently demethylated using BBr_3 to give **47-51** with 48 to 78% yield.



No.	R_1	R_2	R_3
43	H	OCH_3	OCH_3
44	OCH_3	H	OCH_3
45	H	OCH_3	H
46	OCH_3	OCH_3	OCH_3
47	H	OH	OH
48	OH	H	OH
49	H	OH	H
50	OCH_3	OH	OCH_3
51	OCH_3	OH	OH

Scheme 8. Synthesis of acylphloroglucinol derivatives **43**-**51**. Reagents and conditions: (a) isoprene, Amberlyst 15, THF-Hexane; (b) benzoyl chloride, $AlCl_3$, DCM, -5 ℃ to r.t., overnight; (c) BBr_3, DCM, -78 ℃ to r.t., overnight.

(-)-Epigallocatechin-3-O-gallate (EGCG)

(-)-Epigallocatechin-3-O-gallate (EGCG, **52**) is the most abundant catechin in green tee (*Camellia sinensis* L. KUNZE, Theaceae). It is the esterification product of epigallocatechin and gallic acid. Many studies provide evidence that EGCG modulate multiples signal transduction pathways controlling the unwanted proliferation of cells. It inhibits the activation of HIF-1α, NF-κB and VEGF expression, thereby suppressing tumor angiogenesis and cancer progression.[43] Furthermore, EGCG inhibited MMP-2 and MMP-9 (in different cell type), which seem to play

an important role in tumor invasion and metastasis. Also, the inhibition of uPA by EGCG has been observed.[44] uPA has the ability to prevent apoptosis, stimulate angiogenesis, mitogenesis, cell migration, and to modulate cell adhesion. The presence of the 3-galloyl moiety in catechins led to higher biological activities,[45] but an increasing number of aromatic hydroxyl groups results in low stability and the inability of the compound to cross cellular membranes.[46] To prevent oxidation and improve its bioavailability, modifications of EGCG focus on synthesising more stable analogues (**Figure 7**). Anderson et al.(2005) [47] replaced the hydrolytically labile ester bond with a more stable amine and amide bond and evaluated their efficacy as modulators for β-lactam resistance in *S. aureus.* Landis-Piwowar.and Chi-chui Wang et al (2005) [48] protected the hydroxy groups by peracetate. These analogues behave as prodrugs and the acetyl group is removed by cellular cytosolic esterases. Liao (2002) and Huang (2010) et al. [49] acylated the phenol group at 3-position, introduced fatty acids of different size and extensively explored their structure-activity relationship to 5α-reductase. Park and coworkers (2010) [50] also synthesised 3-O-alkyl analogues of epicatechin. They found that the introduction of alkyl groups instead of acyl groups enhanced antimicrobial activities and stability at pH 7.4.

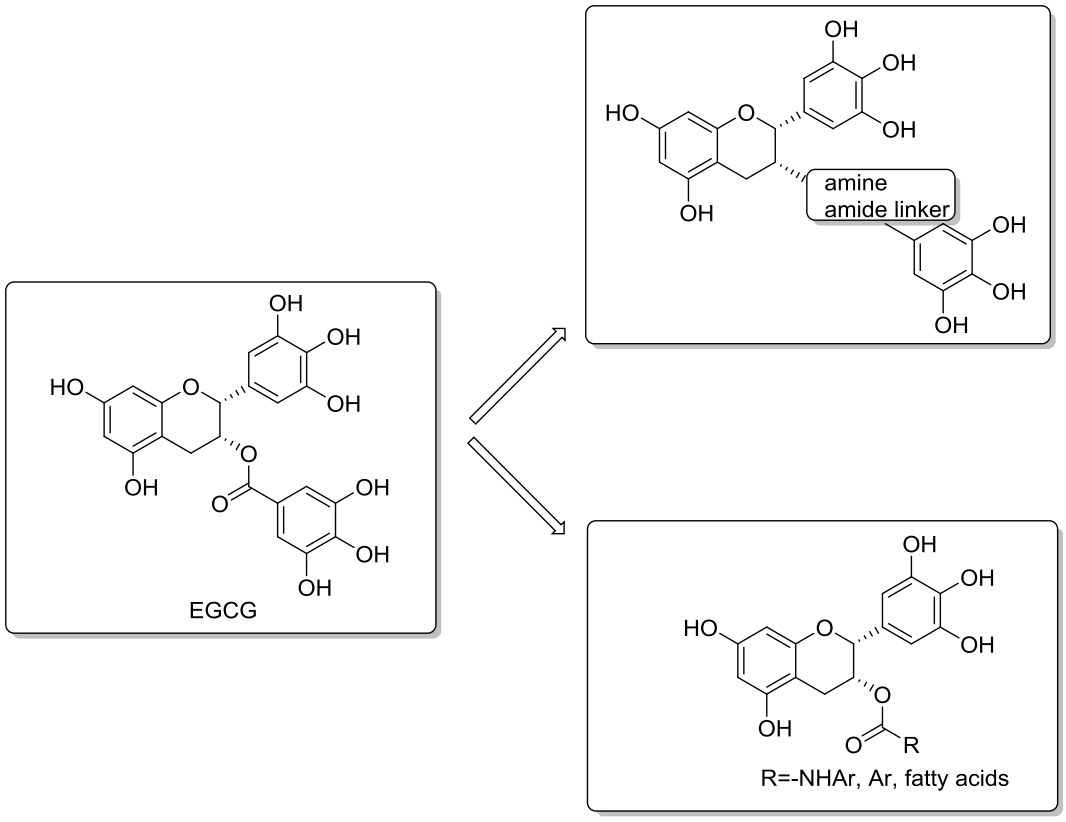
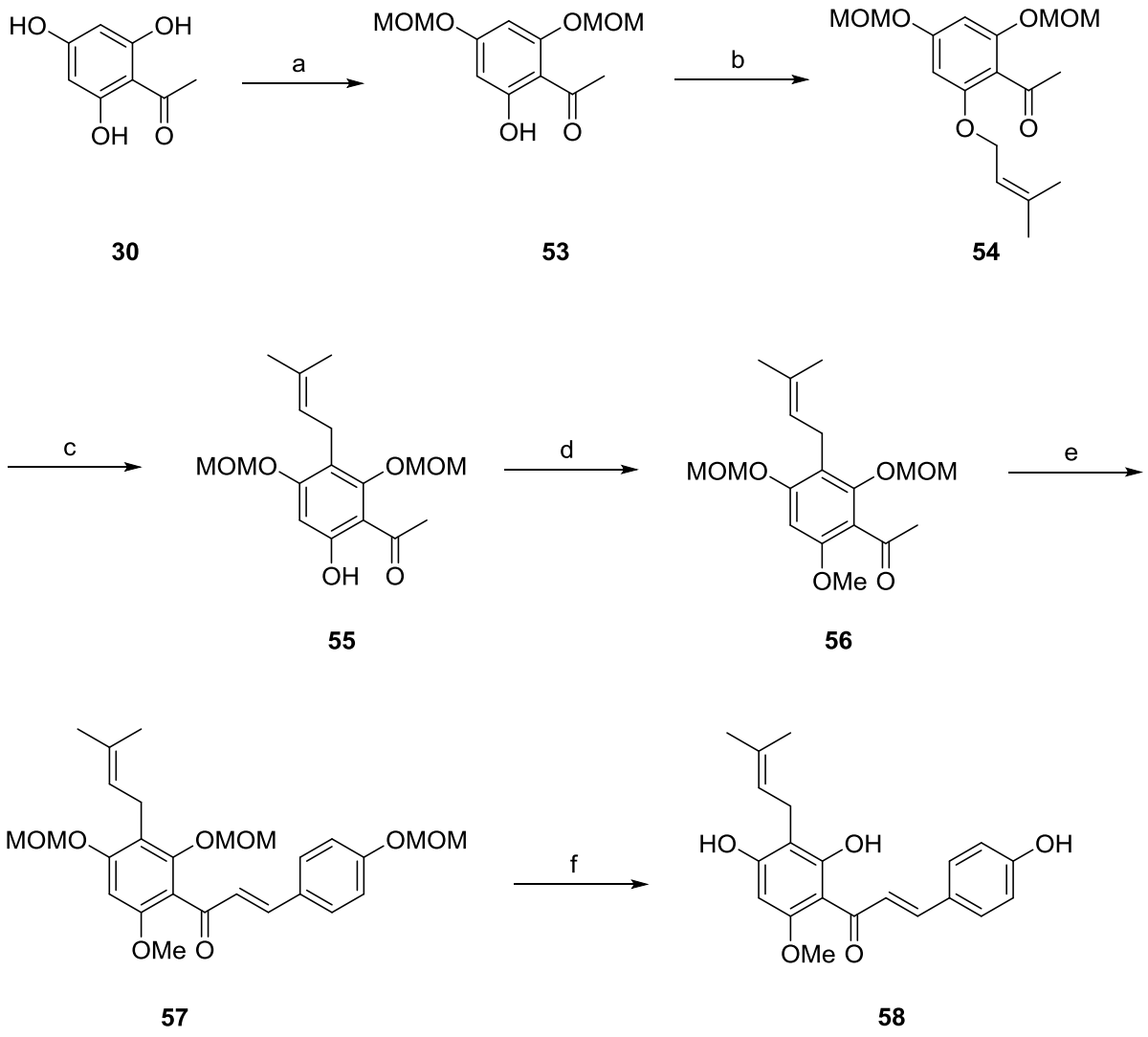


Figure 7. Analogues of (-)-EGCG to prevent oxidation and improve bioavailability of the compounds.

Xanthohumol

Xanthohumol (XN, **58**), a naturally occurring prenylated chalcone in hop plants (*Humulus lupulus* L., Cannabaceae), has been suggested to have potential to halt the development and progression of cancer and is therefore also a compound with a chemopreventive potential.[51] For example, XN shows proliferative inhibition of human breast (MCF-7), colon (HT-29), and ovarian cancer (A-2780) cells *in vitro* with IC_{50} ranging from 0.52 to 13.3 μM. Because most cancer chemopreventive agents have also anti-angiogenic properties *in vitro* and *in vivo*, further investigations [52] showed that XN repressed both the NF-κB and Akt pathways in endothelial cells, inhibited VEGF-A expression in a wound-healing assay and exhibited interference in the angiogenic process. The first total synthesis of xanthohumol was accomplished by Erhardt et al. in six steps with an overall yield of 10% in 2007 (**Scheme 9**).[53] The method was improved by Vogel and Heilmann et al. (2008) [54] to yield also several xanthohumol derivatives occurring as

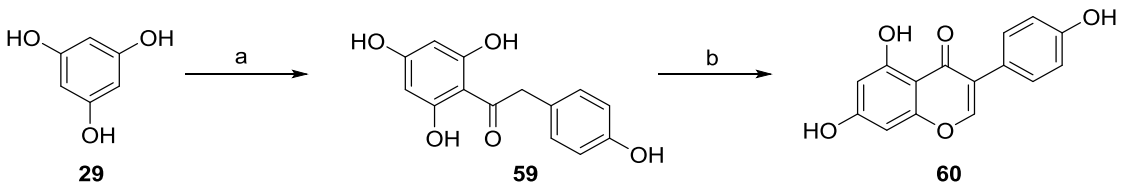
minor compounds in hop cones or as *in vivo* metabolites after xanthohumol intake.[54a] Up to now xanthohumol *in vivo* metabolites are not investigated regarding their anti-agiogenic activity.



Scheme 9. **Scheme 9**. Synthesis of xanthohumol **58**. Reagents and conditions: (a) MOMCl, diisopropyl ethyl amine, CH_2Cl_2; (b) 3-methyl-2-butene-1-ol, diethylazodicarboxylate, PPh_3, toluene/THF; (c) *N*,*N*-dimethylaniline, reflux; (d) $(CH_3O)SO_2$, K_2CO_3, acetone, reflux; (e) 4-methoxymethylbenzaldehyde aqueous NaOH, MeOH, reflux; (f) concentrated HCl (pH=1), MeOH/H_2O, rt.

Genistein

Genistein (**60**) is an isoflavone extracted from soybeans (*Glycine max* (L.) MERR, Fabaceae). It is present as the 7-O-glycoside genistin in the plant, but during the processing of soya products a significant amount of the aglycone genistein is liberated. Genistein was originally described as an exclusive inhibitor of tyrosine-specific protein kinases.[55] These kinases are responsible for the tyrosine-specific protein phosphorylation, which is required for the regulation of cell functions, including cell proliferation and cell transformation. Later on genistein was also found to act as oestrogen receptor agonist.[56] The anti-angiogenic potential of genistein was first reported by Fotsis et al in 1993.[57] Then further studies showed that genistein inhibited angiogenic processes in various *in vitro* and *in vivo* models.[58] The typical synthesis of genistein starts from m-trihydroxybenzene **29** (**Scheme 10**).[59] After Houben–Hoesch reaction or Friedel-crafts acylation, cyclization of the resulting hydroxyketone (**59**) in the presence of BF_3-Et_2O gave genistein (**60**) in good yield.



Scheme 10. Synthesis of genistein **60**. Reagents and conditions: (a) 4-hydroxyphenyl acetonitrile, anhydrous HCl, $ZnCl_2$-Et_2O, then aq HCl, heat or 4-hydroxyphenylacetic acid, BF_3-Et_2O, 120 ℃; (b) BF_3-Et_2O, DMF, $MeSO_2Cl$, 100 ℃, 2 h.

In the Friedel-crafts acylation, BF_3-Et_2O was used as the catalyst and solvent. The following formation of the pyrone was also catalysed by BF_3-Et_2O, a convenient one-pot synthesis of **60** was achieved without isolation of **59**.[60]

Fisetin and Quercetin

The flavonoids fisetin (**67**) and quercetin (**68**) belong to the flavonol subgroup exhibiting a double bond between C-2/C-3 and a hydroxyl group at C-3. Flavonols are the most abundant

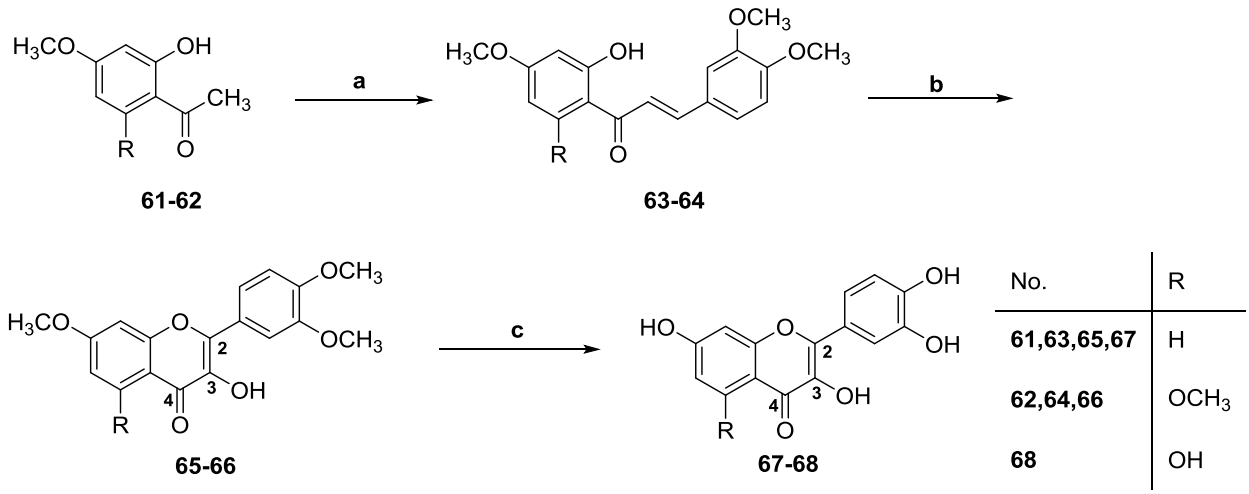
flavonoid subtype in plants which mainly occur as glycosides. Nevertheless, pharmacological testing concentrated (historically based) on the investigation of the aglycones. This has been often criticised, but it is most likely an important aspect with regard to flavonoid metabolism. It has been shown that the flavonoid glycosides are not absorbed after oral intake but are cleaved by lactase-phlorizin hydrolase and absorbed as the corresponding aglycone. The aglycone passes the cell membrane by passive diffusion and undergoes phase-II metabolism in enterocytes and the liver leading to glucuronides as the main metabolites. At least, a release of the aglycones from the glucuronides in tissues or cells with β-glucuronidase activity is possible.[61]

Fisetin can be found in many fruits like strawberries and apples (*Malus* spec. MILL., Rosaceae) as well as in vegetables like onions (*Allium cepa* L., Amaryllidaceae, subfamily Allioideae former family Alliaceae). It possesses anticancer activities in various cancer models, for example, it can inhibit androgen receptor signalling and tumor growth in athymic nude mice,[62] it can cause apoptosis and cell cycle arrest in human prostate cancer LNCaP cells,[63] in HCT-116 human colon cancer cells and it can induce apoptosis associated with an increased level of p53.[64] P.Singh and A.Bhat et al.(2012) [65] tested its anti-angiogenic activity for the first time. Their study revealed that fisetin (10–50 μM) strongly inhibited the growth, proliferation and cell cycle progression in HUVEC by down-regulating the expression of VEGF and eNOS in endothelial cells. Another recent study also demonstrated that fisetin inhibits MMPs and reduces tumor cell invasiveness and endothelial cell tube formation.[66]

Quercetin is besides kaempferol the most abundant aglycone in flavonol glycosides. Quercetin glycosides occur in higher concentrations in onions, red wines, and green tea or in various medicinal plants.[67] In a number of early studies, quercetin showed a strong ability to inhibit tumor growth *in vivo*.[68] Quercetin, inhibits angiogenesis through multiple mechanisms such as inhibition of COX-2 and lipoxygenase (LOX)-5, interference with the EGF receptor, the HER-2 intracellular signaling pathway, and the NF-κB nuclear transcription protein. Chen et al.(2008) [69] reported that quercetin inhibited the proliferation of choroids-retina endothelial cells and the migration and tube formation of RA/6A cells were also significantly inhibited by quercetin in a dose-dependent manner, but in some cell types quercetin is also able to activate the angiogenic pathway by inhibiting HIF-prolyl hydroxylase.[70] Zhao et al.(2014) [71] investigated that the anti-angiogenic activity of quercetin in zebrafish embryos and in human umbilical vein endothelial

cells (HUVECs). The formation of intersegmental vessels was disrupted in transgenic zebrafish embryos. In HUVECs, quercetin inhibited cell viability, the expression of VEGF-2 and tube formation dose-dependently.

The synthesis of fisetin and quercetin can be achieved by two different methods. The first choice for synthesising fisetin and quecetin is by the Allan-Robinson reaction.[72] However, this reaction has some drawbacks as very harsh experimental conditions and the necessity of selective protection and deprotection of the free hydroxyl groups with benzyl and/or benzoyl groups. An alternative method is the Algar-Flynn-Oyamada (AFO) reaction which gives flavone-3-ols directly, but the yield varies depending on different substrates.[73] Simpson and co-workers (1955) [74] improved the reaction conditions of AFO in order to synthesise the flavonol rhamnocitrin by using bismuth carbonate and acetic acid, which increased the yields to 71% and the overall yields to 52% over two steps. According to this, a synthesis of fisetin and quercetin with methyl protected chalcones as starting material was proposed (**Scheme 11**).

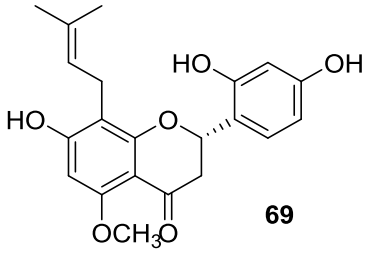


No.	R
61,63,65,67	H
62,64,66	OCH_3
68	OH

Scheme 11. Synthesis of fisetin **67** and quercetin **68**. Reagents and conditions: (a) 3,4-dimethoxybenzaldehyde, KOH, DMF, 0 ℃; (b) $BiCO_3$, AcOH, 2-ethoxyethanol, Δ; (c) BBr_3, DCM, -78 ℃ →r.t.

(2S)-7,2',4'-Trihydroxy-5-methoxy-8-dimethylallyl flavanone

(2S)-7,2',4'-Triihydroxy-5-methoxy-8-dimethylallyl flavanone (**69, Figure 8**) is a prenylated flavanone isolated from *Sophora flavescens* by Wang and Yuan et al in 2013.[75] It displays inhibitory effects on cell proliferation, cell migration, cell adhesion and tube formation with the human umbilical vein endothelial cell line ECV304, which are the four important steps in angiogenesis process. The mechanistic study showed that compound **69** is able to regulate ROS levels and VEGF expression in a dose-depended manner down, and induce cell cycle arrested in G0/G1 phase.



69

Figure 8. Structure of (2S)-7,2',4'-triihydroxy-5-methoxy-8-dimethylallyl flavanone **69**.

Conclusions

As pathological angiogenic processes are supposed to contribute to several diseases, compounds with anti-angiogenic activity have been intensively investigated. Besides antibodies also several low-molecular weight compounds have been chemically and pharmacologically characterized among them several secondary metabolites of natural origin. In anti-angiogenic strategies natural products with phenolic substructures or belonging to the polyphenols are of special importance as they occur in several food and medicinal plants important for human diet and health. Despite the fact that phenolic compounds often show specific interactions in biological systems most of them are pleiotropic substances with an effect on different cellular networks or targets. Several natural phenolic angiogenic inhibitors like curcumin (**3**), epigallocatechin-3-O-gallate (**52**) and xanthohumol (**58**) also showed remarkable chemopreventive activity. However, the stability, availability from natural sources and bioactivity of the natural compounds are typically limited with so far no example of very strong anti-angiogenic activity in the nano-molar range. Thus,

synthetic approaches for the production, diversification and optimization are mandatory. The modification of the structures of polyphenols improving stability and bioactivity, and further enhancing their anti-angiogenic activity, is the main goal of current research in the field. Analogues of the natural phenols with improved drug properties may be promising candidates for future oncology treatment.

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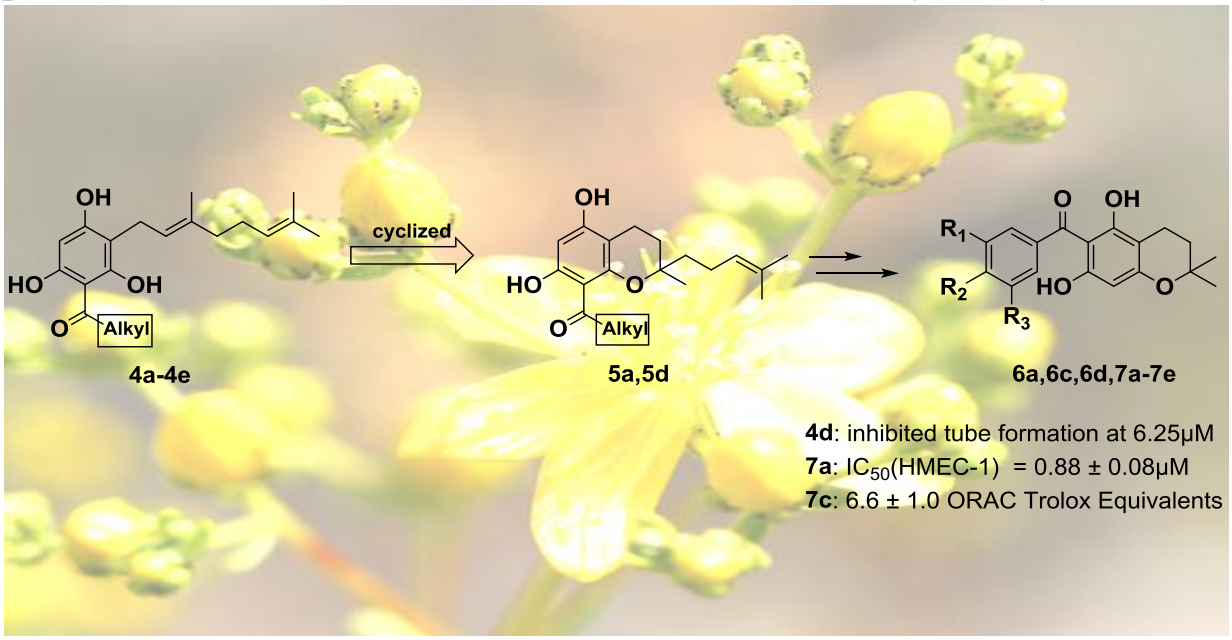
Chapter 2

Synthesis of natural and natural-like acylphloroglucinols with anti-proliferative, anti-oxidative and tube-formation inhibitory activity



Abstract

Two series of natural and natural-like mono- and bicyclic acylphloroglucinols derived from secondary metabolites in the genus *Hypericum* (Hypericaceae) were synthesised and tested *in vitro* for anti-proliferative and tube-formation inhibitory activity in human microvascular endothelial cells (HMEC-1). In addition, their anti-oxidative activity was determined via an ORAC-assay. The first series of compounds (**4a-e**) consisted of geranylated monocyclic acylphloroglucinols with varying aliphatic acyl substitution patterns, which were subsequently cyclised to the corresponding 2-methyl-2-prenylchromane derivatives (**5a** and **5d**). The second series involved compounds containing a 2,2-dimethylchromane skeleton with differing aromatic acyl substitution (**6a-d** and **7a-e**). Compound **7a**, (5,7-dihydroxy-2,2-dimethylchroman-6-yl)-(3,4-dihydroxyphenyl)methanone), showed the highest *in vitro* anti-proliferative activity with an

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IC_{50} of 0.88 ± 0.08 µM and a remarkable anti-oxidative activity of 2.8 ± 0.1 TE from the ORAC test. Interestingly, the high anti-proliferative activity of these acylphloroglucinols was not associated with tube-formation inhibition. Compounds (*E*)-1-(3-(3,7-dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxyphenyl)-2-methylbutan-1-one (**4d**) and (5,7-dihydroxy-2,2-dimethyl-chroman-6-yl)(3,4-dimethoxyphenyl)methanone (**6a**) exhibited moderate to weak anti-proliferative effects (IC_{50} 11.0 ± 1 µM and 48.0 ± 4.3 µM, respectively) and inhibited the capillary-like tube formation of HMEC-1 *in vitro*, whereas **7a** was inactive. The most active compound in the ORAC assay was **7c**, which exhibited an anti-oxidative effect of 6.6 ± 1.0 TE. However, this compound showed only weak activity during the proliferation assay (IC_{50} 53.8 ± 0.3) and did not inhibit tube-formation.

Keywords

acylphloroglucinol; chromane; HMEC-1; anti-proliferative activity; tube formation; ORAC

Introduction

Acylphloroglucinols are typical secondary metabolites biosynthetically derived from the polyketide pathway that accumulate in Hypericaceae [1] and Clusiaceae. Their biosynthesis begins with three malonyl-CoA (coenzyme A) molecules reacting with an activated acyl-CoA to form an intermediate polyketide. A Claisen-like reaction enzymatically cyclises this intermediate to an acylphloroglucinol.[2] Depending on the starting material, the acyl moiety of a natural acylphloroglucinol can be aliphatic, as observed in the Genus *Hypericum*, or aromatic, as in the Genus *Garcinia*. The structural diversity of acylphloroglucinols results mainly from substitutions on the phloroglucinol core with several prenyl or geranyl moieties. Both substituents can cyclise and oxidise, which results in bicyclic, tricyclic or even more complex compounds.[3]

Acylphloroglucinols have attracted attention due to their broad pharmacological activity. Some of these compounds have been reported to exhibit anti-bacterial,[4] cytotoxic,[5] anti-oxidative [6] and anti-depressant [7] effects. Hyperforin, likely the most prominent acylphloroglucinol derivative, has been recently reported to exhibit strong anti-proliferative effects [8] and inhibit angiogenesis *in vivo.* Several key steps of this anti-angiogenic effect have been determined pharmacologically *in vitro.*[9] Due to its instability in aqueous solutions,[10] complex structure and limited availability, hyperforin is neither a drug candidate nor a good model compound. Thus, synthesising pharmacologically active acylphloroglucinol derivatives with higher stability and better solubility is an interesting challenge.

Recently, monocyclic and bicyclic acylphloroglucinol derivatives with anti-proliferative activity for a human microvascular endothelial cell line (HMEC-1) were isolated from *H. empetrifolium* (**Figure 1**),[11] [12] a plant commonly used in traditional Greek medicine. Structurally simpler than hyperforin, the natural monocyclic compounds **4c**, **4d** and **5d** exhibited potent anti-proliferative activity at low micromolar concentrations (**Table 1**). Due to the limited availability of the compound in plant materials, our first step was to synthesise geranylated acylphloroglucinol, **4d**, from **3d** to enable further *in vitro* pharmacological testing. Because non-geranylated monocyclic acylphloroglucinols (**3a-e**) were inactive in the proliferation assay (data not shown), the remarkable activity of the geranylated monocyclic compound, **4d**, encouraged us to more

systematically investigate this class of molecules. First, the acyl group length was varied, and the geranyl moiety was subsequently cyclised, which provided the first series of geranylated monocyclic and bicyclic aliphatic acylphloroglucinols **4a-e, 5a** and **5d** (**Scheme 1**). Of the 2-methyl-2-prenyl-8yl chromane derivatives obtained, compound **5d** had been previously isolated from *H. empetrifolium* [12] and *H. amblycalyx*,[12-13] and **5a** is a natural-like analogue with a shortened acyl side chain.

Figure 1. *Hypericum empetrifolium* WILLD. (Photos by Eirini Aplada, Biologist.-M.Sc.)

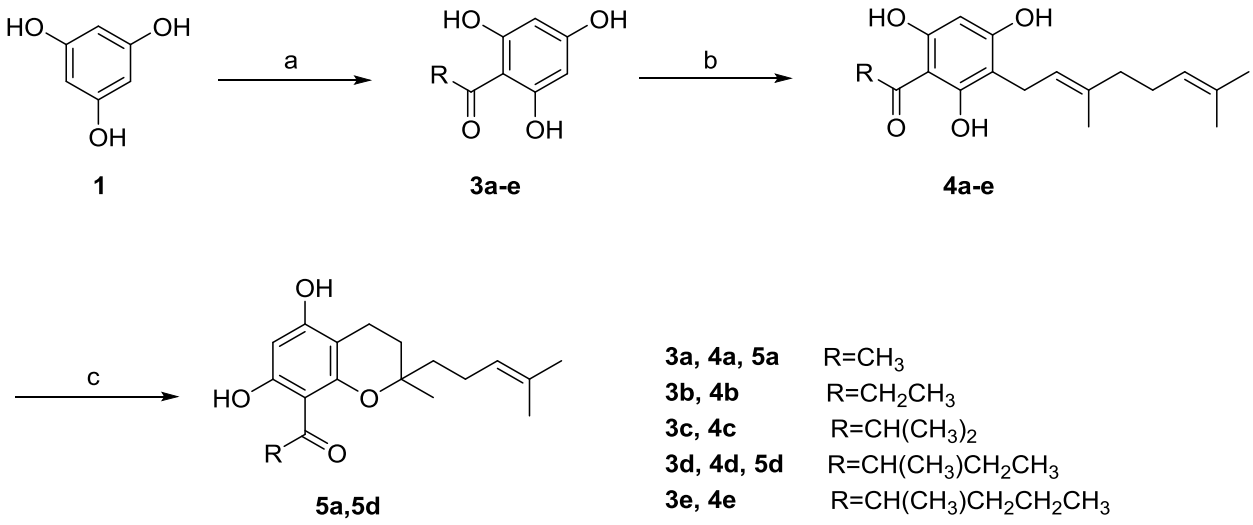
A second series of compound syntheses (**Scheme 2**) was inspired by the aromatic acyl moieties in several natural acylphloroglucinols with chromane skeletons.[14] The bicyclic acylphloroglucinols obtained (**6a-d, 7a-e**) bear aromatic acyl groups at C-6 in the chromane skeleton with different hydroxyl or methoxy substitution patterns. Several pharmacologically active natural acylphloroglucinols containing aromatic substitutions, such as rottlerin and its derivatives [15] or (-)-3-deoxy-MS-II,[16] have a 2,2-dimethyl-chromane core. Therefore, we used this skeleton to synthesise **6a-d** and **7a-e** instead of 2-methyl-2-prenyl-chromane, which simplifies the structure.

Results and discussion

Synthesis

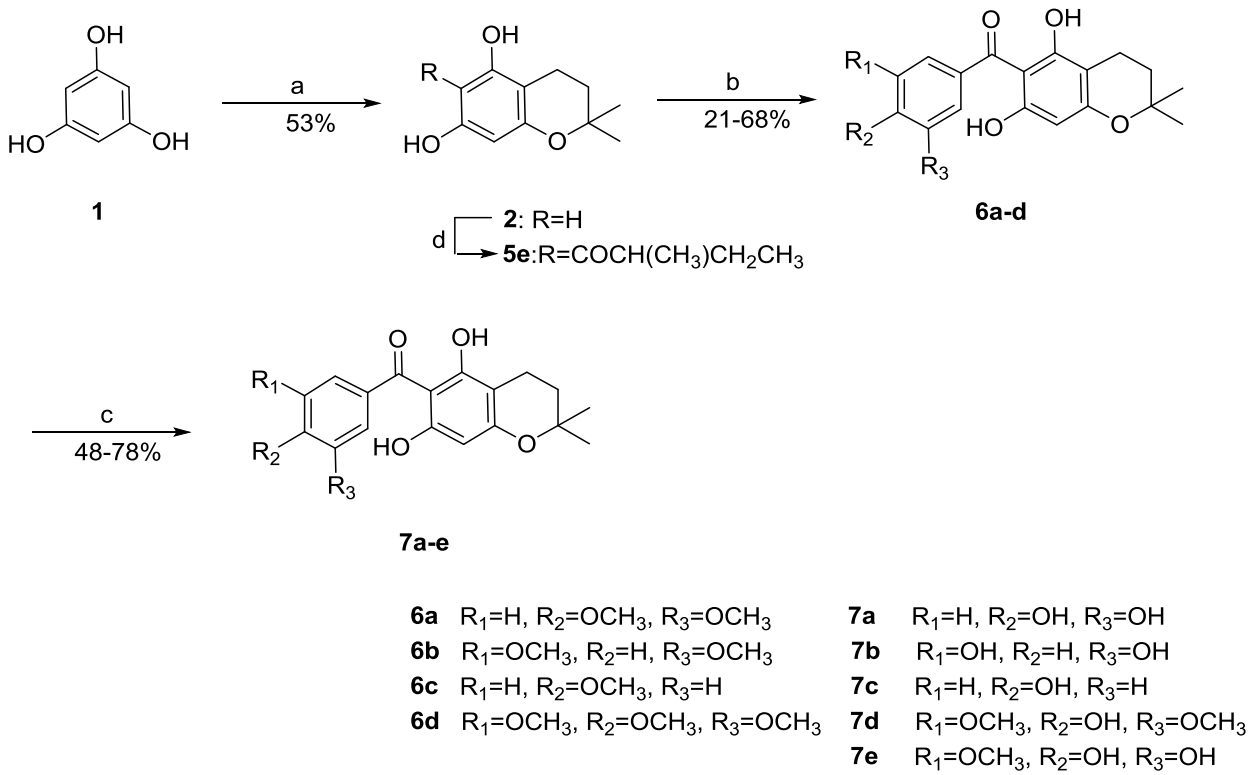
Compounds **3a-e** were synthesised via a Friedel-Crafts acylation (**Scheme 1**) with a 55 to 81% yield. Treating **3a-e** and geranyl bromide with anhydrous potassium carbonate in acetone yielded

the alkylation products **4a-e** with moderate yields from 55 to 60%. Purifying the mixed compounds was difficult because geranyl bromide reacts non-selective with the hydroxide groups or aromatic ring to yield over three side products. Finally, catalysing the cyclisation using para-toluenesulfonic acid (pTSA) afforded the target compounds **5a** and **5d** in 53 and 65% yield, respectively. The 2D NMR analysis of the products and a comparison of their spectroscopic data to literature values revealed that they selectively cyclised at the 2-OH position not the 4-OH position, which was unambiguously determined by comparing the 1D NMR data to literature.



Scheme 1. Synthesis of compounds **4a-e** and **5a, 5d**; Reagents and conditions: (**a**) acyl chloride, $AlCl_3$, CS_2-$PhNO_2$, 55 ℃, 2 h; (**b**) geranyl bromide, K_2CO_3, acetone, reflux overnight; (**c**) pTSA, benzene, reflux, 2 h.

To synthesise the second series, Amberlyst 15 was used to efficiently catalyse the condensation of 1,3,5-trihydroxybenzene (**1**) with isoprene (**2**) in 53% yield. The subsequent Friedel-Crafts acylation yielded intermediates **6a-d**, which were subsequently demethylated with BBr_3 to form **7a-e** with 48 to 78% yield. Compound **5e** was prepared as an aliphatic 2,2-dimethyl-chroman-6yl analogue of the 2-methyl-2-prenyl-chroman-8yl derivative **5d**.



6a R_1=H, R_2=OCH_3, R_3=OCH_3
6b R_1=OCH_3, R_2=H, R_3=OCH_3
6c R_1=H, R_2=OCH_3, R_3=H
6d R_1=OCH_3, R_2=OCH_3, R_3=OCH_3

7a R_1=H, R_2=OH, R_3=OH
7b R_1=OH, R_2=H, R_3=OH
7c R_1=H, R_2=OH, R_3=H
7d R_1=OCH_3, R_2=OH, R_3=OCH_3
7e R_1=OCH_3, R_2=OH, R_3=OH

Scheme 2. Synthesis of compounds **5e**, **6a-d** and **7a-e**; Reagents and conditions: (**a**) isoprene, Amberlyst 15, THF/hexane; (**b**) benzoyl chloride, $AlCl_3$, CH_2Cl_2, -5 ℃ to r.t., overnight; (**c**) BBr_3, CH_2Cl_2, -78 ℃ to r.t., overnight; (**d**) $AlCl_3$, CH_2Cl_2, r.t.

Biological evaluation

The anti-proliferative activity of monocyclic aliphatic acylphloroglucinols (**4a-e**) in HMEC-1 increased with increasing logP (Table 1). The IC_{50} values decreased from 22.5 ± 8.7 µM (**4a**) to 8.7 ± 3.2 µM (**4e**) upon extending the acyl-side chain. The anti-proliferative activity of the bicyclic aliphatic acylphloroglucinols (**5a** and **5d**) had the same range, and their activity likewise increased with the logP to yield IC_{50} values of 20.3 ± 5.2 and 13.3 ± 3.8 µM, respectively. Increasing the number of carbon atoms in the acyl group provides higher lipophilicity, which allows the compound to better penetrate and cross cellular membranes. Accordingly, changing the 2-prenyl (in **5d**) to a methyl group (in **5e**) decreased the lipophilicity (**5d** logP 5.5 versus **5e** logP 3.77) and anti-proliferative effect (**5d** 13.3 ± 3.8 and **5e** 29.3 ± 5.2 µM). The reduced anti-proliferative effect for **5e** matches the slight activity reduction for aliphatic chromanes acylated

at C-6 relative to the C-8 acyl derivatives, as reported for natural acylphloroglucinols by Schmidt et al.[12]

Except for compounds **7a** and **7e**, which exhibited a strong anti-proliferative activity with IC_{50} values of 0.88 ± 0.08 and 7.6 ± 1.5 µM, respectively, acylphloroglucinols in the second series with an aromatic acyl side chain and 2,2-dimethyl-chromane-6yl core exhibited lower anti-proliferative activities relative to their aliphatic 8yl analogues (**Table 1**) and the 6yl derivative **5e**. The strong activity for **7a** resulted from its 3,4-dihydroxy-phenyl substitution, whereas protecting the hydroxyl groups with methyl groups or changing the hydroxyl substitution pattern dramatically decreased the activity. Chromane **7a** is ≈50-fold more active than the 3,4-dimethoxy-benzoyl **6a** (48.0 ± 4.3 µM) and ≈30-fold more active than the 3,5-dihydroxybenzoyl derivative **7b** (25.5 ± 3.4 µM). Furthermore, dimethoxylation significantly reduced the water solubility and hampered using aqueous solutions *in vitro*. Thus, compound **6b**, which bears a 3, 5-dimethoxy-benzoyl group was not tested further in our assays. In contrast to the aliphatic series, the logPs for compounds **6a**-**d** (without **6b**) and **7a**-**e** did not correlate to their anti-proliferative activity *in vitro*, which implies they may act via a different mechanism than acylphloroglucinols **4a-e**, especially for the highly active compounds **7a** and **7e**.

The anti-oxidative activity of the acylphloroglucinols was tested via an ORAC (Oxygen Radical Absorbance Capacity) assay. Phloroglucinols containing aromatic acyl groups generally showed higher anti-oxidative effects (1.1 ± 0.1 – 6.6 ± 1.0 TE) relative to their monocyclic and bicyclic counterparts with aliphatic acyl substitution (0.3 ± 0.1 – 1.2 ± 0.2 TE). We also determined the ORAC activity of natural 8-hydroxy-6-yl chromanes, 1-[5,7-dihydroxy-2-methyl-2-(4-methylpent-3-enyl)-chroman-6-yl]-2-methylpropan-1-one and 1-[5,7-dihydroxy-2-methyl-2-(4-methylpent-3-enyl)-chroman-6-yl]-2-methylbutan-1-one, which were recently isolated from *H. empetrifolium* [11], relative to the aliphatic 6-hydroxy-8-yl **5a**. Their activities were 0.3 ± 0.1 and 0.4 ± 0.1 TE, which indicated that the anti-oxidative activity of the aliphatic 8-hydroxy-6-yl chromanes was reduced further relative to the 6-hydroxy-8-yl chromanes in our series. The strongest activity was observed for compound **7c** with 6.6 ± 1.0 TE, which is comparable to strong anti-oxidants such as caffeic acid or protocateichuic acid as reported by Davalos et al.[17]

Table 1. Anti-proliferative activity of aliphatic monocyclic (**4a-e**), bicyclic (**5a**, **5d** and **5e**) acylphloroglucinols and aromatic bicyclic (**6a-7e**) acylphloroglucinols on HMEC-1 (mean value ± SD, n = 3), status, calculated values for logP and redox-potential (Eh). Trolox equivalents (TE) were determined in an ORAC assay (1-5 µM) for all acylphloroglucinols with the exception of **6d**.

type	Compound	IC_{50} [µM]	LogP[a]	Trolox equivalents (TE)	Redox potential [V][b]
aliphatic sidechain	**4a**	22.5 ± 8.7	4.62	1.0 ± 0.1	1.06
	4b	22.4 ± 3.7	5.13	0.6 ± 0.1	0.86
	4c	21.0 ± 0.4	5.49	0.8 ± 0.1	0.86
	4d	11.0 ± 1.2	6.00	0.4 ± 0.1	0.86
	4e	8.7 ± 3.2	6.50	0.3 ± 0.1	0.86
	5a	20.3 ± 5.2	4.22	0.8 ± 0.1	1.23
	5d	13.3 ± 3.8	5.59	0.3 ± 0.1	1.23
	5e	29.3 ± 5.2	3.77	1.2 ± 0.2	0.92
aromatic sidechain	**6a**	48.0 ± 4.3	4.94	1.3 ± 0.1	0.98
	6c	43.4 ± 15.3	5.54	1.1 ± 0.1	0.96
	6d	51.5 ± 3.2	4.42		0.95
	7a	0.88 ± 0.08	3.68	2.8 ± 0.1	0.80
	7b	25.5 ± 3.4	5.30	3.0 ± 0.1	0.87
	7c	53.8 ± 0.3	5.07	6.6 ± 1.0	1.10
	7d	23.9 ± 2.4	3.66	3.0 ± 0.4	0.75
	7e	7.6 ± 1.5	4.53	2.1 ± 0.2	0.67
	xanthohumol	11.4 ± 1.1		2.3 ± 0.2[30]	
	Trolox				0.49

[a] The partition coefficient (LogP) of compounds were calculated with software ACD (Advanced Chemistry Development)/Lab 12.0.

[b] determined via cyclic voltammetry.

A high redox potential and radical scavenger activity were correlated for several phenolic compounds,[18] we also determined the redox potential for the acylphloroglucinols using cyclic voltammetry measurements with Trolox as the reference compound. However, there was no correlation between anti-oxidative activity measured in the ORAC assay and redox potential

within the tested acylphloroglucinols. The aliphatic 6-hydroxy-8-yl chromanes **5a** and **5d** (E_h=1.23 V each) were unable to reach the lower redox potential of the Trolox reference compound (E_h=0.49 V), and surprisingly, the 6-acyl chromanes with aromatic substitutions typically exhibited lower redox-potentials than the aliphatic 8-yl derivatives.

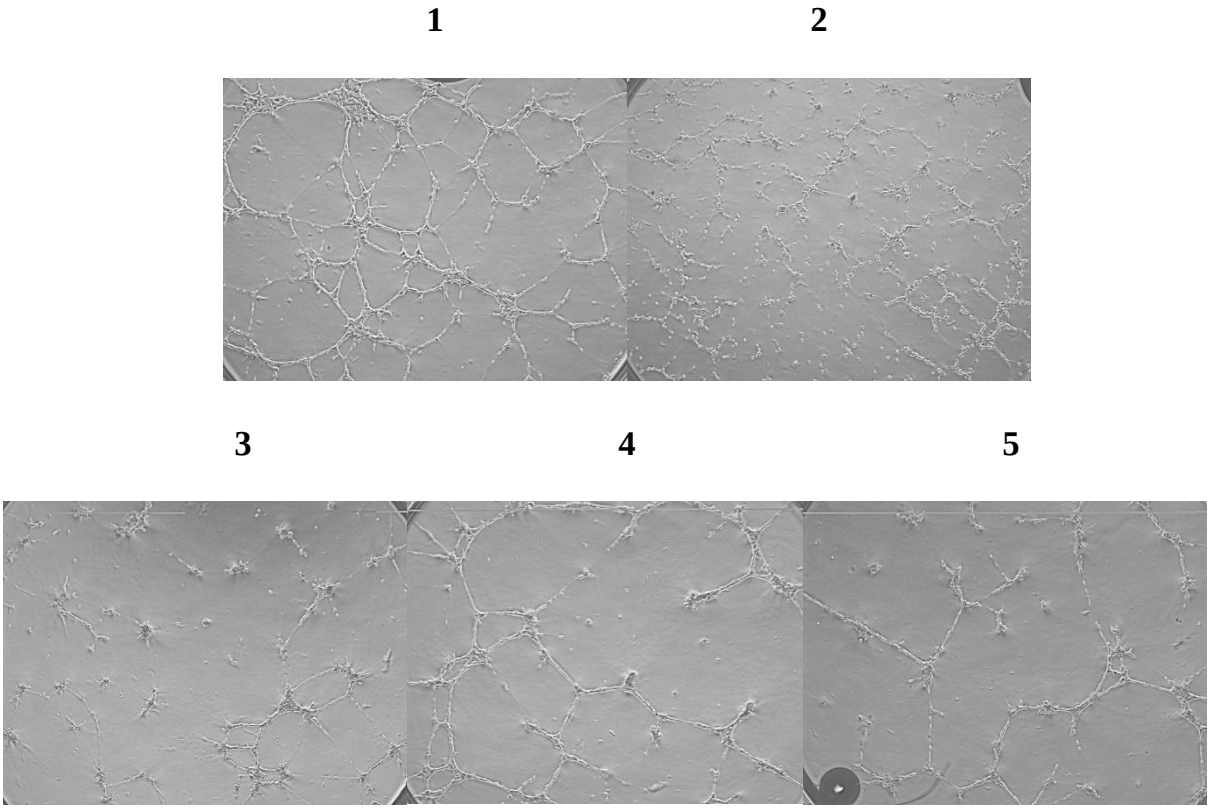


Figure 2: Tube-formation assay using compound **4d** in different concentrations: **1.** negative control (cells untreated), **2.** 50 µM, **3.** 25 µM, **4**. 12.5 µM, **5**. 6.25 µM

Interestingly, the high anti-proliferative activity of acylphloroglucinols was not associated with tube-formation inhibition. Compound **7a**, which showed the strongest activity during the proliferative assay, was inactive during the tube-formation assay. Only compounds **4d** and **6a** maintained their activity across the same concentration range in the proliferative assay in both series. Thus, the less specific anti-proliferative effect, which is potentially connected to a nonspecific cytotoxic activity, can be decoupled from the more specific tube-formation inhibition. Tube-formation is inhibited by compound **4d** at 50, 25, 12.5 and 6.25 µM (**Figure 2**) and by compound **6a** at 100, 50, 25 and 12.5 µM (**Figure 3**). Untreated wells and inactive acylphloroglucinols exhibited branching and interconnectivity, but endothelial cells treated with

4d and **6a** were dispersed and formed small cell clumps without significant network formation. The effect of **4d** was superior to that of **6a** at the same concentration.

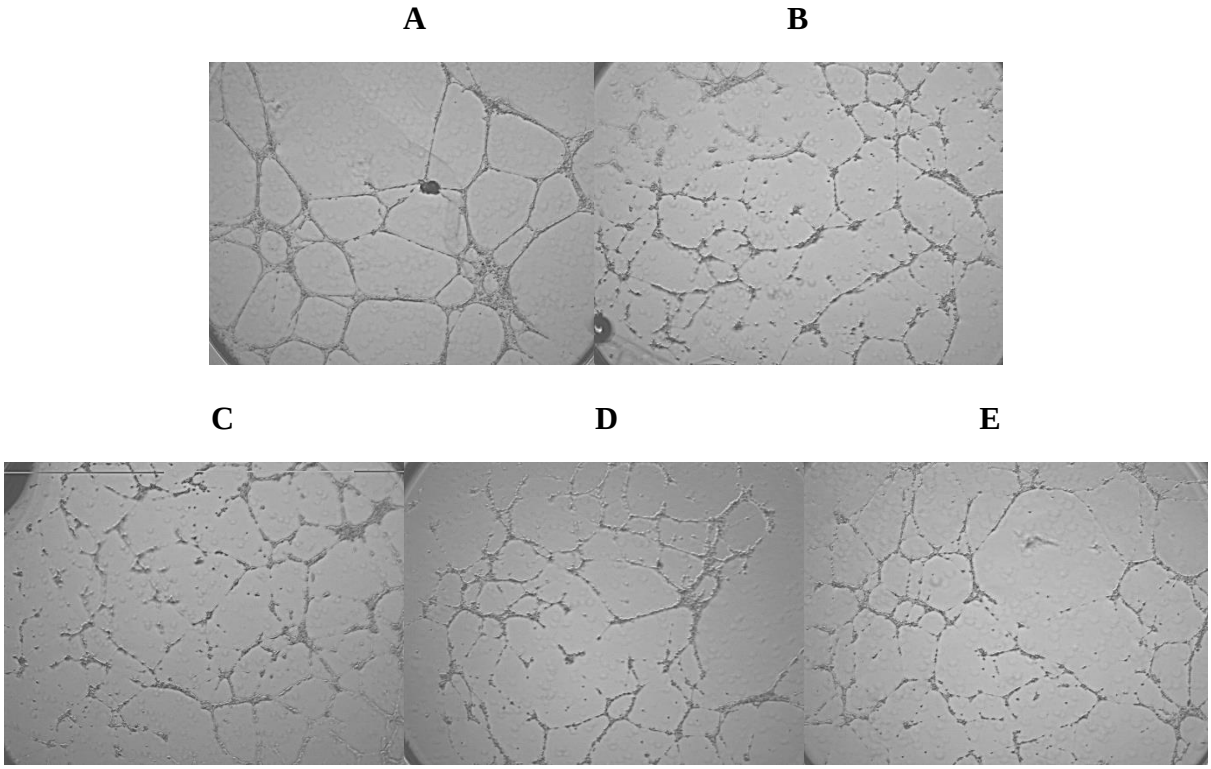


Figure 3: Tube-formation assay with of compound **6a** in different concentrations: **A**. negative control (cells untreated), B. 100 µM, **C**. 50 µM, **D**. 25 µM, **E**. 12.5 µM

Conclusions

Overall, the results suggest acylphloroglucinols containing simpler substitution patterns than hyperforin can show strong anti-proliferative effects and remarkable tube formation inhibition, which are not correlated. For monocyclic and bicyclic compounds containing aliphatic side-chains, the anti-proliferative effect is influenced by the side chain length, prenyl substitution and increasing lipophilicity. A simple correlation to logP was not observed for acylphloroglucinol with an aromatic acyl substitution. The prerequisite for a strong activity during the ORAC assay was the presence of an aromatic acyl-substituent especially one with a 3,4-dihydroxy-substitution. The ORAC assay activity did not correlate to the cyclic voltammetry redox potential.

The recently described biological importance of different terpene substituents at the hydroxyl group monocyclic acylphloroglucinols [11] makes the systematic synthesis of a library of O-substituted mono- and bicyclic acylphloroglucinols interesting. Furthermore, investigating the pharmacological activity of monocyclic phloroglucinols with aromatic acyl moieties is in progress.

Experimental

Chemistry

^{1}H, ^{13}C and 2D NMR spectra were obtained at 298 K using a Bruker AVANCE 300 spectrometer (operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C), Bruker AVANCE 400 spectrometer (operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) and Bruker AVANCE 600 spectrometer (operating at 600.25 MHz for ^{1}H and 150.93 MHz for ^{13}C) (Bruker, Karlsruhe, Germany). The spectra were obtained using chloroform-d (99.8%, Deutero GmbH) or methanol-d_4 (99.8%, Deutero GmbH) and referenced against non-deuterated (^{1}H) / deuterated (^{13}C) solvents. The shift values (δ_H and δ_C) are always given in ppm with *J* values in Hz. The melting points were measured using a Stanford Research Systems OptiMelt MPA 100. The high-resolution mass spectra were obtained using a Finnigan MAT SSQ 710 A spectrometer at 70 eV (HREIMS, positive and negative mode) or an Agilent 6540 UHD (HRESIMS, positive and negative mode). Automated flash chromatography was performed on a Biotage® Isolera™ Spektra One device. Silica gel 60 M (40-63 µm, Merck) was used for the flash column chromatography. The starting materials and reagents were purchased from commercial suppliers and used without further purification. The solvents were p.a. grade for the reaction mixtures and industrial grade for the flash column chromatography. Analytical TLC was performed on silica gel coated alumina plates (MN TLC sheets ALUGRAM® Xtra SIL G/UV_{254}). The visualisation was performed using UV-light (254 and 366 nm). The Log P values were calculated using ACD/Lab 12.0 software. The redox potential experiments were performed using an Autolab PGSTAT3202N to determine the E_h (in V).

The purity of the tested compounds was determined via analytical HPLC, Elite LaChrom (VWR, Darmstadt) using EZChromElite 3.1.7 Software to measure the purity in percent. Column:

Hibar® 2504 Purospher Star RP18e (5 µm), wavelength: peak maximum, gradient: FA 0.1%-MeCN 95%, 5→100 (MeCN 95%) within 30 min before washing and equilibrating the column to the starting conditions for a further 10 min, flow: 0.750 mL/min. The concentration of the tested compounds was 100 µM, and the injection volume was 20 µl. The compound purities were 90% or higher.

2,2-Dimethyl-5,7-dihydroxychromane (**2**)

The sulfonic acid cation-exchange resin Amberlyst 15 (10.0 g) was stirred in dry THF (120 mL) while refluxing at 65–75 ℃. Then, 1,3,5-trihydroxybenzene (5.0 g) was added to the resin and followed with 3.0 g of isoprene in hexane (40 mL) over 2 h. The reaction mixture was stirred for 1 hour under reflux before removing the heat source and adding 100 mL of Et_2O. The resin was filtered via vacuum filtration and rinsed with acetone (75 mL). The crude product mixture was purified via flash chromatography (PE (bp.50–70 ℃) /EtOAc 1:1) to afford compound **2** as white solid. Yield: 4.08 g, 53%. Mp. 156–157 ℃ (162 ℃[19]); ^{1}H NMR (300 MHz, MeOD) δ 5.86 (d, J = 2.4 Hz, 1H), 5.73 (d, J = 2.3 Hz, 1H), 2.52 (t, J = 6.8 Hz, 2H), 1.71 (t, J = 6.8 Hz, 2H), 1.26 (s, 6H). ^{13}C NMR (75 MHz, MeOD) δ 157.5, 157.3, 156.5, 101.5, 96.4, 95.6, 74.9, 33.7, 27.0, 17.7. These spectroscopic data are in accordance with the literature values.[20]

*General procedure for the synthesis of **3a-e***

$AlCl_3$ (4.0 equiv) was added to a stirred phloroglucinol suspension (1.0 equiv) in CS_2. Nitrobenzene was added to the solution over 30 min. The solution was refluxed at 55 ℃ for 30 min. Acyl chloride (1.0 equiv) dissolved in nitrobenzene was added to the reaction mixture over 30 min before heating for another 30 min. The reaction mixture was allowed to cool with stirring and then poured into an ice-water bath. Afterwards, 3M HCl was added. The organic solvents were removed under a reduced pressure, and the oily residue containing the acylphloroglucinol was extracted with Et_2O. After removing the Et_2O, the crude product was purified via flash chromatography (PE (bp.50–70 ℃) / EtOAc 5:1 → 3:1).[21]

1-(2,4,6-Trihydroxyphenyl)ethanone (**3a**)

Yield: 1.89 g, 75%. Yellow solid. Mp. 219–221 ℃ (216–218 ℃[22]); ^{1}H NMR (300 MHz, MeOD) δ 5.79 (s, 2H), 2.59 (s, 3H). The spectroscopic data are in accordance with the literature values.[23]

1-(2,4,6-Trihydroxyphenyl)propan-1-one (**3b**)

Yield: 826 mg, 75%. Pale yellow solid. Mp. 175–176 ℃ (174 ℃[24]); ^{1}H NMR (300 MHz, MeOD) δ 5.79 (s, 2H), 3.06 (q, J = 7.3 Hz, 2H), 1.12 (t, J = 7.3 Hz, 3H). The spectroscopic data are in accordance with the literature values.[23]

2-Methyl-1-(2,4,6-trihydroxyphenyl)propan-1-one (**3c**)

Yield: 1.35 g, 81%. Pale yellow solid. Mp. 138–140 ℃ (140 ℃[24]); ^{1}H NMR (300 MHz, MeOD) δ 5.80 (s, 2H), 3.97 (dt, J = 13.5, 6.8 Hz, 1H), 1.12 (d, J = 6.7 Hz, 6H). The spectroscopic data are in accordance with the literature values.[25]

2-Methyl-1-(2,4,6-trihydroxyphenyl)butan-1-one (**3d**)

Yield: 3.12 g, 55%. Pale yellow oil. ^{1}H NMR (300 MHz, MeOD) δ 5.81 (s, 2H), 3.83 (sext, J = 6.7 Hz, 1H), 1.89 – 1.67 (m, 1H), 1.47 – 1.26 (m, 1H), 1.09 (d, J = 6.8 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H). The spectroscopic data are in accordance with the literature values.[21]

2-Methyl-1-(2,4,6-trihydroxyphenyl)pentan-1-one (**3e**)

Yield: 1.32 g, 60%. Pale yellow oil. ^{1}H NMR (300 MHz, MeOD) δ 5.79 (s, 2H), 3.96 (dt, J = 13.2, 6.6 Hz, 1H), 1.85 – 1.63 (m, 1H), 1.43 – 1.18 (m, 3H), 1.10 (d, J = 6.7 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H). HRMS (EI-MS) calcd for $C_{12}H_{16}O_4$ $[M+H]^+$ 225.1121, found 225.1123.

*General procedure for synthesising **4a-e***

A mixture of **3a-e** (1 equiv), geranyl bromide (1 equiv), and K_2CO_3 (2 equiv) in acetone was refluxed for 24 h. Evaporating the acetone, adding a 2 N HCl solution, extracting with EtOAc, and removing the solvent were followed by a flash column chromatography on silica gel with PE （bp.50–70 ℃)/EtOAc (5:1) to yield the corresponding products.[26]

(E)-1-(3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxyphenyl)ethanone (**4a**).

Yield: 385 mg, 58%. Pale yellow solid. Mp. 112–114 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.41(s, br, 1H), 8.62 (s, br, 1H), 6.35 (s, 1H), 5.86 (s, 1H), 5.25 (dd, *J* = 7.2, 6.0 Hz, 1H), 5.13 – 4.97 (m, 1H), 3.37 (d, *J* = 7.1 Hz, 2H), 2.67 (s, 3H), 2.14 – 2.04 (m, 4H), 1.81 (d, *J* = 0.9 Hz, 3H), 1.67 (s, 3H), 1.59 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.8, 162.4, 161.4, 160.4, 140.0, 132.2, 123.6, 121.4, 105.4, 150.2, 95.3, 39.7, 32.9, 26.3, 25.7, 21.5, 17.7, 16.2. HRMS (EI-MS) calcd for $C_{18}H_{24}O_4$ $[M+H]^+$ 305.1747, found 305.1745.

(E)-1-(3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxyphenyl)propan-1-one (**4b**).

Yield: 182 mg, 60%. Pale yellow solid. Mp. 114–116 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.40 (s, br, 1H), 8.60 (bs, 1H), 6.01 (s, 1H), 5.84 (s, 1H), 5.25 (td, *J* = 7.2, 1.1 Hz, 1H), 5.05 (m, 1H), 3.38 (d, *J* = 7.2 Hz, 2H), 3.09 (q, *J* = 7.2 Hz, 2H), 2.17 – 2.04 (m, 4H), 1.81 (d, *J* = 0.7 Hz, 3H), 1.68 (d, *J* = 0.6 Hz, 3H), 1.60 (s, 3H), 1.17 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.8, 162.3, 160.7, 160.1, 140.2, 132.2, 123.6, 121.4, 105.5, 104.9, 95.4, 39.7, 37.3, 26.3, 25.7, 21.6, 17.7, 16.2, 8.6. HRMS (EI-MS) calcd for $C_{19}H_{26}O_4$ $[M–H]^-$ 317.1758, found 317.1752.

(E)-1-(3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxyphenyl)-2-methylpropan-1-one (**4c**).

Yield: 219 mg, 55%. Pale yellow solid. Mp. 109–111 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.55 (s, br, 1H), 8.35 (s, br, 1H), 5.93 (s, 1H), 5.83 (s, 1H), 5.32 – 5.20 (m, 1H), 5.12 – 4.97 (m, 1H), 3.92 – 3.84 (m, 1H), 3.38 (d, *J* = 6.9 Hz, 2H), 2.10 (t, *J* = 5.0 Hz, 4H), 1.81 (s, 3H), 1.69 (s, 3H), 1.60 (s, 3H), 1.18 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.6, 162.6, 160.7, 159.8, 140.2, 132.2, 123.6, 121.4, 105.6, 104.2, 95.5, 39.7, 39.3, 26.3, 25.7, 21.6, 19.3, 19.3, 17.7, 16.2. HRMS (EI-MS) calcd for $C_{20}H_{28}O_4$ $[M–H]^-$ 331.1915, found 331.1906.

(E)-1-(3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxyphenyl)-2-methylbutan-1-one (**4d**).

Yield: 214 mg, 60%. Pale yellow solid. Mp. 96–97 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.67 (s, br, 1H), 8.20 (s, br, 1H), 5.90 (s, 1H), 5.78 (s, 1H), 5.21 (t, *J* = 6.0 Hz, 1H), 5.11-4.91 (m, 1H), 3.75-3.64 (m, 1H), 3.33 (d, *J* = 7.1 Hz, 2H), 2.05 (s, br, 4H), 1.77 (s, 3H), 1.63 (s, 3H), 1.55 (s, 3H), 1.36 (tt, *J* = 14.5, 7.3 Hz, 2H), 1.11 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.8, 162.7, 161.2, 160.3, 139.4, 132.1, 123.7, 121.7, 105.7, 104.7, 95.4,

45.9, 39.7, 27.0, 26.3, 25.7, 21.6, 17.7, 16.7, 16.2, 12.0. HRMS (EI-MS) calcd for $C_{21}H_{30}O_4$ $[M+H]^+$ 347.2215, found 347.2217.

(E)-1-(3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxyphenyl)-2-methylpentan-1-one (**4e**).

Yield: 78 mg, 55%. Pale yellow solid. Mp. 77–79 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (s, 1H), 5.26 (t, J = 6.0 Hz, 1H), 5.14 – 4.91 (m, 1H), 3.90-3.79 (m, 1H), 3.37 (d, J = 7.1 Hz, 2H), 2.15 – 2.03 (m, 4H), 1.81 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.42 – 1.24 (m, 4H), 1.15 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.7, 162.8, 160.8, 159.6, 140.1, 132.2, 123.6, 121.5, 105.7, 104.6, 95.5, 44.2, 39.7, 36.2, 26.3, 25.7, 21.6, 20.7, 17.7, 17.2, 16.2, 14.2. HRMS (EI) calcd for $C_{22}H_{32}O_4$ $[M–H]^-$ 359.2228, found 359.2229.

1-(5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)chroman-8-yl)ethanone (**5a**).

Yield: 107 mg, 53%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.87 (s, 1H), 6.33 (s, 1H), 5.95 (s, 1H), 5.09 (ddd, J = 7.1, 4.1, 1.2 Hz, 1H), 2.64 (s, 3H), 2.63 – 2.55 (m, 2H), 2.10-2.02 (m, 2H), 1.95 – 1.63 (m, 5H), 1.60 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.6, 164.8, 160.7, 157.6, 132.2, 123.7, 106.3, 99.9, 95.2, 78.4, 39.6, 33.4, 29.4, 25.7, 24.2, 22.6, 17.6, 16.0. HRMS (EI-MS) calcd for $C_{18}H_{24}O_4$ $[M+H]^+$ 305.1747, found 305.1749.

1-(5,7-Dihydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)chroman-8-yl)-2-methylbutan-1-one (**5d**).

Yield: 33 mg, 65%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.12 (s, 1H), 5.97 (s, 1H), 5.09 (t, J = 6.8, 1H), 3.75 (sext, J = 6.8 Hz, 1H), 2.58 (m, 2H), 2.10 − 2.03 (m, 2H), 1.87 – 1.77 (m, 3H), 1.70 (m, 2H), 1.68 (s, 3H), 1.60 (s, 3H), 1.40 (m, 1H), 1.36 (s, 3H), 1.14 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.7, 165.0, 160.5, 157.0, 132.2, 123.6, 106.0, 100.1, 95.5, 78.4, 46.3, 39.7, 29.1, 27.0, 25.6, 24.0, 22.6, 17.5, 16.6, 16.2, 12.0. HRMS (EI-MS) calc. for $C_{21}H_{30}O_4$ $[M+H]^+$ 347.2217, found 347.2216. The spectroscopic data are in accordance with the literature.[11].

*General procedure for synthesis of **5e** and **6a-d**.*[27]

Anhydrous $AlCl_3$ (3.0 equiv) was added portionwise to a solution of **2** (1.0 equiv) in CH_2Cl_2. The corresponding chloride (1.0 equiv) was then added dropwise to keep the reaction temperature below −5 ℃. After stirring overnight at room temperature, the mixture was poured into ice-water and extracted with CH_2Cl_2. The combined organic layer was successively washed with saturated $NaHCO_3$ and brine, and then dried over anhydrous Na_2SO_4. The corresponding products were obtained after flash column chromatography (PE（bp.50-70 ℃）/EtOAc 5:1 → 3:1).

1-(5,7-Dihydroxy-2,2-dimethylchroman-6-yl)-2-methylbutan-1-one (**5e**).

Yield: 52 mg, 26%. Pale yellow solid. Mp. 145–146 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.70 (s, br, 1H), 6.60 (s, br, 1H), 5.74 (s, 1H), 3.81 – 3.70 (m, 1H), 2.58 (t, J = 6.8*Hz*, 2H), 1.78 (t, J = 6.8 Hz, 2H), 1.46 – 1.34 (m, 2H), 1.32 (s, 6H), 1.16 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 210.3, 164.1, 160.2, 157.7, 103.8, 101.7, 95.7, 76.0, 45.8, 32.1, 27.0, 26.7, 16.7, 16.1, 12.0. The spectroscopic data are in accordance with the literature.[28]

6-(3,4-Dimethoxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**6a**).

Yield: 60 mg, 68%. White solid. Mp. 127–128 ℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.73 (s, br, 1H), 7.31 (dd, J = 8.3, 2.0 Hz, 1H), 7.23 (d, J = 1.9 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H), 5.90 (s, 1H), 3.94 (d, J = 14.3 Hz, 6H), 2.61 (t, J = 6.8 Hz, 2H), 1.81 (t, J = 6.8 Hz, 2H), 1.36 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.0, 161.7, 160.9, 158.1, 152.9, 149.6, 131.9, 122.2, 110.9, 110.9, 103.9, 101.8, 96.9, 76.0, 56.2, 56.1, 32.1, 26.8, 16.3. HRMS (EI-MS) calcd for $C_{20}H_{22}O_6$ $[M-H]^-$ 357.1345, found 357.1344.

6-(3,5-Dimethoxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**6b**)

Yield: 200 mg, 52%. Pale yellow solid. Mp. 145–147 ℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 11.02 (s, 1H), 7.28 (s, br, 1H), 6.70 (d, J = 2.3 Hz, 2H), 6.62 (t, J = 2.2 Hz, 1H), 5.87 (s, 1H), 3.83 (s, 6H), 2.60 (t, J = 6.8 Hz, 2H), 1.80 (t, J = 6.8 Hz, 2H), 1.35 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ

196.7, 162.4, 161.6, 158.8, 141.9, 104.5, 104.1, 103.7, 101.8, 97.1, 76.2, 55.6, 32.0, 26.7, 16.1. HRMS (EI-MS) calcd for $C_{20}H_{22}O_6$ $[M+H]^+$ 359.1495, found 359.1491.

6-(4-Methoxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**6c**).

Yield: 270 mg, 48%. Pale yellow solid. Mp. 107–108 ℃. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.81 (s, 1H), 7.64 (d, J = 8.8 Hz, 2H), 7.13 (s, 1H), 6.96 – 6.92 (m, 2H), 5.86 (s, 1H), 3.85 (s, 3H), 2.60 (t, J = 6.8 Hz, 2H), 1.79 (s, 2H), 1.34 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 196.4, 163.0, 161.4, 160.8, 157.8, 132.0, 130.7, 114.1, 104.0, 101.6, 96.7, 75.9, 55.4, 32.0, 26.7, 16.2. HRMS (EI-MS) calcd for $C_{19}H_{20}O_5$ $[M+H]^+$ 329.1386, found 329.1384.

6-(3,4,5-Trimethoxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**6d**).

Yield: 135 mg, 21%. White solid. Mp. 208–209 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (s, 1H), 7.03 (s, br, 1H), 6.88 (s, 2H), 5.89 (s, 1H), 3.91 (s, 3H), 3.88 (s, 6H), 2.62 (t, J = 6.8 Hz, 2H), 1.81 (t, J = 6.8 Hz, 2H), 1.36 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.3, 162.2, 161.4, 158.3, 153.9, 141.5, 134.8, 104.9, 103.7, 101.9, 97.0, 76.2, 61.0, 56.4, 32.1, 26.8, 16.2. HRMS (EI-MS) calcd for $C_{21}H_{24}O_7$ $[M–H]^-$ 387.1449, found 387.1455.

*General procedure for synthesis of **7a-e***.[29]

BBr_3 (2.5 mmol) was added to a solution of **6a-d** (0.84 mmol) in DCM (30 mL) at −78 ℃. The reaction mixture was stirred for 30 min, warmed to room temperature and stirred overnight under an N_2 atmosphere. The mixture was quenched by carefully pouring into iced water. The aqueous layer was extracted three times with EtOAc and washed with 5% $NaHSO_3$ and water before drying the organic layer over anhydrous Na_2SO_4 and removing the solvent under reduced pressure to yield the target products.

6-(3,4-Dihydroxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**7a**).

Yield: 93 mg, 78%. Pale yellow solid. Mp: 79–81 ℃. ^{1}H NMR (600 MHz, MeOD) δ 7.06 (d, J = 2.0 Hz, 1H), 7.02 (dd, J = 8.2, 2.0 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H), 5.95 (s, 1H), 2.55 (s, 2H), 1.65 (t, J = 6.8 Hz, 2H), 1.04 (s, 6H). ^{13}C NMR (151 MHz, MeOD) δ 199.5, 161.6, 160.7, 156.3, 150.6, 145.5, 134.4, 123.2, 117.2, 115.3, 107.6, 101.5, 95.3, 76.0, 33.1, 26.6, 17.6. HRMS (EI-MS) calcd for $C_{18}H_{18}O_6$ $[M+H]^+$ 331.1182, found 331.1181.

6-(3,5-Dihydroxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**7b**).

Yield: 38 mg, 53%. Pale yellow solid. Mp. 99–101 ℃. ^{1}H NMR (300 MHz, MeOD) δ 6.48 (d, J = 2.2 Hz, 2H), 6.40 – 6.32 (m, 1H), 5.74 (s, 1H), 2.59 (t, J = 6.8 Hz, 2H), 1.80 (t, J = 6.8 Hz, 2H), 1.33 (s, 6H). ^{13}C NMR (75 MHz, MeOD) δ 201.1, 162.7, 162.2, 162.2, 160.1, 160.1, 159.5, 159.0, 154.2, 107.5, 106.1, 105.9, 101.6, 96.6, 76.7, 33.2, 27.0, 17.2. HRMS (EI-MS) calcd for $C_{18}H_{18}O_6$ $[M+H]^+$ 331.1182, found 331.1174.

6-(4-Hydroxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**7c**).

Yield: 83 mg, 48%. Pale brown solid. Mp.157–159 ℃. ^{1}H NMR (300 MHz, MeOD) δ 7.60 – 7.41 (m, 2H), 6.81 – 6.63 (m, 2H), 5.77 (s, 1H), 2.59 (t, J = 6.8 Hz, 2H), 1.80 (t, J = 6.8 Hz, 2H), 1.33 (s, 6H). ^{13}C NMR (75 MHz, MeOD) δ 199.4, 162.5, 161.8, 161.3, 159.2, 133.7, 132.7, 115.3, 106.2, 101.8, 96.6, 76.5, 33.3, 27.0, 17.4. HRMS (EI-MS) calcd for $C_{18}H_{18}O_5$ $[M+H]^+$ 315.1232, found 315.1225.

6-(3,5-Dimethoxy-4-hydroxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**7d**).

Yield: 68 mg, 43%. Pale yellow solid. Mp. 182–184 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.86 (s, 1H), 6.95 (s, 3H), 5.99 (s, 1H), 5.90 (s, 1H), 3.91 (s, 6H), 2.61 (t, J = 6.8 Hz, 2H), 1.80 (t, J = 6.8 Hz, 2H), 1.35 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.9, 161.9, 161.0, 158.0, 147.4, 138.9, 130.3, 105.3, 103.8, 101.9, 97.0, 76.1, 56.6, 32.1, 26.8, 16.2. HRMS (EI-MS) calcd for $C_{20}H_{22}O_7$ $[M+H]^+$ 375.1440, found 375.1438.

6-(3,4-Dihydroxy-5-methoxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran (**7e**).

Yield: 113 mg, 64%. Pale yellow solid. Mp. 95–97 ℃. ^{1}H NMR (300 MHz, MeOD) δ 6.88 (dd, J = 10.1, 1.9 Hz, 2H), 5.78 (s, 1H), 3.84 (s, 3H), 2.60 (t, J = 6.8 Hz, 2H), 1.80 (t, J = 6.8 Hz, 2H), 1.32 (d, J = 6.0 Hz, 6H). ^{13}C NMR (75 MHz, MeOD) δ 199.5, 161.3, 161.2, 159.0, 148.8, 145.7, 139.8, 132.8, 112.1, 106.5, 106.0, 101.8, 96.7, 76.4, 56.7, 33.3, 27.0, 17.4. HRMS (EI-MS) calcd for $C_{19}H_{20}O_7$ $[M+H]^+$ 361.1287 found 361.1277.

Redox Potential Experiments. [30]

The potentials were recorded using a glassy carbon working electrode, platinum counter electrode and silver wire pseudo reference electrode. All compounds were measured in CH_3CN with tetrabutyl ammonium tetrafluoroborate as the supporting electrolyte. The solvent was degassed via vigorous argon bubbling prior to the measurements. All experiments were performed under an argon atmosphere. Ferrocene was used as the internal reference for the reduction and oxidation potentials.

Proliferation Assay. [31]

The proliferation assay was performed using a SV-40T transfected human microvascular endothelial cell line (HMEC-1).[32] The cells were incubated at 37 ℃ under a 5% CO_2 air atmosphere with a constant humidity (95%). HMEC-1 were seeded into 96-well microplates (100 µl, 1.5×10^3 cells/well) in Endothelial Cell Growth Medium (ECGM) with 10% FCS, Supplement Mix and Antibiotics (all from Provitro). After 24 h, the medium in a reference plate was removed, and these cells were stained with a crystal violet solution for 10 min to obtain a baseline. The cells in the other plates were treated with increasing concentrations of each test compound (dissolved in DMSO as a stock solution). After incubating for 72 h, the cells were stained as previously described. After washing with distilled water, 100 µl of a soluble buffer was added and the absorbance was measured using a Tecan Spectra Fluor Plus at 540 nm. The IC_{50} values ± SD were calculated in µM using the GraphPad software (from three independent experiments with different passages and each concentration in hexaplicates). Xanthohumol was

used as the positive control. Pure ECGM containing 0.1% DMSO was used as the negative control.

Tube Formation Assay. *[31]*

HMEC-1 build capillary-like structures on Matrigel™ (BD Biosience, Heidelberg Germany), which is a solubilised basement membrane extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma. After polymerising 10 µl of Matrigel™ in µ-slides (ibidi, Martinsried Germany) within 30 min at 5% CO_2, 37 ℃, and 95% humidity, the gel was overlaid with 1×10^4 HMECs in 25 µl ECGM. The cells were either left untreated (+ 25 µl ECGM containing 0.1% DMSO) or stimulated with 25 µl of ECGM including four concentrations of the synthesised acylphloroglucinols in their IC_{50} range. After 19 h, the cells were photographed using a PrimoVert microscope (Zeiss, Oberkochen Germany). Each experiment was performed in triplicate. One representative picture is shown.

ORAC-fluorescein assay.

The antioxidant activity was determined via an oxygen radical absorbance capacity-fluorescein (ORAC-FL) assay[16, 17] as previously described.[33] In brief, the ORAC-FL assay was performed in a 96-well plate containing fluorescein (final concentration 300 nM) as a fluorescent probe and using a 75 mM phosphate buffer (pH 7.4) for all dilution steps and as a reaction milieu. The antioxidant (test compounds or Trolox, 20 µL) was incubated to different concentrations (test compounds: 1–5 µM, Trolox: 1–8 µM) with a fluorescein solution (120 mL) at 37 ℃ for 15 min. The reaction began upon the addition of 60 µL AAPH (2,2'-azobis-(2-methylpropionamide)-dihydrochloride, final concentration: 12 mM) for a final volume of 200 µL. After adding AAPH, the fluorescence was recorded every minute in a Tecan 96-plate reader (λ_{ex} 485 nm, λ_{em} 536 nm, 37 ℃) for 80 min. The reaction mixtures were prepared in quadruplicate, and at least four independent assays were performed for each sample. The samples were measured at five different concentrations (1–5 µM). Eight calibration curves were obtained for each assay using 1–8 µM Trolox as the antioxidant. The controls were measured without an antioxidant or without AAPH and antioxidant. The ORAC values were expressed as Trolox equivalents (mean ± SD)

using the standard curve calculated for each assay. The Regression coefficient between the AUC and antioxidant concentration was calculated for all samples (r^2 >0.93). Further positive control measurements were performed using xanthohumol.

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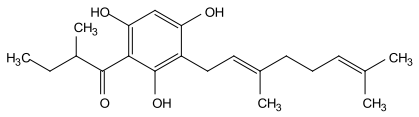
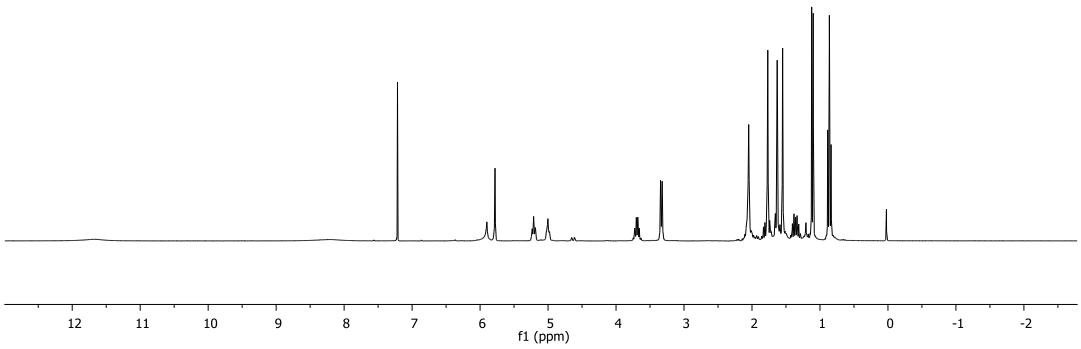
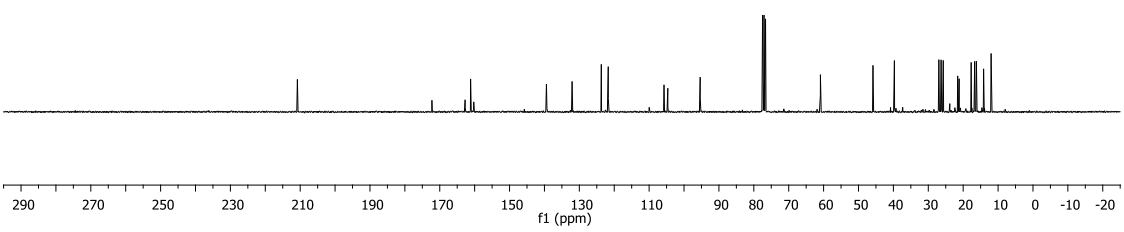
^{1}H and ^{13}C NMR spectra of selected final compounds

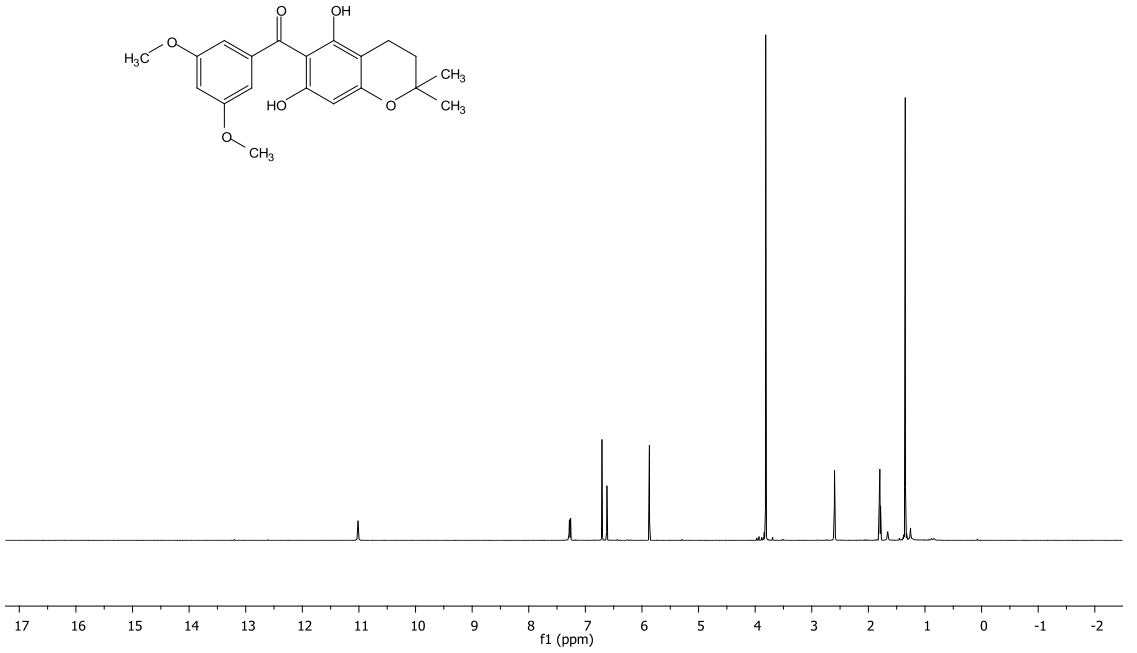
^{1}H and ^{13}C NMR spectra for (E)-1-(3-(3,7-Dimethylocta-2,6-dien-1-yl)-2,4,6-trihydroxy phenyl)-2-methylbutan-1-one **(4d)** (300 MHz, $CDCl_3$)

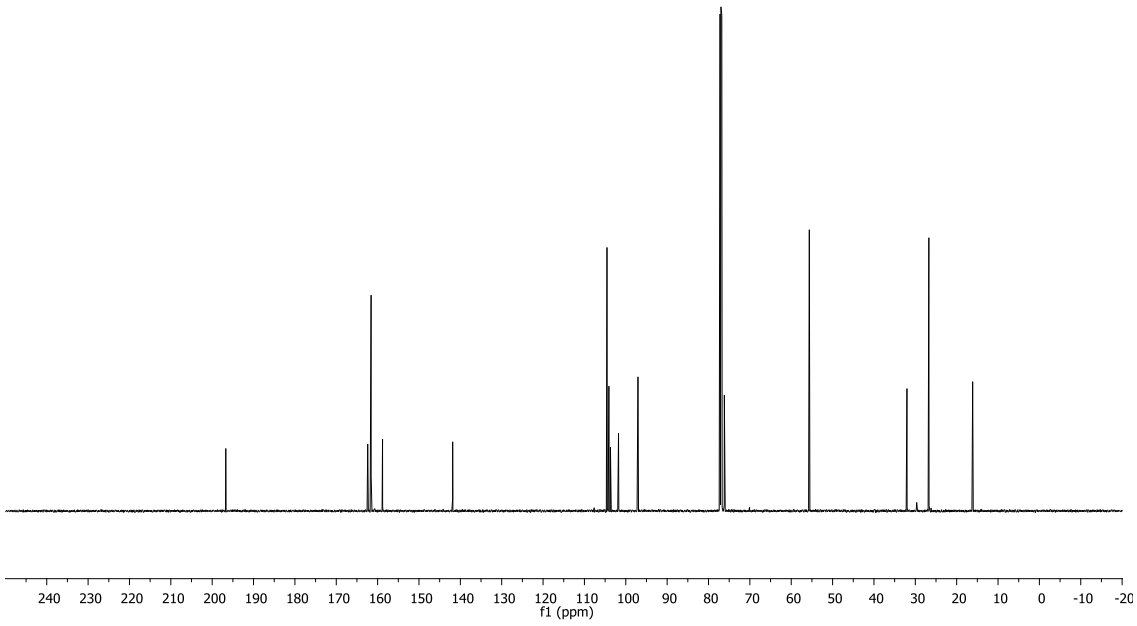




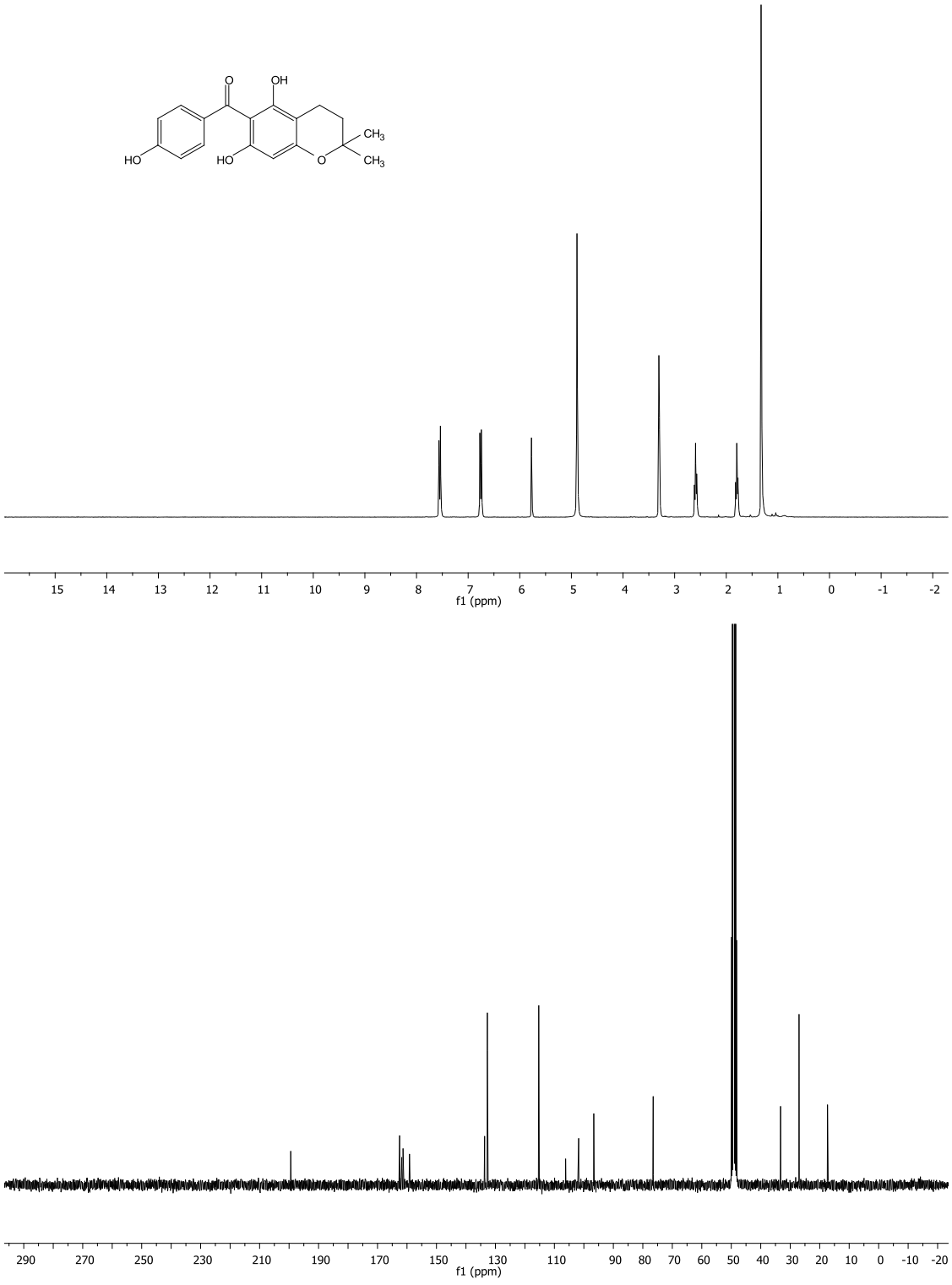


^{1}H and ^{13}C NMR spectra for 6-(3,5-Dimethoxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran **(6b)** (600 MHz, $CDCl_3$)



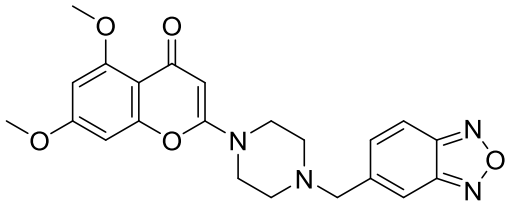


^{1}H and ^{13}C NMR spectra for 6-(4-Hydroxy)benzyl-5,7-dihydroxy-2,2-dimethyl-3,4-dihydro-2H-1-benzopyran **(7c)** (300 MHz, MeOD)



Chapter 3

Flavonoid derivatives as selective ABCC1 modulators: synthesis and functional characterization



ABCC1: IC_{50}=11.3 uM; inactive at ABCB1 and ABCG2

Abstract

A series of chromones, bearing substituted amino groups or N-substituted carboxamide moieties in 2-position, was synthesised and characterized in cellular assays for modulation of the ABC transporters-ABCC1 (MDCKII-MRP1 cells), ABCB1 (Kb-V1 cells) and ABCG2 (MCF-7/Topo cells). The most potent ABCC1 modulators identified among these flavonoid-type compounds were comparable to reversan regarding potency, but superior in terms of selectivity concerning ABCB1 and ABCG2 (IC_{50} values: reversan: ABCC1, 4.3 μM; ABCB1, 5.6 μM, ABCG2, 109 μM; 2-[4-(Benzo[c][1,2,5]oxadiazol-5-ylmethyl)piperazin-1-yl]-5,7-dimethoxy-4*H*-chromen-4-one (**51**): ABCC1: 11.3 μM; inactive at ABCB1 and ABCG2). Compound **51** was as effective as reversan in reverting ABCC1-mediated resistance to cytostatics in MDCKII-MRP1 cells and proved to be stable in mouse plasma. Modulators, such as compound **51**, are of potential value as pharmacological tools for the investigation of the (patho)physiological role of ABCC1.

Keywords

ABC transporters, multidrug resistance-associated protein 1, inhibitors, synthesis, flavonoid.

Manuscript in preparation.
Q.Sun synthesized all the compounds and wrote the chemistry part of the manuscript.

Introduction

The superfamily of *human ATP-binding cassette* (ABC) proteins comprises 49 members divided into 7 subfamilies (ABCA − ABCG).[1] The proteins ABCB1 (p-glycoprotein, p-gp), ABCG2 (BCRP, MXR) and ABCC1 (MRP1) are amply expressed efflux pumps in various tissues related with absorption, metabolism and excretion as lung, gut, kidney and liver. In addition, these transporters are important components of physiological barriers such as the blood-brain barrier and the blood-cerebrospinal fluid barrier, mediating the transport of endogenous compounds and playing a protective role against a wide range of structurally diverse xenobiotics.[2] ABC transporters are known to lower the bioavailability of orally administered drugs and to limit the access of pharmacotherapeutics to the central nervous system (CNS). Moreover, ABCB1, ABCG2 and ABCC1 are the most prominent efflux transporters expressed in cancer cells and related to resistance against numerous antitumor agents such as anthracyclines, vinca alcaloids or epipodophyllotoxins, resulting in poor outcome of chemotherapy.[1c, 2-3] This phenomenon is known as *multidrug resistance* (MDR).[3] Since its discovery[4] in 1992, ABCC1 has been the subject of many investigations on its role in physiological processes and MDR.[5] Besides non-small-lung cancer cells,[4] ABCC1 overexpression was detected in acute myeloblastic leukemia, acute lymphoblastic leukemia, prostate cancer, some types of breast cancer and especially neuroblastoma in early childhood.[6] Based on this evidence, the coadministration of selective ABCC1 modulators and cytostatic, which is substrate of ABCC1, might improve the outcome in cancer therapy. Several substances were described as modulators of ABCC1 (**Figure 1**), including MK-571, flavonoids, imidazothiazole derivatives, dihydropyridines, pyrrolopyrimidines, indolopyrimidines, anellated isoxazoles such as LY465803 and LY455776, and reversan.[6-7] Nevertheless, most of them are non-selective inhibitors or substrates of the ABCC1 transporter.

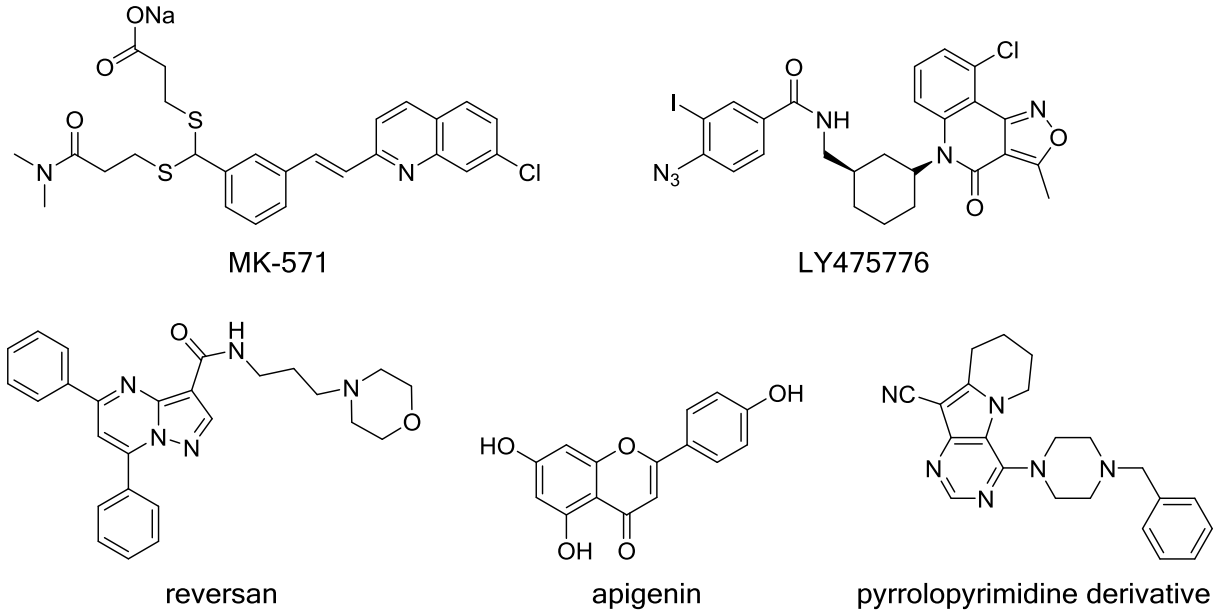


Figure 1. Structure of several ABCC1 (MRP1) modulators.

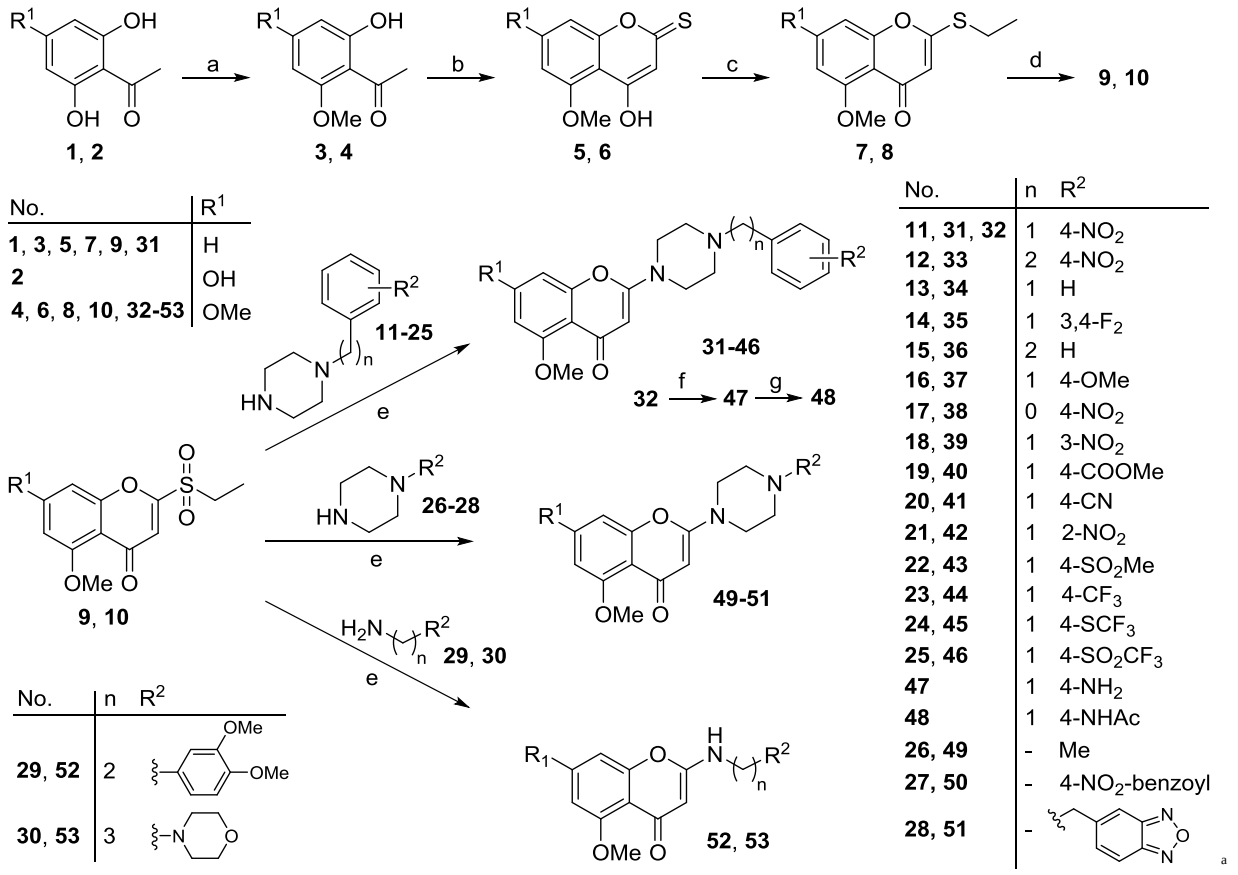
Selective modulators are required as pharmacological tools to investigate their role of ABC transporters in health and disease, to improve access to the brain or to overcome chemoresistance. Previously, potent and selective ABCB1 and ABCG2 modulators were developed in our laboratory.[8] Here we report on the synthesis and characterization of a new class of ABCC1 modulators based on the core structure of flavonoids.

Results and discussion

Chemistry

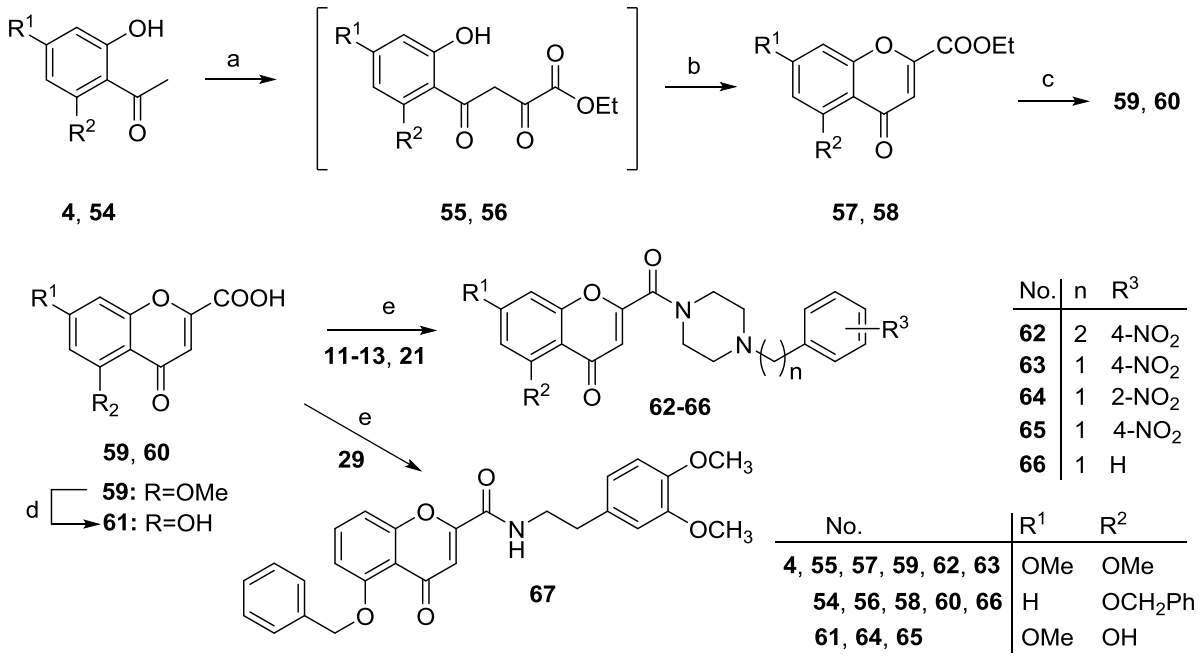
The design of the title compounds was inspired by different studies reporting flavonoids[7a, 7b] as substrates or inhibitors of ABC transporters and by recent studies, which used computational approaches such as docking and pharmacophore models to elucidate structure-activity relationships.[7e, 7f]

Our synthetic strategy for compounds **31-53** (**Scheme 1**) follows a published procedure.[9] Commercially available hydroxylated acetophones (**1, 2)** were methylated with Me_2SO_4 in good yields. Ring closure of the obtained compounds (**3, 4**) was accomplished by treatment with CS_2 in the presence of H_2SO_4 as a catalyst to give the chromene-2-thiones **5** and **6**. Subsequent S-alkylation with ethyl iodide under basic conditions followed by the oxidation of the ethylsulfanyl-4-chromones (**7, 8)** with m-CPBA led to the corresponding sulfoxides (**9, 10**). Finally, the reaction of **9-10** with appropriate N-substituted piperazines **11-28** or primary amines **29**, **30** at ambient conditions afforded **31-46**, **49-53** in 20–45% yield. The aromatic amine **47** was obtained in 80 % yield by reduction of **32** using Fe/HCl/H_2O according to a known protocol [10] and chromatographic purification in 80 % yield. Subsequent amidation with acetyl chloride gave **48** as a colorless solid in 46% yield.



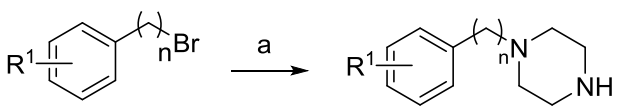
Scheme 1. Synthesis of final compounds **31-53.** Reagents and conditions: (a) Me_2SO_4, acetone, 65 ℃. (b) (1) tBuOK, CS_2, $PhCH_3$, r.t; (2) 10% H_2SO_4, rt. (c) EtI, K_2CO_3, acetone, reflux. (d) mCPBA, DCM, reflux. (e) DIPEA, N-substituted piperazines, EtOH, 80 ℃. (f) iron powder, conc. HCl, EtOH, reflux. (g) acetyl chloride, Et_3N, DCM, r.t.

Compounds **62-65** and the reference compounds **66** and **67**[11] were synthesised as shown in **Scheme 2**. In the first step, by analogy with a described procedure,[12] Claisen condensation was conducted yielding intermediates **55, 56** followed by acid-catalysed cyclisation reaction, which gave esters 1**4, 15**. After hydrolysis of the ester, products **62-67** were prepared under standard amide coupling conditions with HBTU.[13]

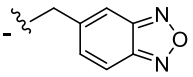


Scheme 2. Synthesis of chromone-2-carboxamides **62-67**. Reagents and conditions: (a) NaOEt, diethyl oxalate. (b) conc.HCl, EtOH, reflux. (c) K_2CO_3, EtOH, THF, r.t. (d)HBr, HOAc, reflux. (e) DIPEA, HBTU, DCM, r.t.

Compound **17** and **26** were commercially available; the synthesis of other analogues has already been reported.[14] The N-substituted piperazine analogues **11-16, 18-25** and **28** (**Scheme 3**) were obtained by nucleophilic substitution reaction with piperazine gaving 45%–75% yields.

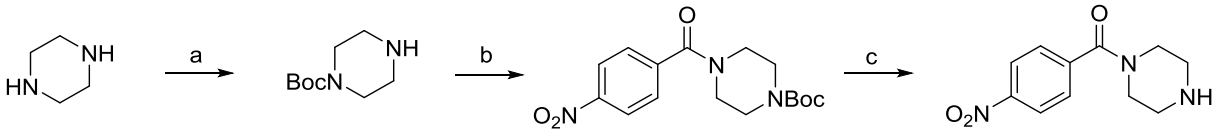


68-82 **11-16, 18-25, 28**

No.	n	R^2
11, 68	1	4-NO_2
12, 69	2	4-NO_2
13, 70	1	H
14, 71	1	3,4-F_2
15, 72	2	H
16, 73	1	4-OMe
18, 74	1	3-NO_2
19, 75	1	4-COOMe
20, 76	1	4-CN
21, 77	1	2-NO_2
22, 78	1	4-SO_2Me
23, 79	1	4-CF_3
24, 80	1	4-SCF_3
25, 81	1	4-SO_2CF_3
28, 82	-	

Scheme 3. Synthesis of N-substituted piperazine analogues **11-16, 18-25 and 28**. Reagents and conditions: (a) piperazine, K_2CO_3, THF, reflux.

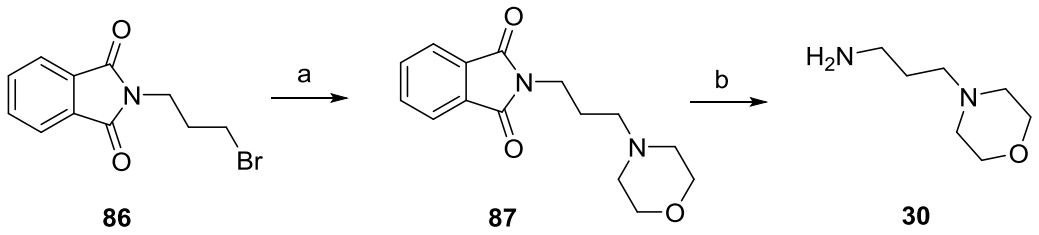
To avoid of acylation on both NH groups, mono-protection of piperazine was performed with *tert*-butyl dicarbonate. Followed by peptide coupling and deprotecting the Boc group (**Scheme 4**), compound **27** was obtained as yellow crystals in a good yield.[13]



83 **84** **85** **27**

Scheme 4. Synthesis of (4-nitrophenyl) (piperazine-1-yl)methanone **27**. Reagents and conditions: (a) Boc_2O, DCM, r.t; (b) DIPEA, HBTU, DCM, r.t. (c) (1) TFA, DCM, r.t; (2) 10% NaOH.

As shown in **Scheme 5**, 3-morpholinopropan-1-amine **30** was synthesised via nucleophilic substitution, followed by subsequent deprotection of phthalimide by hydrazine hydrate.[15]



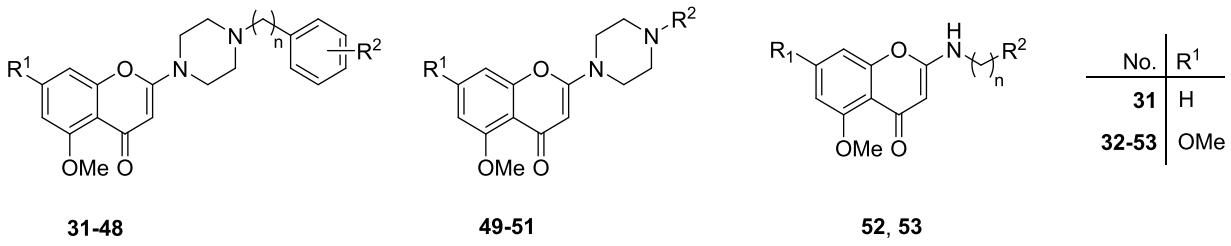
Scheme 3. Synthesis of 3-morpholinopropan-1-amine **30**. Reagents and conditions: (a) morpholine, Et_3N, DCM, reflux; (b) hydrazine hydrate, EtOH, reflux.

Biological evaluation

Inhibition of ABCC1-, ABCB1- and ABCG2-Transporters

The synthesised compounds **31-53**, **62-67** and reference substances were investigated for modulation of ABCC1, ABCB1 and ABCG2 using a calcein-AM and Hoechst 33342 efflux assays in the microtiter plate format. The data are summarized in **Table 2** and **Table 3**.

Table 2. Inhibition of ABCC1, ABCB1 and ABCG2 transporters by the new flavonoid-type modulators **31-53** and reference compounds



Compound	n	R^2	ABCC1[a]		ABCB1[b]		ABCG2[c]	
			IC_{50} µM[d]	I_{max} % [d,e]	IC_{50} µM[c]	I_{max} % [c,f]	IC_{50} µM[d]	I_{max} % [d,g]
31	1	4-NO_2	7.3 ±2	38.8 ± 3.5	inactive	-	inactive[h]	-
32	1	4-NO_2	13.2 ±2.5	130.1 ± 7.7	inactive	-	87.8 ±6.7	56.9 ± 2.5
33	2	4-NO_2	10.9 ±1.8	118.5 ± 6.4	33.0 ± 4.6	54.8 ± 3.6	36.9 ±6.7	31.9 ± 2.7
34	1	H	>100	-	inactive	-	inactive[h]	-

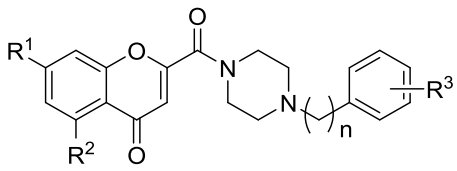
-**Table 1** continued-

35	1	3,4-F_2	38.0 ±16	140.6 ± 20	58.5 ±6	14.3 ±1	66.6 ±11.3	50.4 ± 5
36	2	H	6.8 ±4.2	44.5 ± 7.6	inactive	-	>100	-
37	1	4-OMe	>100	-	inactive	-	>130 [h]	-
38	0	4-NO_2	12.1 ±2.2	64.1 ± 3.5	inactive	-	18.3 ± 2.2[h]	15.9 ± 0.9
39	1	3-NO_2	98.7 ± 39.2 [h]	149.9 ± 21.3	32.8 ± 3.9	29.2 ± 1.9	38.2 ±6.4	44.0 ± 3.8
40	1	4-COOMe	58.3 ± 10.3	118.5 ± 11.8	>100	-	>100	-
41	1	4-CN	26.7 ±3.5	114.9 ± 5.2	110.7 ± 3.9	48.1 ± 1.2	99.7 ±8.6	59.1 ± 4.0
42	1	2-NO_2	63.8 ± 23.1[h]	82.5 ± 12.9	>100	-	44.8 ±5.1	93.1 ± 5.7
43	1	4-SO_2Me	66.4 ± 30.7	98.5 ± 31.2	inactive	-	inactive	-
44	1	4-CF_3	19.8 ±2.6	158.6 ± 7.3	31.3 ±83	21.8 ± 1.5	98.1 ±28	99.2 ± 23.5
45	1	4-SCF_3	20.8 ± 11.2	136.5 ± 24.6	36.6 ± 3.2	29.4 ±1	91 ±14.2	202 ± 32.3
46	1	4-SO_2CF_3	22.7 ±5.8	98.8 ± 8.6	>100	-	92 ±30.4	127 ± 34.8
47	1	4-NH_2	>100	-	inactive	-	inactive[h]	-
48	1	4-NHAc	>100	-	inactive	-	inactive[h]	-
49	1	Me	inactive	-	inactive	-	inactive[h]	-
50	1	4-NO_2-benzoyl	30.1	19.7 ± 1.5	inactive	-	inactive	-
51		benzo[c][1,2,5]oxadiazol-5-ylmethyl	11.3 ±1.8	119.6 ± 6.4	inactive	-	inactive	-
52	2	3,4-dimethoxyphenyl	>100	-	inactive	-	>100 [h]	-
53	3	Morpholin-4-yl	53.3 ± 17.8	14 ±2.7	inactive	-	inactive	
Reversan			4.3 ±0.2	100	6.8 ± 0.3[f]	143.3 ± 3.3	n.d.	-
Fumitremorgin C			inactive	-	n.d.	-	0.73 ± 0.09[i]	100
Tariquidar			inactive	-	0.22 ± 0.008[j]	100	0.52± 0.085[j]	69±5

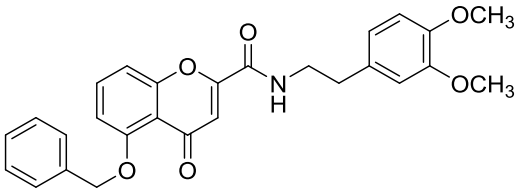
[a] Calcein-AM microplate assay using ABCC1-overexpressing MDCK-MRP1 or [b] ABCB1-overexpressing Kb-V1 cells. [c] Hoechst 33342 assay using ABCG2-overexpressing MCF-7/Topo cells. [d] Mean values ± SEM from two to four independent experiments performed in triplicate or sextuplicate. [e] Inhibitory effect (I_{max}) relative to the maximum response to reversan at a concentration of 30 µM (100%). [f] I_{max} expressed as percent inhibition relative to tariquidar at a concentration of 1 µM (100%). [g] I_{max} relative to FTC at a concentration of 10 µM (100%). [h] N=1. [i] Ref.[16] [j] Ref.[17] IC_{50} values were calculated using SigmaPlot 11.0, four parameter logistic curve fitting; inactive: no transporter inhibition up to a concentration of 100 µM; n.d. not determined.

The most potent ABCC1 inhibitors identified among the compounds **31**-**53** and **62**-**67** were comparable with reversan in terms of IC_{50} values (**31**, **32**, **36**, **38**, **51**, **63**-**65**). Interestingly, these compounds were different from the reference substance regarding maximal response (I_{max}) and especially, selectivity. Based on recent studies [7e, 7f] we initially used **33** and **53** as scaffolds to explore a new class of potential ABCC1 modulators. Chromone derivative **33** exhibited modulator activity in the two-digit micromolar range and showed a slight preference for ABCC1 compared to ABCB1 and ABCG2. Solubility and ABCC1 selectivity were improved with compound **32**, the smaller homologue of **33**, which was inactive at ABCB1 and much less potent at ABCG2. Insertion of a carbonyl group between the phenylchromone moiety and N-substituted piperazines led to a slight decrease in the inhibitory effect (**62**, **63**), whereas the replacement of the methylene linker in **32** by a carbonyl group (**50**) dramatically decreased the maximal response from 130% to approximately 20%. Shortening of the ethylene spacer (**33**) to methylene (**32**) did not influence the activity, but increased the solubility of the compound. In addition, **64** and **65** were prepared to study the contribution of a H-bond donor in position 5 of the phenylchromone core. Compared with the compounds bearing two methoxy groups, the IC_{50} value at ABCC1 decreased from 8.2 µM (**63**) to 1.8 µM (**65**); however a moderate inhibitory activity at ABCB1 or ABCG2 was observed. Changing the position of the nitro group on the phenyl ring from *para* (**32**) to *meta* reduced the ABCC1 inhibitory activity (**39**). The shift of the nitro group to *ortho* position in the phenyl ring decreased the inhibition of ABCC1, while the activity at ABCG2 increased (**42**). Keeping in mind that nitro groups can be reduced *in vivo*, resulting in potentially toxic reactive metabolites,[18] we replaced the nitro group by different substituents.

Table 3. Inhibition of ABCC1-, ABCB1-, and ABCG2-transporters by the new flavonoid-type modulators **62–65** and reference compounds **66**, **67**



62-66



67

Compound	n	R^1	R^2	R^3	ABCC1[a] IC_{50} µM[d]	ABCC1[a] I_{max} %[d,e]	ABCB1[b] IC_{50} µM[c]	ABCB1[b] I_{max} %[c,f]	ABCG2[c] IC_{50} µM[d]	ABCG2[c] I_{max} %[d,g]
62	2	OMe	OMe	4-NO_2	18.5 ±3.4	139.4 ± 7.6	83.9 ± 11.7	37.7 ±3.4	>300	-
63	1	OMe	OMe	4-NO_2	8.2 ±1.6	75.0 ±3.8	58.2 ±3.3	33.4 ±2.0	>100	-
64	1	OMe	OH	2-NO_2	8.3±1.1	68.8 ±4.0	20	15.9	34.9 ±2.5	90.5 ±2.7
65	1	OMe	OH	4-NO_2	1.8 ±0.3	70.5 ±4.8	inactive	-	12 ±1.2	23.1 ±0.9
66	1	H	OCH_2Ph	H	>200	-	15.8 ±1.4	34.3 ±1.7	16.3 ± 4.7[h]	84.1 ±9.9
67	-	-	-		inactive	-	1.9 ±0.9	7.5 ±0.9	1.8 ±0.3[i]	108 ±4.6

[a] Calcein-AM microplate assay using ABCC1-overexpressing MDCK-MRP1 or [b] ABCB1-overexpressing Kb-V1 cells. [c] Hoechst 33342 assay using ABCG2-overexpressing MCF-7/Topo cells. [d] Mean values ± SEM from two to four independent experiments performed in triplicate or sextuplicate. [e] Inhibitory effect (I_{max}) relative to the maximum response to reversan at a concentration of 30 µM (100%). [f] I_{max} expressed as percent inhibition relative to tariquidar at a concentration of 1 µM (100%). [g] I_{max} relative to FTC at 10 µM (100%). [h] Ref[11]: ABCG2 inhibition reported for **66**: 9.8 ± 2.5 at a concentration of 5 µM, determined on mitoxantrone flow cytometric assay using ABCG2-transfected HEK293 cells; [i] Ref.[11]: ABCG2 inhibition reported for **67**: IC_{50}=0.17 µM, determined on mitoxantrone flow cytometric assay using ABCG2-transfected HEK293 cells. IC_{50} values were calculated using SigmaPlot 11.0, four parameter logistic curve fitting; inactive: inactive up to a concentration of 100 µM; n.d. not determined.

In general, electron-withdrawing groups on the phenyl ring markedly contributed to their inhibition of ABCC1 transporter. Compounds (**32**, **33**, **35**, **41**, **44**, **46**, **51**) with strong electron withdrawing group in para position showed higher potencies and maximum effects. In contrast, lack of substituents or introduction of electron-donating groups(**34, 36, 37** and **47**) resulted in a strong decrease in the inhibitory activity. Examples of concentration-response curves are shown in the **Figure 2**.

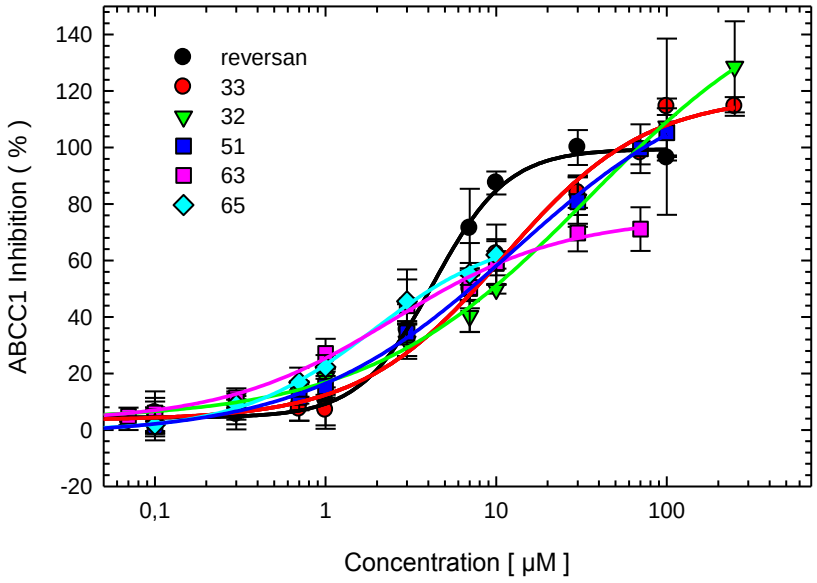


Figure 2. Concentration dependent inhibition of ABCC1 transporter in MDCKII-MRP1 cells (calcein-AM assay) by reveresan, **32**, **33**, **51**, **63**, and **65**. The inhibition is expressed relative to the maximal effect in the presence of 30 µM of reversan set to 100%.

The flavanoids **66** and **67** were described as selective inhibitors of ABCG2.[11] As the scaffold of **66** and **67** is similar to that of **31**-**53** and **62**-**65** we synthesised and investigated these substances for comparison. Compound **66** showed negligible inhibition and **67** was inactive at ABCC1(**Table 2**). In our assays systems, compound **66** inhibited ABCB1 with an IC_{50} value of 15.8 µM (I_{max} 34%). In case of **67**, the potency determined with MCF-7/Topo cells was 10-fold lower than that reported for HEK293-ABCG2 cells, whereas the selectivity of **67** for ABCG2

was confirmed (no IC_{50} values for inhibition of ABCB1 and ABCC1 by **66** and **67** provided in ref.).

Taken together, the maximum inhibitory effect was strongly dependent both on the substituents (R^1, R^2) at the chromone and the presence of an electron-withdrawing substituent at the phenyl moiety, in particular NO_2 or CF_3. Whereas reversan was equipotent at ABCB1 and ABCC1, several of the new modulators revealed preference for ABCC1 with highest selectivity residing in compound **51**.

Chemosensitivity and reversal of drug resistance of ABCC1 expressing MDCKII-MRP1 cells

On the basis of the results obtained from the functional efflux assays, the effect of the most potent and selective ABCC1 modulator (**51**) on the proliferation as well as the ability to overcome drug resistance of MDCKII-MRP1 cells was investigated in a kinetic crystal violet chemosensitivity assay, using the modulator or the reference compound reversan alone and in combination with the cytostatic etoposide, an ABCC1 substrate. The results are shown in the **Figure .**

Incubation of MDCKII-MRP1 cells with reversan alone had no effect on cell prolifereation up to a concentration of 10 µM (**Figure 3A**). The combination of etoposide at a weakly toxic concentration of 1.5 µM with reversan at a concentration of 1.0 µM yielded a complete reversal of the ABCC1-mediated resistance, which was not further enhanced by increasing the concentration of the modulator (**Figure 3B**). Furthermore, the cytostatic effect of etoposide at 1.5 µM in combination with reversan was equieffective with application of etoposide alone at a concentration of 10 µM (data not shown). By analogy to reversan, the proliferation of MDCKII-MRP1 cells incubated with ABCC1 modulator **51** alone was not affected (**Figure 3C**). The cytostatic effect of 1.5 µM etoposide was enhanced to a maximal response by combination with the new modulator **51**, already at a concentration of 1.0 µM (**Figure 3D**). In the same way, the ABCC1 overexpressing MDCKII-MRP1 cells were incubated with another ABCC1 substrate,

doxorubicin, at a per se nontoxic concentration of 50 nM in combination with reversan or **51**, leading to complete reversal of ABCC1-mediated chemoresistance (**Figure 4**).

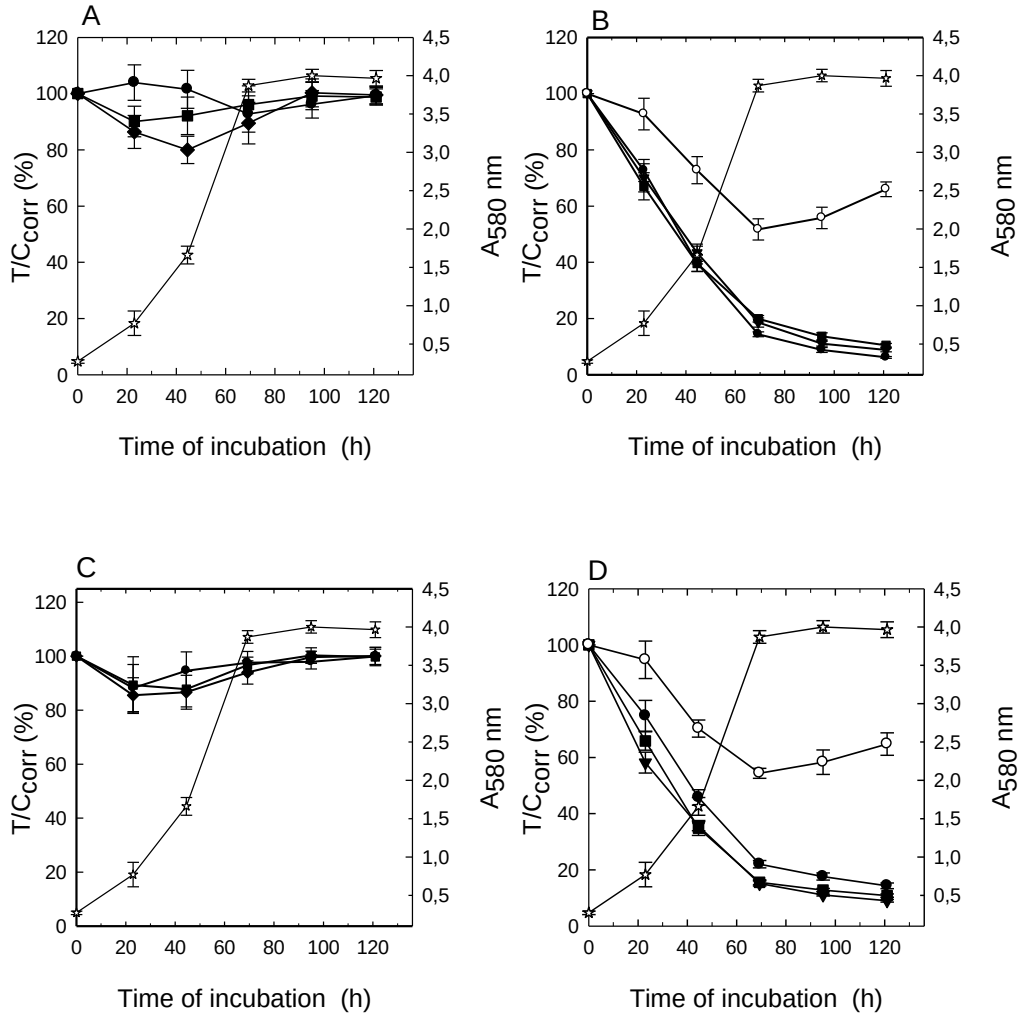


Figure 3. Reversal of ABCC1 mediated drug resistance against etoposide and doxorubicin on proliferating MDCKII-MRP1 cells. Effect of reference compound reversan alone (A) and in combination with 1.5 µM etoposide (B) on proliferating MDCKII-MRP1 cells (long term exposure); vehicle (☆), 1.5 µM etoposide (○) and reversan at different concentrations: 1 µM (●), 2.5 µM (▼), 5 µM (■), and 10 µM (◆). Effect of compound **51** alone (C) and in combination with 1.5 µM etoposide (D) on proliferating MDCKII-MRP1 cells (long term exposure); vehicle (☆), 1.5 µM etoposide (○) and 51 at different concentrations: 1 µM (●), 2.5 µM (▼), 5 µM (■), and 10 µM (◆).Effect of reversan alone and in combination with 50 nM doxorubicin (E); vehicle (☆), 50 nM doxorubicin (○) and reversan at different concentrations: 1 µM (●), 2.5 µM (▼), 5 µM (■), and 10 µM (◆). Effect of compound 51 alone in combination with 50 nM doxorubicin (F) on vehicle (☆), 50 nM doxorubicin (○) and 51 at different concentrations: 1 µM (●), 2.5 µM (▼), 5 µM (■), and 10 µM (◆).

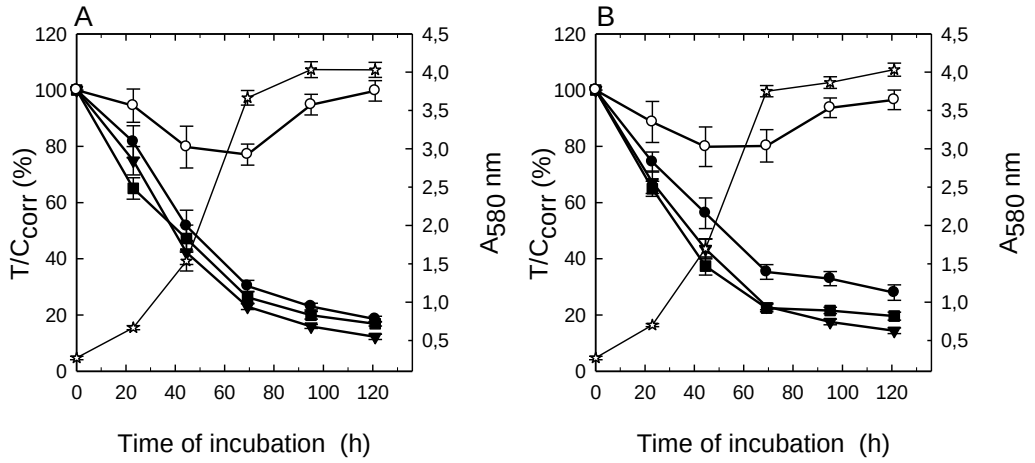


Figure 4. Effect of reference compound reversan in combination with 50 nM doxorrubicin (A) on proliferating MDCKII-MRP1 cells (long term exposure); vehicle (☆), 50 nM doxorubicin (○) and reversan at different concentrations: 1 µM (●), 2.5 µM (▼), 5 µM (■), and 10 µM (◆). Effect of compound **51** alone in combination with 50 nM doxorubicin (B) on proliferating MDCKII-MRP1 cells (long term exposure); vehicle (☆), 50 nM doxorrubicin (○) and 51 at different concentrations: 1 µM (●), 2.5 µM (▼), 5 µM (■), and 10 µM (◆).

Stability in mouse plasma

As stability in plasma is a prerequisite for *in vivo* studies, the most potent modulator of our study was investigated in mouse plasma. Compound **51** was incubated in plasma at a temperature of 37 ℃ and aliquots were analyzed by HPLC over a period of 24 hours. As observed in **Figure 5**, products of cleavage or degradation of compound **51** were not detected.

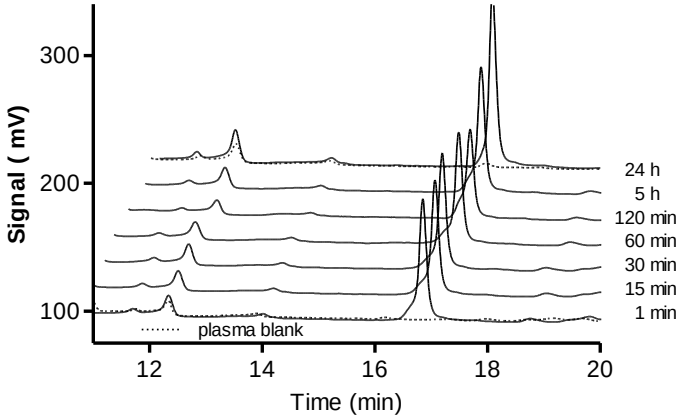


Figure 5. Chromatograms of compound **51** incubated in mouse plasma over a period of 24 h. HPLC analysis, UV detection at 220 nm.

Conclusions

Among the synthesised flavonoids several ABCC1 modulators were identified, which are comparable to reversan in terms of potency, but superior regarding their selectivity over ABCB1 and ABCG2. The new compounds are capable of reverting chemoresistance of ABCC1 overexpressing cells and are expected to be of potential value as pharmacological tools for the investigation of the (patho)physiological role of the ABCC1 transporter.

Experimental

Chemistry

^{1}H, ^{13}C and 2D NMR spectra were measured at 298 K on a Bruker AVANCE 300 spectrometer (operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C), a Bruker AVANCE 400 spectrometer (operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) and a Bruker AVANCE 600 spectrometer (operating at 600.25 MHz for ^{1}H and 150.93 MHz for ^{13}C) (Bruker, Karlsruhe, Germany). Spectra were measured in chloroform-d (99.8%, Deutero GmbH) or methanol-d_4 (99.8%, Deutero GmbH) or DMSO-d_6 (99.9%, Deutero GmbH) and referenced against

undeuterated (^{1}H) /deuterated (^{13}C) solvent. Shift values (δ_H and δ_C) were always given in ppm, *J* values in Hz. Melting points were measured on a Stanford Research Systems OptiMelt MPA 100. High-resolution mass spectra were measured on a Finnigan MAT SSQ 710A spectrometer at 70 eV (HREIMS, positive and negative mode) or recorded on an Agilent 6540 UHD (HRESIMS, positive and negative mode). Automated flash chromatography was performed on a Biotage® Isolera™ Spektra One device. Silica gel 60M (40-63 µm, Merck) for flash column chromatography was used. Starting materials and reagents were purchased from commercial suppliers and used without further purification. Solvents were used in p.a. grade for reaction mixtures and in industrial grade for flash column chromatography. Analytical TLC was performed on silica gel coated alumina plates (MN TLC sheets ALUGRAM® Xtra SIL G/UV_{254}). Visualization was conducted with UV-light (254 and 366 nm).

*General synthetic procedure of N-substituted piperazines **11-16**, **18-25** and **28**.*

Piperizine (3.0 equiv.) and K_2CO_3 were mixed in THF under reflux condition, then **68-82** was added. After three more hours of refluxing, the reaction mixture was concentrated *in vacuo* and partitioned between water and DCM. The organic phase was washed with brine and dried over Na_2SO_4. After purification by flash chromatography (DCM : MeOH = 8:1) the products **11-16**, **18-25** and **28** were obtained.

1-(4-Nitrobenzyl)piperazine **(11)**.

Pale yellow solid. Yield: 353 mg, 69%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.7 Hz, 2H), 7.50 (d, *J* = 7.6 Hz, 2H), 3.56 (s, 2H), 2.95 – 2.81 (m, 4H), 2.41 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.1, 146.4, 129.5, 123.5, 62.7, 54.5, 46.0. The spectroscopic data are in accordance with literature.[19]

1-(4-Nitrophenethyl)piperazine **(12)**.

Yellow solid. Yield: 245 mg, 48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.7 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 2H), 2.84 – 2.99 (m, 6H), 2.66–2.57 (m, 2H), 2.50 (s, 2H), 1.61 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6, 146.3, 129.5, 123.6, 60.1, 54.4, 46.1, 33.3. HRMS (EI-MS)

calculated for $C_{12}H_{17}N_3O_2$ $[M+H]^+$ 237.1423, found 237.1421. The spectroscopic data are in accordance with literature.[20]

1-Benzylpiperazine **(13)**.

Colorless solid. Yield: 376 mg, 73%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.13 – 7.44 (m, 5H), 3.54 (s, 2H), 3.30 (dt, J = 3.3, 1.6 Hz, 4H), 2.82 – 3.04 (m, 4H), 2.53 (s, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ 138.2, 130.7, 129.4, 128.6, 64.1, 53.1, 45.7. The spectroscopic data are in accordance with literature.[21]

1-(3,4-Difluorobenzyl)piperazine **(14)**.

Pale yellow solid. Yield: 333 mg, 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 – 6.96 (m, 3H), 3.42 (s, 2H), 2.99 – 2.77 (m, 4H), 2.40 (s, br, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.9, 151.1, 148.7, 148.5, 135.4, 124.7, 117.6, 116.8, 62.4, 54.2, 46.0. The spectroscopic data are in accordance with literature.[22]

1-Phenethylpiperazine **(15)**.

Colorless solid. Yield: 380 mg, 74%. ^{1}H NMR (300 MHz, CD_3OD) δ 7.37 – 7.06 (m, 5H), 2.98 – 2.90 (m, 4H), 2.84-2.73 (m, 2H), 2.63-2.58 (m, 6H). The spectroscopic data are in accordance with literature.[23]

1-(4-Methoxybenzyl)piperazine **(16)**.

Yellow solid. Yield: 200 mg, 39%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 – 7.19 (m, 2H), 6.87 – 6.82 (m, 2H), 3.79 (s, 3H), 3.42 (s, 2H), 2.92 – 2.83 (m, 4H), 2.40 (s, 4H). The spectroscopic data are in accordance with literature.[24]

1-(3-Nitrobenzyl)piperazine **(18).**

Pale yellow solid. Yield: 236 mg, 46%. ^{1}H NMR (300 MHz, CD_3OD) δ 8.24 (s, 1H), 8.14 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 7.57 (t, *J* = 7.9 Hz, 1H), 3.65 (d, *J* = 5.5 Hz, 2H), 2.96 – 2.82 (m, 4H), 2.49 (s, 4H). The spectroscopic data are in accordance with literature.[24]

Methyl 4-(piperazin-1-ylmethyl)benzoate **(19).**

Pale yellow solid. Yield: 210 mg, 41%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.3 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 3.91 (s, 3H), 3.53 (s, 2H), 2.95 – 2.85 (m, 4H), 2.43 (s, 4H).). The spectroscopic data are in accordance with literature.[25]

4-(Piperazin-1-ylmethyl)benzonitrile **(20)**.

Pale yellow solid. Yield: 282 mg, 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 – 7.47 (m, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 3.46 (s, 1H), 2.88 – 2.72 (m, 2H), 2.35 (s, 2H). The spectroscopic data are in accordance with literature.[26]

1-(2-Nitrobenzyl)piperazine **(21).**

Pale yellow solid. Yield: 220 mg, 43%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.62 – 7.57 (m, 1H), 7.52 (td, *J* = 7.5, 1.2 Hz, 1H), 7.43 – 7.33 (m, 1H), 3.77 (s, 2H), 2.90 – 2.78 (m, 4H), 2.46 – 2.32 (m, 4H). The spectroscopic data are in accordance with literature.[27]

1-(4-(Methylsulfonyl)benzyl)piperazine **(22).**

Pale yellow solid. Yield: 184 mg, 36%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 8.3 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 3.57 (s, 2H), 3.05 (s, 3H), 2.92 (t, *J* = 6.0 Hz, 4H), 2.45 (s, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0, 139.3, 129.7, 127.4, 62.8, 54.0, 45.8, 44.6. The spectroscopic data are in accordance with literature.[28]

1-(4-(Trifluoromethyl)benzyl)piperazine **(23)**.

Pale yellow solid. Yield: 261 mg, 51%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 3.52 (s, 2H), 2.89 (t, J = 4.0 Hz, 4H), 2.42 (s, 4H), 2.18 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.4, 142.4, 129.8, 129.5, 129.2, 129.2, 128.9, 125.6, 125.2, 125.2, 125.1, 125.1, 122.9, 63.0, 54.2, 45.9. The spectroscopic data are in accordance with literature.[28]

*1-(4-(Trifluoromethylthio)benzyl)piperazine (**24**).*

Pale yellow oil. Yield: 438 mg, 86%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 3.51 (s, 2H), 3.12 (s, 1H, NH), 2.99 – 2.76 (m, 4H), 2.39 (s, br, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.5, 136.3, 130.0, 62.8, 53.8, 45.7. HRMS (EI-MS) calculated for $C_{12}H_{15}F_3N_2S$ $[M+H]^+$ 277.0981 found 277.0987.

1-(4-((Trifluoromethyl)sulfonyl)benzyl)piperazine **(25)**.

Pale yellow solid. Yield: 173 mg, 34%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 8.3 Hz, 2H), 3.61 (s, 2H), 2.92 (d, J = 8.0 Hz, 4H), 2.45 (s, 4H), 2.30 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.7, 130.9, 130.0, 62.7, 53.9, 45.7. HRMS (EI-MS) calculated for $C_{12}H_{15}F_3N_2O_2S$ $[M+H]^+$ 309.0879 found 309.0885.

5-(Piperazin-1-ylmethyl)benzo[c][1,2,5]oxadiazole **(28).**

Pale yellow solid. Yield: 236 mg, 46%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, J = 9.3 Hz, 1H), 7.70 (s, 1H), 7.49 (dd, J = 9.3, 1.2 Hz, 1H), 3.56 (d, J = 0.8 Hz, 2H), 2.93 (t, J = 6.0 Hz, 4H), 2.49 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.3, 149.0, 142.9, 133.5, 116.1, 114.3, 63.1, 54.2, 45.9. The spectroscopic data are in accordance with literature.[29]

tert-Butyl piperazine-1-carboxylate **(84)**.

To a solution of 1,4-piperazine (1.38 g, 16.0 mmol, 1.0 equiv.) in 40 mL DCM, a solution of Boc_2O (1.92 g, 8.8mmol, 0.55 equiv.) in 20 mL DCM was added dropwise at room temperature. The reaction mixture was stirred overnight, and then evaporated. Then residue was dissolved in 40mL of water, and the precipitated product was collected by filtration. The filtrate was extracted

with DCM three times and the combined organic fraction was dried over Na_2SO_4 and evaporated to obtain N-Boc piperazine. Colorless solid. Yield: 1.01 g, 34%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.47 – 3.32 (m, 4H), 2.86 – 2.76 (m, 4H), 1.95 (s, 1H), 1.46 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.8, 28.4. The spectroscopic data are in accordance with literature.[30]

tert-Butyl 4-(4-nitrobenzyl)piperazine-1-carboxylate **(85)**.

4-Nitrobenzoic acid (674 mg, 4.03 mmol, 1.5 equiv.), DIPEA (3.46 g, 26.8 mmol, 10.0 equiv.) and HBTU (3.05 g, 8.04 mmol, 3.0 equiv.) were dissolved in dry DCM under N_2 atmosphere and cooled to 0 ℃. N-Boc piperazine (500 mg, 2.68 mmol, 1.0 equiv.) was added portion wise. Then the reaction mixture was heated up to room temperature and stirred for 24 h. The organic phase was washed with water and brine, dried over Na_2SO_4 and concentrated. The further purification was done by column chromatography (PE (50−70°C) : EtOAc = 1: 1). Yellow solid. Yield: 321 mg, 69%.^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 – 8.15 (m, 2H), 7.70 – 7.48 (m, 2H), 3.51 (m, 8H), 1.47 (s, 9H). The spectroscopic data are in accordance with literature.[31]

(4-Nitrophenyl) (piperazine-1-yl)methanone **(27)**.

To a solution of tert-butyl 4-(4-nitrobenzyl)piperazine-1-carboxylate **85** (330 mg, 0.98 mmol, 1 equiv.) in 2.5 mL DCM, 2.5 mL TFA was added dropwise at room temperature. The reaction mixture was stirred overnight, and then evaporated to obtain the trifluoroacetic acid salt. Then 10% NaOH was added and extracted three times with EtOAc to give (4-nitrophenyl)(piperazine-1-yl)methanone **27**. Yellow solid. Yield: 203 mg, 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 – 8.22 (m, 2H), 7.70 – 7.49 (m, 2H), 3.78 (s, 2H), 3.34 (s, 2H), 2.97 (s, 2H), 2.78 (s, 2H).The spectroscopic data are in accordance with literature.[32]

2-(3-Morpholinopropyl)isoindoline-1,3-dione **(87)**.

In a 100 mL flask 2-(3-bromopropyl)isoindoline-1,3-dione (**86**, 1.0 g, 3.73 mmol, 1.0 equiv.), morpholine (325 mg, 3.73 mmol, 1.0 equiv.) and triethylamine (906 mg, 8.96 mmol, 2.4 equiv.) were dissolved in 25 mL of DCM. This mixture was refluxed for 30 h at 40 ℃ and purified by chromatography column (PE (50−70°C) : EtOAc = 1:1) to obtain product **87**. Yellow oil. Yield: 1.23 g, 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, J = 5.4, 3.1 Hz, 2H), 7.71 (dd, J = 5.5, 3.1

Hz, 2H), 3.78 (t, *J* = 6.9 Hz, 2H), 3.60 – 3.44 (m, 4H), 2.47 – 2.24 (m, 6H), 1.86 (p, *J* = 6.8 Hz, 2H). The spectroscopic data are in accordance with literature.[32]

3-Morpholinopropan-1-amine **(30)**.

To a round bottom flask **87** (1.0 g, 3.65 mmol, 1.0 equiv.) and hydrazine hydrate (292 mg, 9.13 mmol, 2.5 equiv.) were added and dissolved in 60 mL of ethanol. After refluxing at 80 ℃ overnight, the solid was filtered and the filtrate was evaporated. Finally the crude product was dissolved in DCM and concentrated *in vacuo*, giving compound **30**, which can be used directly in the next step without further purification. Yield: 432 mg, 82%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.75 – 3.62 (m, 4H), 2.75 (t, *J* = 6.8 Hz, 2H), 2.47 – 2.32 (m, 6H), 1.63 (dt, *J* = 14.0, 6.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 67.0, 56.9, 53.8, 40.7, 30.1. The spectroscopic data are in accordance with literature.[33]

*General synthetic procedure for intermediates **3** and **4**.*

A round bottom flask was charged with **1** or **2** (1.0 equiv.) and potassium carbonate (1.0 equiv. or 2.0 equiv.). After addition of acetone (C_1 or C_2 = 0.1mol·L^{-1}), the solution was refluxed at 65 ℃. Then dimethyl sulfate (1.0 equiv. or 2.0 equiv.) was added in three portions every 2 h. The temperature was decreased to 40 ℃ and the reaction mixture was stirred overnight, the solid was filtered off and the filtrate was evaporated *in vacuo*. The residue was dissolved in DCM and filtered again. After concentration, product 1-(2-hydroxy-6-methoxyphenyl)ethanone **3** or 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone **4** were obtained as a yellow solid.

1-(2-Hydroxy-6-methoxyphenyl)ethanone **(3)**.

Yellow solid. Yield: 1.66 g, 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.26 (s, 1H), 7.35 (t, *J* = 8.4 Hz, 1H), 6.57 (dd, *J* = 8.4, 0.9 Hz, 1H), 6.42 – 6.35 (m, 1H), 3.90 (s, 3H), 2.68 (s, 3H). The spectroscopic data are in accordance with literature.[34]

1-(2-Hydroxy-4,6-dimethoxyphenyl)ethanone **(4).**

Yellow solid. Yield: 5.72 g, 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 14.03 (d, *J* = 0.8 Hz, 1H), 6.04 (dd, *J* = 3.1, 2.4 Hz, 1H), 5.90 (t, *J* = 2.3 Hz, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 2.59 (s, 3H). ^{13}C

NMR (75 MHz, $CDCl_3$) δ 203.2, 167.6, 166.1, 162.9, 106.0, 93.5, 90.7, 55.5, 33.0. The spectroscopic data are in accordance with literature.[35]

*General synthetic procedure for intermediates **5** and **6**.*

A solution of potassium tert-butoxide (3.2 equiv.) was slowly treated with a mixture of **3** or **4** (1.0 equiv.) and CS_2 (1.0 equiv.) in toluene at 10 ℃ under nitrogen atmosphere. The solution was stirred 16 h at room temperature. Then the mixture was extracted with water and the aqueous phase was washed with ether. The solution was brought with 10 % sulfuric acid to pH 4 and stirred at room temperature for 16 h under nitrogen atmosphere. Finally the solution was extracted with DCM und the organic phase was dried over Na_2SO_4. After concentration, 4-hydroxy-5-methoxy-2*H*-chromene-2-thione **5** or 4-hydroxy-5,7-dimethoxy-2*H*-chromene-2-thione **6** were obtained as a yellow solids.

4-Hydroxy-5-methoxy-2H-chromene-2-thione **(5).**

Yellow solid. Yield: 714 mg, 38%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (t, J = 8.4 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.75 (s, 1H), 4.10 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.8, 161.1, 158.4, 156.3, 133.4, 111.4, 110.1, 106.3, 77.2, 57.3. HRMS (EI-MS) calculated for $C_{10}H_8O_3S$ $[M+H]^+$ 209.0267, found 209.0270.

4-Hydroxy-5,7-dimethoxy-2H-chromene-2-thione **(6)**.

Yellow solid. Yield: 2.87 g, 43%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.74 (d, J = 2.2 Hz, 1H), 6.53 (d, J = 2.2 Hz, 1H), 6.46 (s, 1H), 3.87 (d, J = 2.0 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 194.7, 164.1, 163.8, 160.5, 158.6, 106.3, 100.6, 96.4, 93.1, 56.4, 56.1. HRMS (EI-MS) calculated for $C_{11}H_{10}O_4S$ $[M+H]^+$ 240.0404, found 240.0403.

*General synthetic procedure for intermediates **7** and **8**.*

A mixture of **5** or **6** (1.0 equiv.), potassium carbonate (1.13 equiv.) and iodoethane (3.6 equiv.) in acetone was heated to reflux for 2 h. Then the solution was concentrated *in vacuo*, and the residue was partitioned between water and DCM. The aqueous phase was extracted with DCM, dried over Na_2SO_4 and evaporated *in vacuo* to yield product **7** or **8**.

2-(Ethylthio)-5-methoxy-4H-chromen-4-one **(7)**.

Brown solid. Yield: 708 mg, 96%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (t, J = 8.4 Hz, 1H), 6.96 (dd, J = 8.5, 0.7 Hz, 1H), 6.80 (d, J = 8.3 Hz, 1H), 6.19 (s, 1H), 3.97 (s, 3H), 3.03 (q, J = 7.4 Hz, 2H), 1.42 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 166.2, 159.8, 158.3, 133.5, 110.3, 109.5, 106.8, 89.6, 56.6, 25.5, 14.2. HRMS (EI-MS) calculated for $C_{12}H_{12}O_3S$ [$M^{+\cdot}$] 236.0507, found 236.0505.

2-(Ethylthio)-5,7-dimethoxy-4H-chromen-4-one **(8).**

Brown solid. Yield: 2.77 g, 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.39 (d, J = 2.3 Hz, 1H), 6.32 (d, J = 2.3 Hz, 1H), 6.09 (s, 1H), 3.90 (d, J = 4.6 Hz, 3H), 3.85 (s, 3H), 2.99 (q, J = 7.4 *Hz*, 2H), 1.40 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 165.2, 163.7, 160.9, 160.4, 110.4, 108.6, 96.2, 92.5, 56.5, 55.8, 25.5, 14.2. HRMS (EI-MS) calculated for $C_{13}H_{14}O_4S$ $[M+H]^+$ 267.0686, found 267.0687.

*General synthetic procedure for intermediates **9** and **10**.*

A mixture of **7** or **8** (1.0 equiv.) and m-chloroperbenzoic acid (5.0 equiv.) in DCM was refluxed for 3 h, then cooled to −15 ℃ and filtered. The filtrate was collected and washed with sat. Na_2SO_3 and sat. $NaHCO_3$, concentrated *in vacuo* to give product **9** or **10** as light yellow solid.

3-(Ethylsulfonyl)-5-methoxy-4H-chromen-4-one **(9)**.

Yellow solid. Yield: 556 mg, 70%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (t, J = 8.5 Hz, 1H), 7.11 (dd, J = 8.5, 0.6 Hz, 1H), 6.97 (s, 1H), 6.90 (d, J = 8.3 Hz, 1H), 4.00 (s, 3H), 3.34 (q, J = 7.5Hz, 2H), 1.42 (t, J = 7.5Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.6, 159.8, 157.8, 157.5, 135.3, 115.2, 114.7, 110.1, 107.8, 56.7, 47.4, 6.8. HRMS (EI-MS) calculated for $C_{12}H_{12}O_5S$ $[M+H]^+$ 269.0478, found 269.0488.

2-(Ethylsulfonyl)-5,7-dimethoxy-4H-chromen-4-one **(10).**

Yellow solid. Yield: 2.18 g, 78%.^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.54 (d, J = 2.3 Hz, 1H), 6.42 (d, J = 2.3 Hz, 1H), 3.94 (s, 3H), 3.90(s, 3H), 3.32 (q, J = 7.5 Hz, 2H), 1.41 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 165.2, 161.2, 159.5, 156.9, 115.6, 109.5, 97.3, 93.0, 56.6, 56.0, 47.4, 6.9. HRMS (EI-MS) calculated for $C_{13}H_{14}O_6S$ $[M+H]^+$ 299.0584, found 299.0590.

Ethyl 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylate **(57)**.

Sodium (1.14 g, 49.4 mmol, 4.85 equiv.) was dissolved in absolute ethanol (60 mL) and 2'-hydroxy-4', 6'-dimethoxyacetophenone **4 (**2.0 g, 10.2 mmol, 1.0 equiv) and diethyl oxalate (3.6 mL, 26.5 mmol, 2.6 equiv.) was added. The mixture was stirred for 2 h under reflux conditions. A large amount of solid appeared shortly after heating. The reaction mixture was cooled and 6N HCl (5 mL) was added. The mixture was concentrated, diluted with water (30 mL) and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4. The residue **55** was dissolved in ethanol (60 mL) and treated with conc.HCl (6 mL) overnight. The solution was concentrated *in vacuo*. Purification by chromatography (PE (50−70°C) : EtOAc = 1:1) provided compound **57**. Colorless solid. Yield: 1.56 g, 55%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.59 (d, J = 2.3 Hz, 1H), 6.37 (d, J = 2.3 Hz, 1H), 4.42 (q, J = 7.2 Hz, 2H), 3.93 (s, 3H), 3.88 (d, J = 5.5 Hz, 3H), 1.40 (t, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.1, 164.8, 160.9, 160.6, 159.7, 149.8, 116.7, 110.1, 96.8, 93.1, 62.8, 56.4, 55.9, 14.1. The spectroscopic data are in accordance with literature.[36]

Ethyl 5-(benzyloxy)-4-oxo-4H-chromene-2-carboxylate (**58**).

Sodium (1.087 g, 47.24 mmol, 4.85 equiv.) was dissolved in absolute ethanol (60 mL) and 1-(2-(benzyloxy)-6-hydroxyphenyl)ethanone **54 (**2.36 g, 9.74 mmol, 1.0 equiv.) and diethyl oxalate (2.62 mL, 25.3 mmol, 2.6 equiv.) was added. The mixture was stirred for 2 h under reflux condition. A large amount of solid appeared shortly after heating. The reaction mixture was cooled and 6N HCl (4.65 mL) was added. The mixture was concentrated, diluted with water (26.5 mL) and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4. The residue **56** was dissolved in ethanol (53 mL) and treated with conc.HCl (5.3 mL) overnight. The solution was concentrated *in vacuo*. Purification by chromatography (PE (50−70°C) : EtOAc = 1:1) provided compound **58**. Beige solid. Yield: 2.15 g, 68%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 – 7.50 (m, 3H), 7.46 – 7.23 (m, 4H), 7.16 (dd, J = *8.5*, 0.7 Hz, 1H), 7.00 (s, 1H), 5.28 (s, 2H), 4.45 (q, J = *7.1 Hz*, 2H), 1.43 (t, J = *7.1 Hz*, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 160.7, 158.6, 158.0, 136.3, 134.6, 128.7, 127.8, 126.6, 116.5, 110.9, 108.7, 70.9, 62.9, 14.1. The spectroscopic data are in accordance with literature.[37]

6,8-Dimethoxy-4-oxo-4H-chromene-2-carboxylic acid (**59**).

In a 500 mL flask ethyl 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylate **57** (1.5 g, 5.39 mmol, 1.0 equiv.) was dissolved in 120 mL of THF and 30 mL of ethanol. Afterwards the solution of potassium carbonate (2.24 g, 16.17 mmol, 3.0 equiv.) in 60 mL of water was slowly added, the mixture was stirred for 24 h at room temperature. Then 30 mL of water and 2N HCl were added until pH≈2. After evaporating the solvents, the residue was filtered, washed with 50 mL of water and dried at 45 ℃ in the oven to give product **59**. Colorless solid. Yield: 1.29 g, 96%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 6.69 (d, J = 2.3 Hz, 1H), 6.62 (s, 1H), 6.52 (d, J = 2.3 Hz, 1H), 3.88 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 175.5, 164.4, 161.4, 160.4, 159.0, 150.7, 115.3, 109.1, 96.7, 93.3, 56.14, 54.9. The spectroscopic data are in accordance with literature.[38]

5-(Benzyloxy)-4-oxo-4H-chromene-2-carboxylic acid **(60)**.

In a 500 mL flask **58** (2.15 g, 6.63 mmol, 1.0 equiv.) was dissolved in 147 mL of THF and 38 mL of ethanol. Afterwards the solution of potassium carbonate (2.75 g, 19.89 mmol, 3.0 equiv.) in 74 mL of water was slowly added, the mixture was stirred for 24 h at room temperature. Then 40 mL of water and 2N HCl were added until pH≈2. After evaporating the solvents, the residue was filtered, washed with water and dried at 45 ℃ in the oven to give product **60**. Colorless solid. Yield: 1.66 g, 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74 (t, J = 8.4 Hz, 1H), 7.62 (d, J = 7.2 Hz, 2H), 7.48 – 7.24 (m, 3H), 7.17 (dd, J = 26.2, 8.1 Hz, 2H), 6.74 (s, 1H), 5.27 (s, 2H). The spectroscopic data are in accordance with literature.[39]

5-Hydroxy-7-methoxy-4-oxo-4H-chromene-2-carboxylic acid **(61)**.

Compound **59** (500 mg, 2.00 mmol, 1.0 equiv.) was dissolved in 5 mL of HBr and 10 mL HOAc (0.13mol L^{-1} of compound **59**) and the reaction mixture was refluxed for 2 h. After cooling, the solution was extracted with ethyl acetate (30 mL × 3), washed three times with water (30 mL × 3) and brine (30 mL × 3) and dried over Na_2SO_4. After evaporation compound **61** was obtained. Yellow solid. Yield: 237 mg, 50%. ^{1}H NMR (600 MHz, CD_3OD) δ 6.92 (s, 1H), 6.64 (d, J = 2.3 Hz, 1H), 6.39 (d, J = 2.3 Hz, 1H), 3.89 (s, 3H). The spectroscopic data are in accordance with literature.[40]

*General synthetic procedure for final compounds **31-53**.*

To a solution of **9** or **10** (1.0 equiv.) in ethanol N-subtituted piperazine derivatives (1.0 equiv.) and DIPEA (5.0 equiv.) were added at 0 ℃. Then the mixture was refluxed for 24 h at 80 ℃. After purification by flash column, the final products **31-53** were obtained in moderate yields.

5-Methoxy-2-(4-(4-nitrobenzyl)piperazin-1-yl)-4H-chromen-4-one **(31)**.

Pale yellow solid. Yield: 119 mg. 54%. Mp. 167−168 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 – 8.17 (m, 2H), 7.54 (d, J = 8.7 Hz, 2H), 7.43 (t, J = 8.4 Hz, 1H), 6.87 (dd, J = 8.4, 0.8 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 5.44 (s, 1H), 3.96 (s, 3H), 3.66 (s, 2H), 3.55 – 3.45 (m, 4H), 2.69 – 2.50 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.8, 161.1, 159.6, 155.9, 147.4, 145.4, 132.2, 129.5, 123.7, 113.1, 108.9, 106.8, 88.9, 61.9, 56.5, 52.2, 44.5. HRMS (EI-MS) calculated for $C_{21}H_{21}N_3O_5$ $[M+H]^+$ 396.1554, found 396.1555.

5,7-Dimethoxy-2-(4-(4-nitrobenzyl)piperazin-1-yl)-4H-chromen-4-one **(32).**

White solid. Yield: 53 mg, 37%. Mp. 190−192 ℃.^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 6.32 (q, J = 2.4 Hz, 2H), 5.33 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.64 (s, 2H), 3.48 – 3.41 (m, 4H), 2.61 – 2.46 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 162.9, 161.1, 160.6, 157.4, 147.3, 145.5, 129.5, 123.7, 107.4, 95.8, 92.4, 88.6, 61.9, 56.4, 55.6, 52.2, 44.62. HRMS (EI-MS) calculated for $C_{22}H_{23}N_3O_6$ $[M+H]^+$ 426.1660, found 426.1663.

5,7-Dimethoxy-2-(4-(3-nitrophenethyl)piperazin-1-yl)-4H-chromen-4-one **(33).**

White solid. Yield: 41 mg, 28%. Mp. 197−198 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (t, J = 8.3 Hz, 2H), 7.38 (d, J = 8.6 Hz, 2H), 6.35 – 6.33 (m, 2H), 5.35 (s, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.50 – 3.37 (m, 4H), 3.00 – 2.86 (m, 2H), 2.72-2.67 (m, 2H), 2.65 – 2.58 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 162.9, 161.1, 160.6, 157.4, 147.8, 146.6, 129.5, 123.7, 107.5, 95.8, 92.4, 88.6, 59.1, 56.5, 55.6, 52.2, 44.5, 33.3. HRMS (EI-MS) calculated for $C_{23}H_{25}N_3O_6$ $[M+H]^+$ 440.1816, found 440.1815.

2-(4-Benzylpiperazin-1-yl)-5,7-dimethoxy-4H-chromen-4-one **(34)**.

Pale yellow solid. Yield: 40 mg, 31%. Mp.90−92 ℃. ^{1}H NMR (300 MHz, CD_2Cl_2) δ 7.37 – 7.22 (m, 5H), 6.38 (d, J = 2.3 Hz, 1H), 6.32 (d, J = 2.3 Hz, 1H), 5.32 (dd, J = 2.9, 1.8 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.54 (s, 2H), 3.47 – 3.39 (m, 4H), 2.58 – 2.48 (m, 4H). ^{13}C NMR (75 MHz, CD_2Cl_2) δ 177.1, 163.3, 161.7, 160.9, 157.9, 138.4, 129.5, 128.7, 127.6, 107.7, 96.0, 92.8, 88.3,

63.1, 56.5, 56.1, 52.5, 45.2. HRMS (EI-MS) calculated for $C_{22}H_{24}N_2O_4$ $[M+H]^+$ 381.1809, found 381.1811.

2-(4-(3,4-Difluorobenzyl)piperazin-1-yl)-5,7-dimethoxy-4H-chromen-4-one **(35)**.

White solid. Yield: 32 mg, 23%. Mp.159−161 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 – 6.99 (m, 3H), 6.36 – 6.27 (m, 2H), 5.34 (s, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.50 (s, 2H), 3.47 – 3.39 (m, 4H), 2.57 – 2.47 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 162.9, 161.1, 160.6, 157.4, 124.6, 117.4, 117.1, 107.9, 95.74, 92.4, 88.6, 61.7, 56.5, 55.6, 52.1, 44.6. HRMS (EI-MS) calculated for $C_{22}H_{22}F_2N_2O_4$ $[M+H]^+$ 417.1620, found 417.1624.

5,7-Dimethoxy-2-(4-phenethylpiperazin-1-yl)-4H-chromen-4-one **(36)**.

Yellow solid. Yield: 36 mg, 27%. Mp. 145−147 ℃. ^{1}H NMR (300 MHz, CD_3OD) δ 7.35 – 7.10 (m, 5H), 6.57 (d, J = 2.4Hz, 1H), 6.45 (d, J = 2.3 Hz, 1H), 5.36 (s, 1H), 3.87 (d, J = 4.2 Hz, 6H), 3.63 – 3.53 (m, 4H), 2.88-2.83 (m, 2H), 2.74 – 2.64 (m, 6H). ^{13}C NMR (75 MHz, CD_3OD) δ 180.1, 165.4, 163.1, 161.7, 158.8, 141.0, 129.8, 129.6, 127.3, 107.2, 97.1, 93.9, 87.7, 61.2, 56.5, 53.3, 45.3, 34.0. HRMS (EI-MS) calculated for $C_{23}H_{26}N_2O_4$ $[M+H]^+$ 395.1965, found 395.1958.

5,7-Dimethoxy-2-(4-(4-methoxybenzyl)piperazin-1-yl)-4H-chromen-4-one **(37).**

White solid. Yield: 50 mg, 36%. Mp. 120−122 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, J = 8.6 Hz, 2H), 6.90 – 6.84 (m, 2H), 6.32 (q, J = 2.3 Hz, 2H), 5.33 (s, 1H), 3.93 – 3.88 (m, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.49 (s, 2H), 3.46 – 3.39 (m, 4H), 2.58 – 2.43 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 162.9, 161.2, 160.6, 158.9, 157.4, 130.4, 129.4, 113.7, 107.5, 95.7, 92.4, 88.4, 62.3, 56.4, 55.6, 55.3, 52.0, 44.6. HRMS (EI-MS) calculated for $C_{23}H_{26}N_2O_5$ $[M+H]^+$ 411.1914, found 411.1918.

5,7-Dimethoxy-2-(4-(4-nitrophenyl)piperazin-1-yl)-4H-chromen-4-one **(38)**.

White solid. Yield: 36 mg, 26%. Mp. 203−205 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 9.4 Hz, 2H), 6.86 (d, J = 9.4 Hz, 2H), 6.38 − 6.35 (m, 2H), 5.41 (s, 1H), 3.93 (s, 3H), 3.87 (s, 3H), 3.66-3.64 (m, 4H), 3.60 − 3.57 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.4, 163.1, 160.8, 160.7, 157.40, 154.2, 139.3, 126.0, 113.0, 107.5, 95.9, 92.5, 88.8, 56.5, 55.6, 46.3, 43.9. HRMS (EI-MS) calculated for $C_{21}H_{21}N_3O_6$ $[M+H]^+$ 412.1503, found 412.1507.

5,7-Dimethoxy-2-(4-(3-nitrobenzyl)piperazin-1-yl)-4H-chromen-4-one **(39)**.

White solid. Yield: 43 mg, 30%. Mp. 148−149 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 8.18 – 8.13 (m, 1H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 8.0 Hz, 1H), 6.34 (s, 2H), 5.40 (s, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.69 (s, 2H), 3.55 – 3.44 (m, 4H), 2.69 – 2.55 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.4, 163.0, 161.1, 160.7, 157.4, 148.5, 139.4, 135.0, 129.4, 123.8, 122.7, 107.3, 95.8, 92.4, 88.6, 61.7, 56.4, 55.6, 52.0, 44.5. HRMS (EI-MS) calculated for $C_{22}H_{23}N_3O_6$ $[M+H]^+$ 426.1660, found 426.1663.

Methyl 4-((4-(5,7-dimethoxy-4-oxo-4H-chromen-2-yl)piperazin-1-yl)methyl)benzoate **(40)**.

White solid. Yield: 62 mg, 42%. Mp.138−140 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.2 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 6.36 (s, 2H), 5.42 (s, 1H), 3.91 (d, *J* = 1.6 Hz, 6H), 3.84 (s, 3H), 3.61 (s, 2H), 3.50 – 3.42 (m, 4H), 2.60 – 2.50 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 167.0, 163.0, 161.2, 160.6, 157.3, 142.9, 129.8, 129.4, 129.0, 107.5, 95.8, 92.4, 88.4, 62.4, 56.5, 55.6, 52.2, 44.6. HRMS (EI-MS) calculated for $C_{24}H_{26}N_2O_6$ 439.1864, found 439.1861.

5,7-Dimethoxy-2-((3-morpholinopropyl)amino)-4H-chromen-4-one **(53)**.

Colorless sticky gum. Yield: 47 mg, 40%. ^{1}H NMR (300 MHz, CD_3OD) δ 6.50 (d, *J* = 2.3 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 5.21 (s, 1H), 3.87 (d, *J* = 4.0 Hz, 6H), 3.73 – 3.68 (m, 4H), 3.34 (s, br, 2H), 2.52-2.47 (m, 6H), 1.97 – 1.73 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 165.1, 161.8, 158.7, 96.8, 93.9, 67.7, 57.2, 56.4, 56.4, 54.7, 40.7, 26.5. HRMS (EI-MS) calculated for $C_{18}H_{24}N_2O_5$ 349.1758, found 349.1761.

4-((4-(5,7-Dimethoxy-4-oxo-4H-chromen-2-yl)piperazin-1-yl)methyl)benzonitrile **(41)**.

White solid. Yield: 40 mg, 31%. Mp. 185−187 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 2H), 6.31 – 6.22 (m, 2H), 5.38 (s, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.56 (s, 2H), 3.45 – 3.32 (m, 4H), 2.54 – 2.41 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 163.0, 161.2, 160.6, 157.4, 143.3, 132.3, 129.5, 118.8, 111.3, 107.3, 95.8, 92.4, 88.4, 62.2, 56.4, 55.6, 52.2, 44.6.HRMS (EI-MS) calculated for $C_{23}H_{23}N_3O_4$ $[M+H]^+$ 406.1761, found 406.1760.

5,7-Dimethoxy-2-(4-(2-nitrobenzyl)piperazin-1-yl)-4H-chromen-4-one **(42)**.

White solid. Yield: 47 mg, 33%. Mp. 164−166 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 7.9 Hz, 1H), 7.49 (d, J = 4.0 Hz, 2H), 7.37 (td, J = 8.6, 3.9 Hz, 1H), 6.27-6.24 (m, 2H), 5.32 (s, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.40 – 3.26 (m, 4H), 2.53 – 2.39 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 163.0, 161.1, 160.5, 157.3, 149.8, 132.9, 132.5, 131.0, 128.4, 124.6, 107.2, 95.8, 92.4, 88.3, 60.4, 59.0, 56.4, 55.6, 52.1, 44.6. HRMS (EI-MS) calculated for $C_{22}H_{23}N_3O_6$ $[M+H]^+$ 426.1660, found 426.1663.

5,7-Dimethoxy-2-(4-(4-(methylsulfonyl)benzyl)piperazin-1-yl)-4H-chromen-4-one (**43**).

White solid. Yield: 45 mg, 29%. Mp. 188−191 ℃.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.3 Hz, 2H), 6.33 (t, J = 2.3 Hz, 2H), 5.43 (s, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.63 (s, 2H), 3.46 (t, J = 6.0 Hz, 4H), 3.06 (s, 3H), 2.55 (t, J = 6.0 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.8, 162.0, 160.1, 159.6, 156.3, 143.2, 138.5, 128.7, 126.5, 106.2, 94.8, 91.3, 87.3, 61.0, 55.3, 54.6, 51.1, 43.5. HRMS (EI-MS) calculated for $C_{23}H_{26}N_2O_6S$ $[M+H]^+$ 459.1584, found 459.1585.

5,7-Dimethoxy-2-(4-(4-(trifluoromethyl)benzyl)piperazin-1-yl)-4H-chromen-4-one (**44**).

White solid. Yield: 41 mg, 27%. Mp. 180−183 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 8.1 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 6.47 – 6.15 (m, 2H), 5.39 (s, 1H), 3.90 (s, 3H), 3.83 (s, 3H), 3.60 (s, 2H), 3.45 (d, J = 3.0 Hz, 4H), 2.54 (t, J = 6.0 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 163.0, 161.2, 160.6, 157.4, 141.7, 129.5, 129.2, 126.0, 125.3, 107.3, 95.8, 92.4, 88.3, 77.5, 77.1, 76.7, 62.2, 56.4, 55.6, 52.1, 44.6. HRMS (EI-MS) calculated for $C_{23}H_{24}F_3N_2O_4$ $[M+H]^+$ 449.1683, found 499.1688.

5,7-Dimethoxy-2-(4-(4-((trifluoromethyl)thio)benzyl)piperazin-1-yl)-4H-chromen-4-one (**45**). White solid. Yield: 34 mg, 21%. Mp. 192−195 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.1 Hz, 2H), 6.32 (s, 2H), 5.40 (s, 1H), 3.91 (s, 3H), 3.84 (s, 3H), 3.58 (s, 2H), 3.46 (t, J = 4.0 Hz, 4H), 2.55 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 162.0, 160.2, 159.6, 156.4, 139.9, 135.4, 130.1, 128.9, 127.1, 106.3, 94.8, 91.4, 87.4, 61.1, 55.4, 54.6, 51.1, 43.6. HRMS (EI-MS) calculated for $C_{23}H_{24}F_3N_2O_4S$ $[M+H]^+$ 481.1403, found 481.1399.

5,7-Dimethoxy-2-(4-(4-((trifluoromethyl)sulfonyl)benzyl)piperazin-1-yl)-4H-chromen-4-one (**46**).

White solid. Yield: 29 mg, 17%. Mp. 168−172 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.2 Hz, 2H), 7.68 (d, *J* = 8.3 Hz, 2H), 6.33 (s, 2H), 5.40 (s, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.69 (s, *J* = 10.6 Hz, 2H), 3.40 – 3.54 (m, 4H), 2.47 – 2.61 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.7, 163.1, 161.2, 160.7, 157.4, 148.0, 131.0, 130.0, 107.4, 95.8, 92.4, 88.5, 62.0, 56.4, 55.6, 52.3, 44.6. HRMS (EI-MS) calculated for $C_{23}H_{24}F_3N_2O_4S$ $[M+H]^+$ 481.1403, found 481.1399.

2-(4-(4-Aminobenzyl)piperazin-1-yl)-5,7-dimethoxy-4H-chromen-4-one (**47**).

Iron powder (236 mg, 4.2 mmol, 10.0 equiv.) and conc. HCl (ca. 2 mg) were added to a solution of **32** (180 mg, 0.42 mmol, 1.0 equiv.) in 9 mL EtOH and 2.25 mL water. The mixture was heated to reflux for 90 min. EtOAc was added to the mixture and dried with Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by chromatography (EtOAc : MeOH = 10:1) to afford pale yellow solid. Yield: 132 mg, 80%. Mp. 174−176 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.09 (d, *J* = 8.3 Hz, 2H), 6.64 (dd, *J* = 8.6, 2.1 Hz, 2H), 6.32-6.30 (m, 2H), 5.31 (s, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.45 – 3.41(m, 6H), 2.57 – 2.45 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 177.5, 162.8, 161.1, 160.5, 157.3, 145.8, 130.4, 114.9, 107.4, 95.7, 92.3, 88.3, 62.3, 56.3, 55.5, 51.8, 44.5. HRMS (EI-MS) calculated for $C_{22}H_{25}N_3O_4$ $[M+H]^+$ 396.1918, found 396.1923.

N-(4-((4-(5,7-Dimethoxy-4-oxo-4H-chromen-2-yl)piperazin-1-yl)methyl)phenyl)acetamide (**48**).

To an ice-cold of **47** (132 mg, 0.33mmol, 1.0 equiv.) in DCM (10mL) was added Et_3N (100 mg, 0.99mmol, 3.0 equiv.) dropwise and the reaction mixture was stirred at the same temperature for 15 min. Acetyl chloride (39 mg, 0.495mmol, 1.5 equiv.) was then added and the reaction was stirred at rt for 1 h. After completion of the reaction, 10 mL water was added and the mixture extracted with DCM (20 mL × 3). The combined organic phase was washed with brine (20 mL × 3), dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by flash chromatography (EtOAc: MeOH=10:1) to give a white solid. Yield: 124 mg, 86%. Mp. 176−178 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.74 (s, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.33 (d, *J* = 2.3 Hz, 1H), 6.29 (d, *J* = 2.3 Hz, 1H), 5.29 (s, 1H), 3.83 (s, 6H), 3.48 (s, 2H), 3.44 – 3.33 (m, 4H), 2.58 – 2.40 (m, 4H), 2.14 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 177.7, 169.1, 163.0, 161.2, 160.5, 157.4, 137.9, 132.5, 129.6, 119.9, 107.2, 95.8, 92.4, 88.1, 62.3, 56.2,

55.7, 51.9, 44.5, 24.4. HRMS (EI-MS) calculated for $C_{24}H_{27}N_3O_5$ $[M+H]^+$ 438.2023, found 438.2028.

5,7-Dimethoxy-2-(4-methylpiperazin-1-yl)-4H-chromen-4-one (**49**).

Pale yellow solid. Yield: 39 mg, 38%. Mp.117−118 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.27 (m, 2H), 5.28 (s, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.43 – 3.34 (m, 4H), 2.49 – 2.39 (m, 4H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 162.9, 161.2, 160.6, 157.4, 107.4, 95.8, 92.3, 88.4, 56.4, 55.6, 54.1, 46.1, 44.5. HRMS (EI-MS) calculated for $C_{16}H_{20}N_2O_4$ $[M+H]^+$ 305.1496, found 305.1499.

5,7-Dimethoxy-2-(4-(4-nitrobenzoyl)piperazin-1-yl)-4H-chromen-4-one (**50**).

White solid. Yield: 43 mg, 29%. Mp. 248−250 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.6 Hz, 2H), 6.33 (s, 2H), 5.36 (s, 1H), 4.03-3.78 (t, J = 19.2 Hz, 8H), 3.66-3.26 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.5, 168.2, 163.2, 160.7, 160.7, 157.4, 148.7, 141.0, 128.2, 124.1, 107.3, 96.0, 92.4, 89.3, 56.5, 55.7, 53.5.HRMS (EI-MS) calculated for $C_{22}H_{21}N_3O_7$ $[M+H]^+$ 440.1452, found 440.1450.

2-(4-(Benzo[c][1,2,5]oxadiazol-5-ylmethyl)piperazin-1-yl)-5,7-dimethoxy-4H-chromen-4-one **(51)**.

White solid. Yield: 35 mg, 25%. Mp. 204−206 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, J = 9.3 Hz, 1H), 7.72 (s, 1H), 7.50 (dd, J = 9.3, 1.1 Hz, 1H), 6.51 – 6.14 (m, 2H), 5.36 (s, 1H), 3.91 (s, 3H), 3.84 (s, 3H), 3.63 (s, 2H), 3.47 (t, J = 3.0 Hz, 4H), 2.60 (t, J = 6.0 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 163.0, 161.1, 160.6, 157.4, 149.3, 149.0, 142.2, 133.2, 116.5, 114.5, 107.4, 95.8, 92.4, 88.5, 62.4, 56.4, 55.6, 52.2, 44.6. HRMS (EI-MS) calculated for $C_{22}H_{22}N_4O_5$ $[M+H]^+$ 423.1663, found 423.1667.

2-((3,4-Dimethoxyphenethyl)amino)-5,7-dimethoxy-4H-chromen-4-one **(52)**.

White solid. Yield: 52 mg, 40%. Mp.80−82 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 – 6.65 (m, 3H), 6.26 (d, J = 2.4 Hz, 1H), 6.22(d, J = 2.4 Hz, 1H), 5.30 (s, 1H), 3.86 (s, 3H), 3.83 (d, J = 1.8

Hz, 6H), 3.79 (s, 3H), 3.39 (s, 2H), 2.86 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 177.4, 162.8, 161.6, 160.5, 157.3, 149.1, 147.9, 130.5, 120.7, 111.9, 111.4, 107.3, 95.7, 92.5, 86.2, 56.4, 55.9, 55.6, 43.0, 34.5, 31.6. HRMS (EI-MS) calculated for $C_{21}H_{23}NO_6$ $[M+H]^+$ 386.1603, found 386.1606.

*General synthetic procedure for final products **62-67**.*

A 50 mL schlenk flask was charged with **59-61** (1.0 equiv.), DIPEA (10.0 equiv.) and HBTU (3.0 equiv.) under nitrogen atmosphere. After dissolving in 10 mL of dried DCM, N-subtituted piperazines **11-13**, **21** and **29** (1.0 equiv.) was slowly added at 0 ℃. Then the mixture was stirred for 24 h at room temperature. The organic phase was washed with water and brine and dried over anhydrous Na_2SO_4. After purification with flash chromatography column (EtOAc: MeOH = 30:1), the final products **62-67** were obtained.

5,7-Dimethoxy-3-(4-(3-nitrophenethyl)piperazine-1-carbonyl)-4H-chromen-4-one **(62)**.

White solid. Yield: 159 mg, 85%. Mp.169−171 ℃. ^{1}H NMR (300 MHz, DMSO-d_6) *δ* 8.15 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 8.6 Hz, 2H), 6.72 (d, *J* = 2.2 Hz, 1H), 6.54 (d, *J* = 2.1 Hz, 1H), 6.24 (s, 1H), 3.85 (d, *J* = 13.8 Hz, 6H), 3.54 (d, *J* = 23.6 Hz, 4H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.63 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) *δ* 32.0, 56.0, 56.1, 58.0, 93.3, 96.5, 108.6, 111.9, 123.2, 129.9, 145.8, 149.0, 154.9, 158.8, 160.3, 164.0, 174.7. HRMS (EI-MS) calculated for $C_{24}H_{25}N_3O_7$ $[M+H]^+$ 468.1765, found 468.1173.

5,7-Dimethoxy-2-(4-(4-nitrobenzyl)piperazine-1-carbonyl)-4H-chromen-4-one **(63)**.

White solid. Yield: 123 mg, 68%. Mp. 191−192 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 8.18 (d, *J* = 8.7 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 6.44 (d, *J* = 2.3 Hz, 1H), 6.36 (d, *J* = 2.3 Hz, 1H), 6.32 (s, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.76 (s, 2H), 3.63 (s, 2H), 3.59 (s, 2H), 2.51 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 176.3, 164.5, 161.0, 161.0, 159.4, 154.7, 147.4, 145.4, 129.5, 123.7, 113.4, 109.6, 96.7, 92.9, 61.8, 56.5, 55.9. HRMS (EI-MS) calculated for $C_{23}H_{23}N_3O_7$ $[M+H]^+$ 454.1609, found 454.1622.

5-Hydroxy-7-methoxy-2-(4-(2-nitrobenzyl)piperazine-1-carbonyl)-4H-chromen-4-one **(64).**

White solid. Yield: 100 mg, 54%. Mp. 144−146 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 12.36 (s, 1H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.61 – 7.48 (m, 2H), 7.47 – 7.38 (m, 1H), 6.38 (q, *J* = 2.1 Hz, 3H), 3.86

(s, 5H), 3.70 (s, 2H), 3.49 (s, 2H), 2.50 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.6, 166.1, 162.2, 160.4, 158.2, 157.2, 149.9, 144.1, 132.5, 131.0, 128.5, 124.7, 110.4, 106.1, 98.8, 92.9, 58.9, 55.9, 53.2, 52.2, 42.4. HRMS (EI-MS) calculated for $C_{22}H_{21}N_3O_7$ $[M+H]^+$ 440.1452, found 440.1450.

5-Hydroxy-7-methoxy-2-(4-(4-nitrobenzyl)piperazine-1-carbonyl)-4H-chromen-4-one **(65)**.

White solid. Yield: 149 mg, 60%. Mp.184−186 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.33 (s, 1H), 8.18 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.6 Hz, 2H), 6.39 (d, *J* = 2.2 Hz, 1H), 6.37 (s, 1H), 6.36 (d, *J* = 2.2 Hz, 1H), 3.85 (s, 3H), 3.67 (d, *J* = 8.4 Hz, 4H), 3.65 (s, 2H), 2.53 (d, *J* = 19.6 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 166.1, 162.3, 160.5, 158.1, 157.2, 147.4, 145.3, 129.5, 123.7, 110.5, 106.1, 98.7, 93.1, 61.8, 55.9, 53.2, 52.4, 47.0, 42.4. HRMS (EI-MS) calculated for $C_{22}H_{21}N_3O_7$ $[M+H]^+$ 440.1452, found 440.1450.

5-(Benzyloxy)-2-(4-benzylpiperazine-1-carbonyl)-4H-chromen-4-one **(66)**.

White solid. Yield: 132 mg, 86%. Mp: 169−171 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.3 Hz, 2H), 7.53 (t, *J* = 8.4 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.36 – 7.27 (m, 6H), 7.06 – 6.99 (m, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.41 (s, 1H), 5.28 (s, 2H), 3.77 (s, 2H), 3.58 (d, *J* = 12.4 Hz, 4H), 2.52 (d, *J* = 19.8 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8, 160.8, 158.6, 157.7, 155.6, 137.2, 136.2, 134.2, 129.0, 128.5, 128.3, 127.7, 127.4, 126.6, 115.4, 113.3, 110.4, 108.9, 70.8, 62.6, 53.0, 52.3. HRMS (EI-MS) calculated for $C_{26}H_{26}N_2O_4$ $[M+H]^+$ 455.1965, found 455.1962.

5-(Benzyloxy)-N-(3,4-dimethoxyphenethyl)-4-oxo-4H-chromene-2-carboxamide **(67).**

Pale yellow solid. Yield: 123 mg, 80%. Mp. 186−188 ℃. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 – 7.48 (m, 3H), 7.43 – 7.22 (m, 3H), 6.98 – 6.71 (m, 6H), 5.26 (s, 2H), 3.87 (d, *J* = 4.2 Hz, 6H), 3.70 (q, *J* = 6.8 Hz, 2H), 2.90 (t, *J* = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.6, 159.3, 158.8, 157.2, 152.6, 149.2, 147.9, 136.2, 134.4, 130.8, 128.7, 127.8, 126.6, 120.7, 115.5, 113.7, 111.9, 111.4, 110.0, 108.9, 70.9, 56.0, 55.9, 41.1, 35.1. The spectroscopic data are in accordance with literature.[39]

Biology

Calcein-AM and Hoechst 33342 assay:

Solutions

Loading buffer was made of 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$ $6H_2O$, 1.5 mM $CaCl_2$ $2H_2O$, 25 mM HEPES, 10 mM glucose, the pH was adjusted to 7.4.

Phosphate buffered saline (PBS) was made of 8 g/L NaCl, 1 g/L Na_2HPO_4, 0.2 g/L KCl, 0,2 g/L KH_2PO_4 and NaH_2PO_4 H_2O, finally the pH was adjusted to 7.4.

4% Paraformaldehyde (PFA) solution in PBS was obtained by stirring 4 g PFA per 100 g total solution while heating on a magnetic stirrer for approximately 30 min. If not otherwise stated, chemicals (p.a. quality) were purchased from Merck (Darmstadt, Germany). Purified water (Milli-Q system, Millipore, Eschborn, Germany) was used.

Chemicals used for cellular assays

Calcein-AM and Hoechst 33342 were from Biotium (Hayward, CA, USA). Calcein-AM was dissolved in DMSO (Merck, Darmstadt, Germany) at a concentration of 1 mM, whereas as solution of Hoechst 33342 (0.8 mM) was prepared in Millipore water. Pluronic® F127 was obtained from Sigma (Munich, Germany) and dissolved to achieve a final concentration of 20% (m/m) in DMSO. Reversan and fumitremorgin C (FTC) were from Merck (Darmstadt, Germany), dissolved in DMSO and diluted to achieve a concentration of 3 mM and 1 mM, respectively. Vinblastine, topotecan and etoposide were from Sigma (Munich, Germany) and dissolved in 70% ethanol. Tariquidar was synthesised according to literature [41] with minor modifications,[42] compounds **66** and **67** were synthesised as reference compounds according to a published protocol.[11] Work solutions of vinblastine and topotecan at a concentration of 0.1 mM were stored at 4 ℃, and all stock solution at −20 ℃.

Cell culture conditions

Human Kb-V1 cells, a subclone of human Kb cells (ATCC® CCL-17™), overexpressing ABCB1 were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma, Munich, Germany) supplemented with 3.7 g/L $NaHCO_3$ (Merck, Darmstadt, Germany) and 110 mg/L sodium pyruvate (Serva, Heidelberg, Germany) containing 10% FCS (Biochrom, Berlin,

Germany) and vinblastine at a final concentration of 330 nM to maintain the ABCB1 transporter expression.[42]

The human breast adenocarcinoma cell line MCF-7/Topo (ABCG2 overexpressing subclone of MCF-7 cells, ATCC® HTB-22™) were incubate in Eagle's Minimum Essential Medium containing 2.2 g/L $NaHCO_3$ (Merck, Darmstadt, Germany) and 110 mg/L sodium pyruvate (Serva, Heidelberg, Germany) and 10% FCS (Biochrom) and 550 nM topotecan to maintain the ABCG2 transport overexpression. The ABCG2 overexpression was described previously.[8b]

The MDCKII-MRP1 cell line (Madin-Darby Canine Kidney, strain II; a canine epithelial cell line; ATCC® CRL-2936™ transfected with the human ABCC1 transporter [43]) was a kind gift from Prof. Dr. P. Borst from Netherland Cancer Institute (Amsterdam, NL). These cells were maintained in DMEM supplemented with 10% FCS (Biochrom).[16]

All cells were cultured in a water-saturated atmosphere (5% CO_2) at 37 °C in cell culture flasks purchased from Sarstedt (Nümbrecht, Germany). Subculturing of cells was performed every 3-7 days after trypsinization (0.05% trypsin / 0.02% EDTA or 0.1% trypsin / 0.04% EDTA, GE Healthcare (PAA Laboratories, Pasching, Austria). Additionally the cells were routinely monitored for *mycoplasma* contamination by PCR (Venor® GeM, Minerva Biolabs, Berlin, Germany) and only *mycoplasma* free cultures were used.

Modulation of ABCB1:

ABCB1 modulation was determined in a microplate assay (calcein-AM assay) as described.[44]

Modulation of ABCG2:

ABCG2 modulation was determined with the Hoechst 33342 microplate assay as described.[44]

Modulation of ABCC1:

ABCC1 modulation was determined with the calcein-AM microplate assay by analogy with the procedure established for ABCB1.[16] Briefly, 3-5 days after passaging, the ABCC1 overexpressing MDCKII-MRP1 cells were trypsinized and resuspended in culture medium at 25 °C. The cells were seeded into flat-bottomed 96-well plates at a density of 20000 – 25000 cells per well. The next day, cells were washed with loading buffer in order to remove unspecific serum esterases. Afterwards, cells were incubated with loading suspension (loading buffer, 5 mg/mL BSA, 1.25 µL/mL pluronic F127 (20% in DMSO) containing 0.5 µM of calcein-AM and vehicle or the test compound at increasing concentrations (10 nM – 100 µM) for 60 min (37 °C / 5% CO_2). Reversan at a final concentration of 30 µM was used as reference compound; the response obtained under these conditions was defined as 100% inhibition of calcein-AM efflux. In general, test compounds were investigated from two to four independent experiments performed in triplicate, in case of controls in sextuplicate. Subsequently, the loading suspension was discarded and cells were fixed under light protection using 100 µL of a PFA solution (4 % in PBS) for 20 min. After three washing circles with 150 µL of loading buffer, fixed cells were overlaid with 100 µL of loading buffer and relative fluorescence intensities were determined at a GENios Pro microplate reader (Tecan Deutschland GmbH, Crailsheim, Germany). Measurement mode: fluorescence top; excitation filter: 485/20 nm; emission filter: 535/25 nm; number of reads: 10; integration time: 40 µs; lag time: 0 µs; mirror selection: Dichroic 3; plate definition file: GRE96ft.pdf; multiple reads per well (Circle): 3x3; time between move and flash: 100 ms. On each plate, the optimal gain was calculated by determination of the fluorescence in absence and presence of the reference compound reversan (30 µM). All values were corrected by subtracting the fluorescence intensity in the absence of ABCC1 modulator (DMSO control value), and the maximal response was referred to the signal obtained using 30 µM reversan (100%). IC_{50} values were calculated using SigmaPlot 11.0, "four parameter logistic curve" fitting. Errors are expressed as standard error of the mean (SEM)

Determination of the stability of compound 51 in mouse plasma

Compound stability in mouse plasma was determined according to a previously established procedure.[8d] The blood from NMRI (nu/nu) mice was collected by heart puncture in deep anesthesia using heparin-coated syringes. Samples were immediately centrifuged for 7 min at 4500 g (Eppendorf centrifuge 5415R, Eppendorf, Hamburg, Germany). The test compound was dissolved in DMSO at a concentration of 7 mM. A 1:50 dilution of substance with mouse plasma was prepared in 1.5 mL polypropylene reaction vessels (Eppendorf, Hamburg, Germany). The samples were shortly vortexed and promptly incubated at 37 ℃. Aliquots were obtained at different incubation times. The samples were deproteinated by mixing with two parts of ice-cold acetonitrile (MeCN). For quantitative precipitation, the samples were vortexed and stored at 4 ℃ for 30 min. Afterwards the samples were centrifuged for 5 min at 14000 g (Eppendorf centrifuge 5415 R) and the supernatants were transferred into new reaction vessels. The samples were diluted (1:1) with MeCN and stored at −80 ℃ until the HPLC analysis.

RP-HPLC analysis was performed with a Eurosphere-100 C18 column (250 x 4 mm, 5 µm, Knauer, Berlin, Germany), maintained to 30 ℃, on a Merck Hitachi system consisting of an AS-2000A , a L-6200-A pump, a L-400A UV-VIS detector. UV-detection was done at 220 nm. The samples were thawed at room temperature and 100 µL were injected. Mixtures of MeCN (A) and 0.05 % aq. TFA (B) were used as mobile phase. The applied gradient was 0 to 30 min (A/B): 10/90 to 80/20 in 30 min.

Chemosensitivity Assays

The assays were performed according to an established protocol [45] with minor modifications.[8d]

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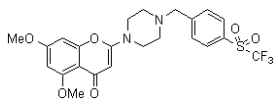
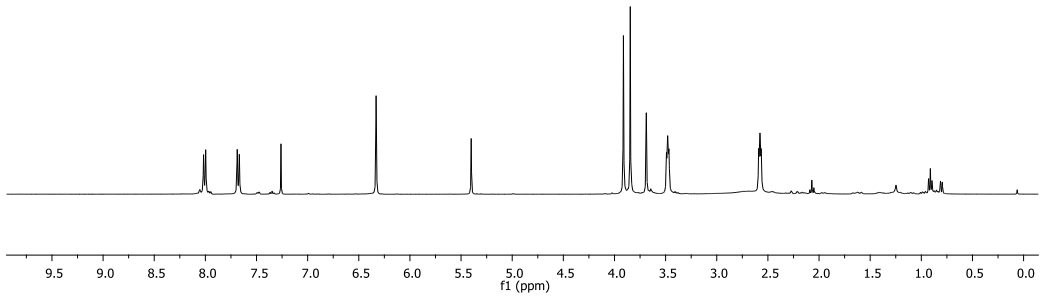
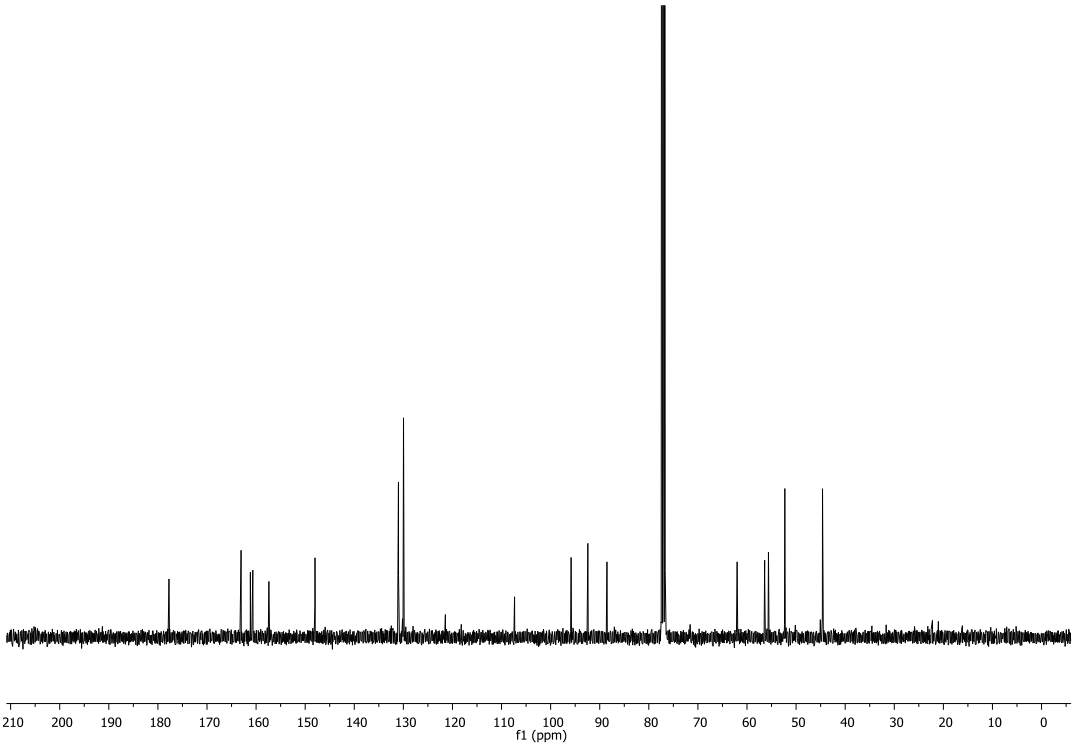
^{1}H and ^{13}C NMR spectra of selected final compounds

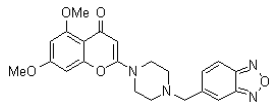
^{1}H and ^{13}C NMR spectra for 5,7-dimethoxy-2-(4-(4-((trifluoromethyl)sulfonyl)benzyl)piperazin-1-yl)-4H-chromen-4-one (**46**) (400 MHz, $CDCl_3$)

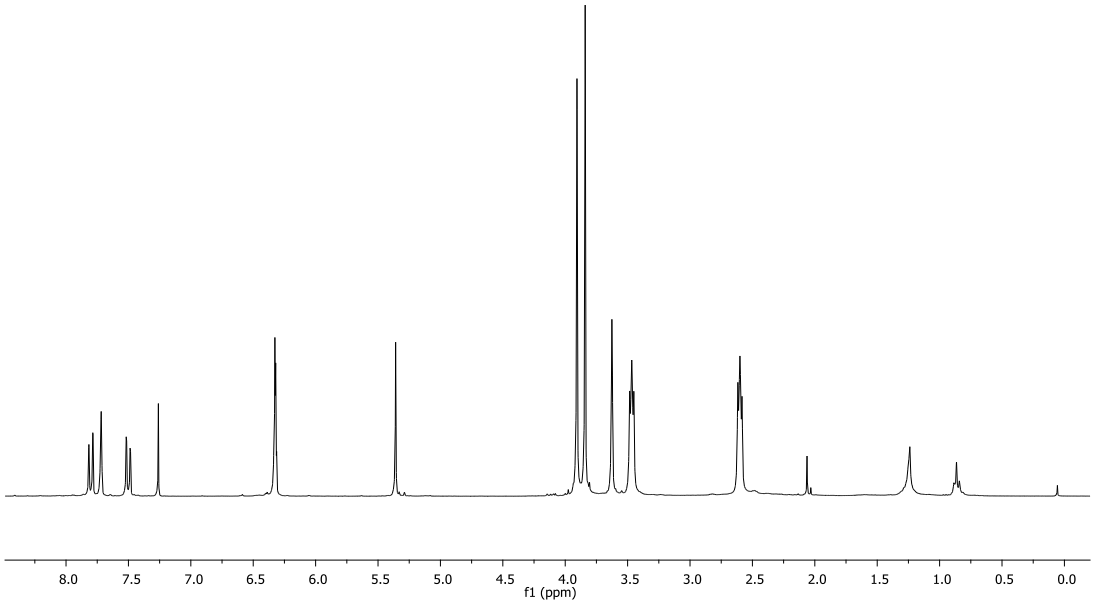


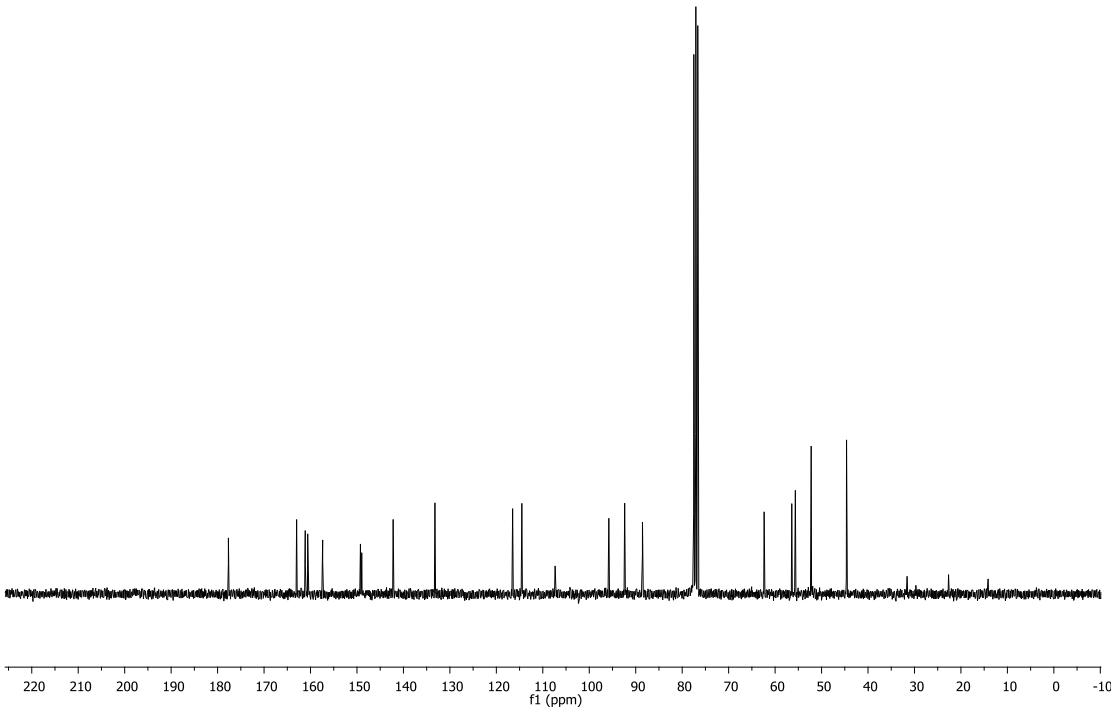




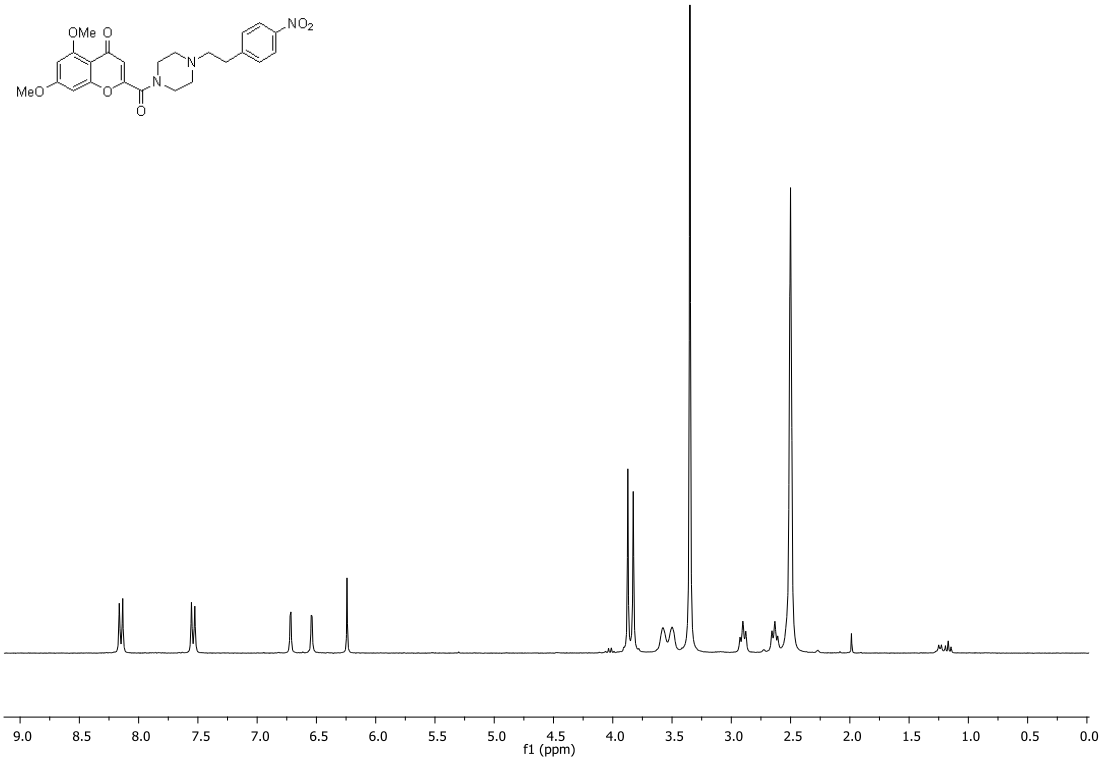
^{1}H and ^{13}C NMR spectra for 2-(4-(benzo[c][1,2,5]oxadiazol-5-ylmethyl)piperazin-1-yl)-5,7-dimethoxy-4H-chromen-4-one (**51**) (300 MHz, $CDCl_3$)

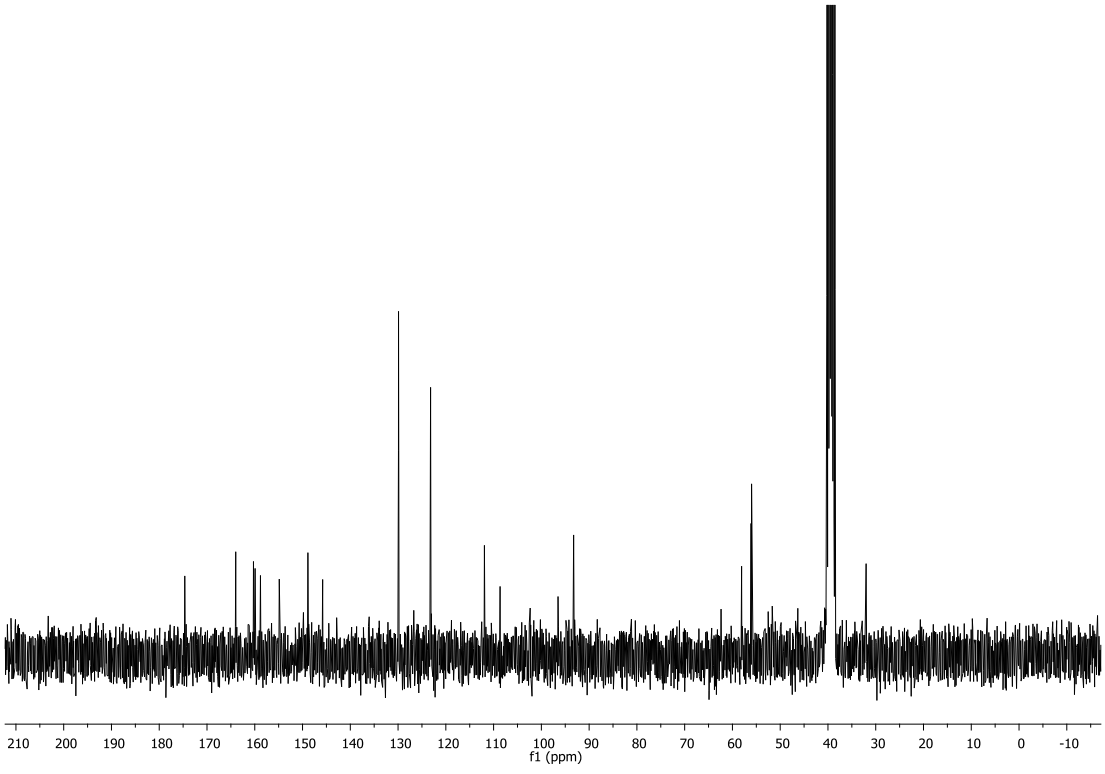






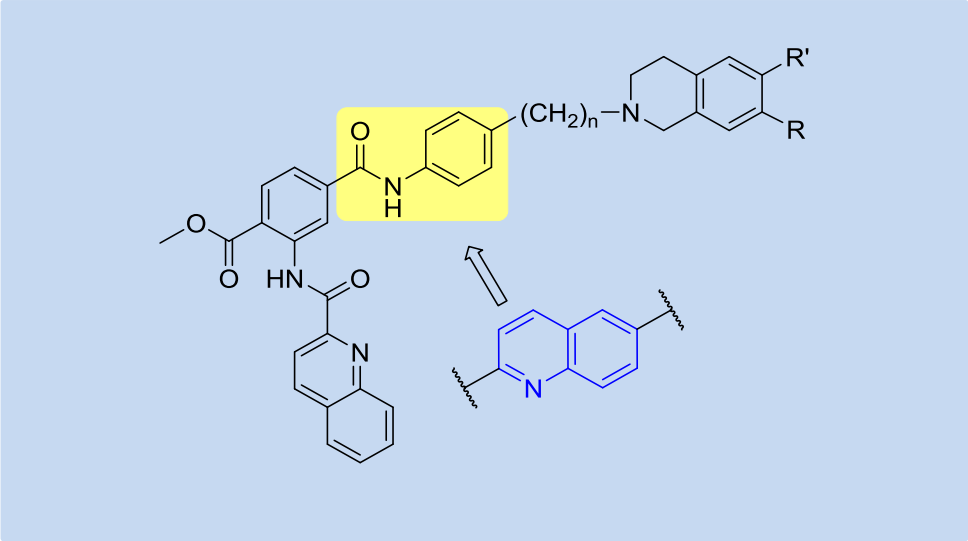
^{1}H and ^{13}C NMR spectra for 5,7-dimethoxy-2-(4-(4-nitrobenzyl)piperazine-1-carbonyl)-4H-chromen-4-one (**62**) (300 MHz, DMSO-d_6)





Chapter 4

Quinoline carboxamide-type ABCG2 modulators: quinoline moiety as anilide replacement



Abstract

A new series of quinoline analogues targeting breast cancer resistance protein derived from tariquidar was synthesised and tested in ABCB1, ABCG2 and ABCC1 assay. The replacement of anilide core by quinoline moiety increased the stability compared with parent compounds UR-ME22-1, UR-COP78 but gave less potent compounds than indole compound UR-COP-25g. The quinoline analogues were less water soluble than UR-ME22-1, UR-COP78 and UR-COP-25g due to coplanar structures. The introduction of amine groups on the tetrahydroisoquinoline moiety increased the water solubility to some extent.

Keywords

ABC transporters; breast cancer resistance proteins; inhibitors; quinoline; syntheses

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Author contributions:
Q.Sun synthesized and characterized quinoline derivatives.

Introduction

The ATP-driven drug efflux transporters ABCB1 (p-glycoprotein, p-gp), ABCC1 (MRP1), and ABCG2 (breast cancer resistance protein, BCRP) play an important role in multidrug resistance (MDR) of cancer.[1] In addition, the chemotherapy of malignant CNS tumors is compromised due to the expression of ABC proteins at the blood-brain barrier, restricting the access of many potent cytostatics to the brain, in the case that these compounds are substrates of the respective pumps. The coadministration of such cytostatics with inhibitors of efflux transporters represents an attractive strategy to overcome the blood-brain barrier and to improve chemotherapy of malignancies in the CNS, as demonstrated in a proof-of-concept study for ABCB1.[2] It is expected that this concept is also applicable to ABCG2, provided that appropriate inhibitors are available.

Recently, we identified selective ABCG2 inhibitors among 3-(quinolinecarbonylamino)benzanilides (**Figure 1**).[3] With an IC_{50} value of 65 nm and a maximal inhibitory effect of 63% (Hoechst 333342 assay, MCF7-Topo cells), UR-ME22-1 turned out to be more potent but less efficient compared to the reference compound fumitremorgin C (FTC). The incorporation of a triethylenglycol ether group at the tetrahydroisoquinoline moiety gave compound UR-COP78, which is comparable to UR-ME22-1 in potency but produces a higher maximal response of 88%, indicating that low water solubility was an efficacy-limiting factor.[3b] Unfortunately, these compounds were unstable in mouse plasma due to complete enzymatic cleavage of the benzamide bond within 30 min, giving the corresponding phenethyl tetrahydroisoquinoline fragment.[4] Aiming at more stable ABCG2 modulators, we replaced the labile benzanilide core structure according to a bioisosteric approach. A biphenylyl moiety was tolerated, but potency and selectivity were reduced compared to the parent compounds.[4a] Replacement of biphenyly moiety with indole moiety largely increased the activity to IC_{50} value = 59 ± 11nm with maximal inhibitory effect of 101%, besides it was stable in mouse plasma for 24 hours.[5] For better understanding the structure-activity relationship on this class of compounds, the modification of fragment C and D (**Figure. 1**) has been intensively studied in our group. Here we report on the replacement of the indole core on fragment B by quinolyl

moieties and introduction of amine on fragment A to increase water solubility rather than triethylenglycol ether group.

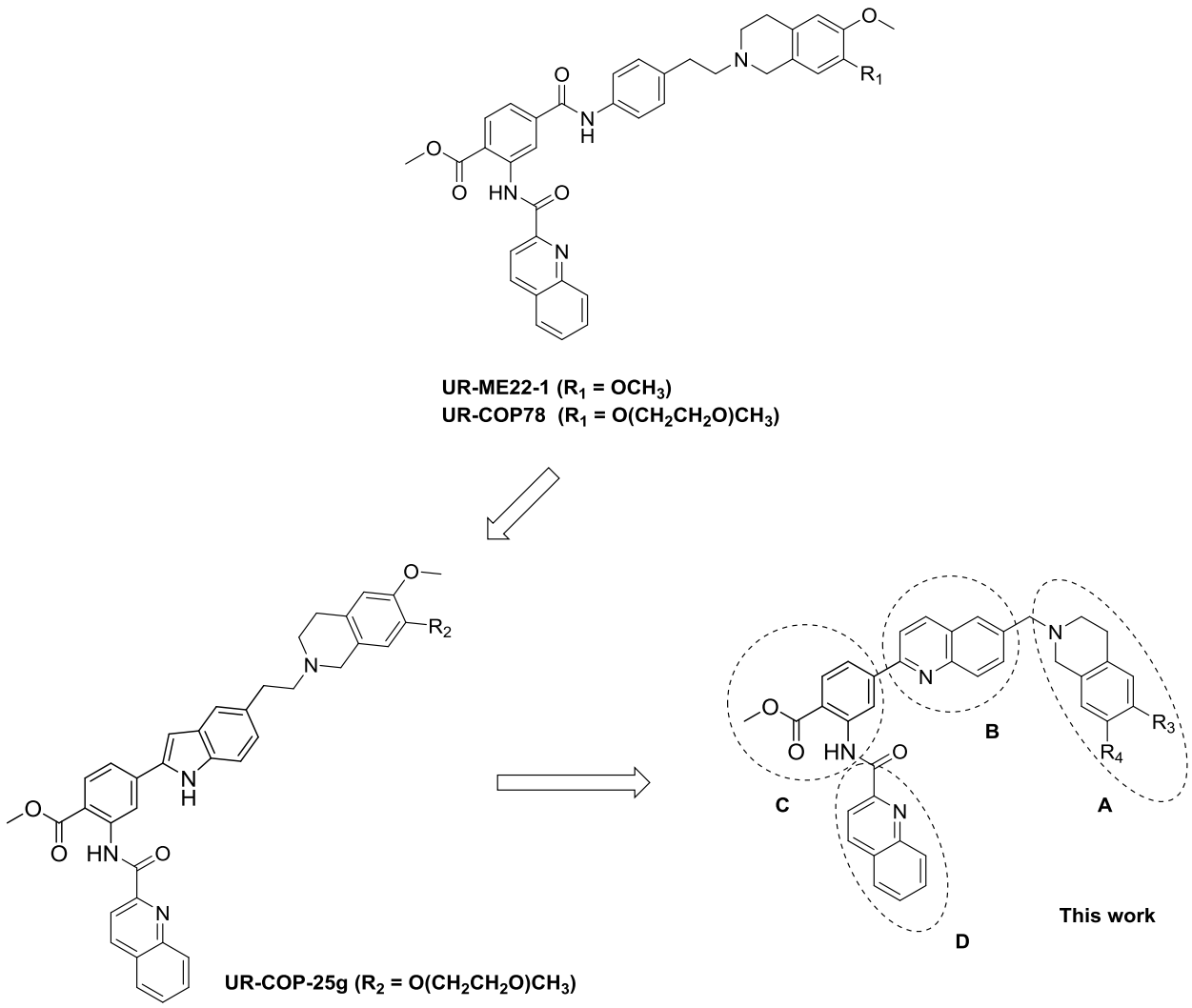


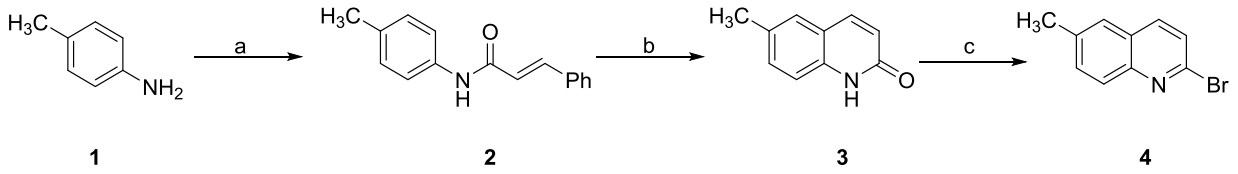
Figure 1. The selective ABCG2 modulators UR-ME22-1, UR-COP78 and UR-COP-25g and the general structures of the title compounds.

Results and Discussion

Synthesis

As shown in **Figure 1**, our target compounds constitute four fragments: the tetrahydroisoquinoline moiety (fragment A), the quinoline motif (fragment B), the methyl

aminobenzoate moiety (fragment C) and the quinoline-2-carbonyl core (fragment D) (dashed ovals, **Figure 1**). At the beginning, we focused on the synthesis of quinoline core. Cottet et al.[6] have developed an ingenious synthesis of the precursor **3** by high-temperature Lewis acid-catalysed cyclization of the N-arylcinnamamide **2** in a reaction that formally generates benzene as a leaving group (**Scheme 1**). Following this route, 4-methylaniline **1** was acylated with cinnamoyl chloride to afford **2** in high yield. Reaction of **2** with $AlCl_3$ at 125 ℃ led to a 41% isolated yield of the quinolin-2-one **3**. Consequently, bromination **3** with $POBr_3$ gave 2-Bromo-6-methylquinoline **4** in 61% yields.



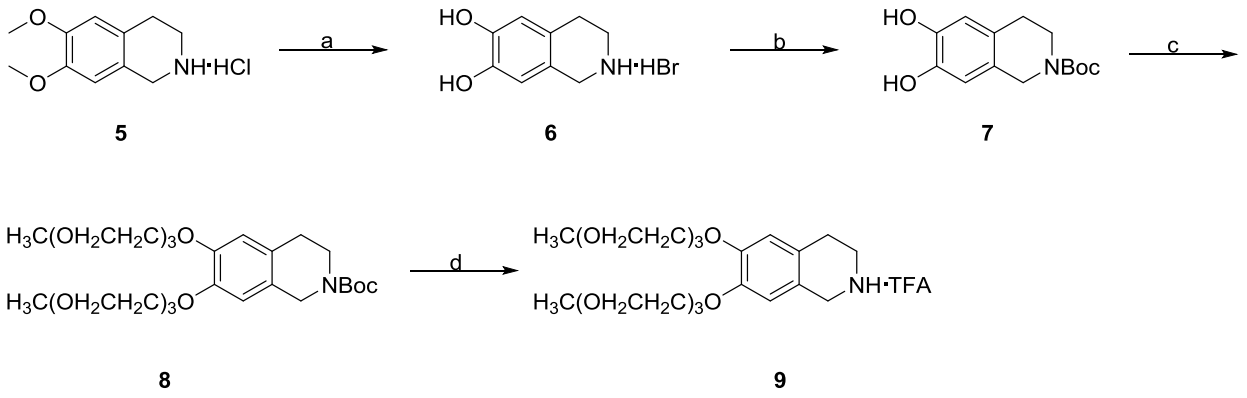
1 **2** **3** **4**

Scheme 1. Synthesis of compound 2-Bromo-6-methylquinoline **4**. Reagents and conditions: (a) *E*-PhCHCHCOCl, K_2CO_3, water, acetone, 0 ℃, 2 h; (b) $AlCl_3$, PhCl, 125 ℃, 24 h; (c) $POBr_3$, 140 ℃, 3 h.

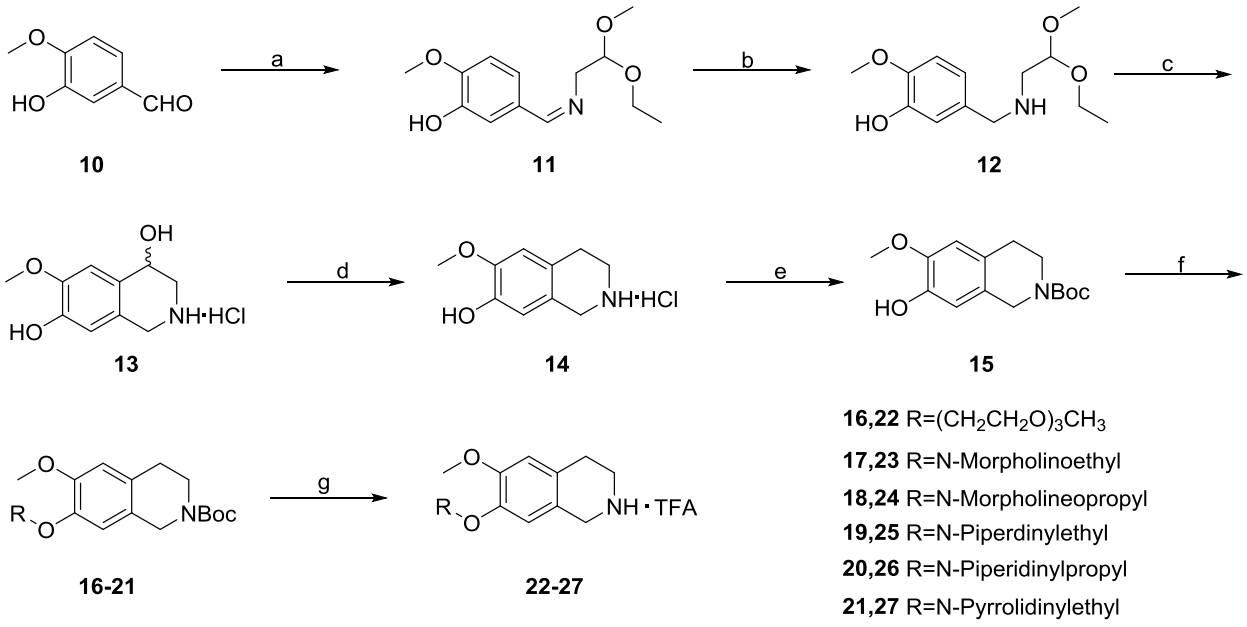
Water solubility plays a very important role in drug absorption and affects the bioavailability of drugs.[7] To increase water solubility, triethylene glycol was introduced to tetrahydroisoquinoline moiety (**Scheme 2**), After efficient cleavage of methoxy groups with HBr/CH_3COOH, free secondary amine in the molecule was protected by boc reagent. The triethylene glycol monomethyl ether was activated as tosylate and then used to form the ether **8** under basic conditions. The deprotection of intermediate **8** by trifluoro acetic acid (TFA) gave free amine as triflate salt **9** in quantitative yield.

Substitution of two triethylene glycol chains largely increased the solubility of the compounds, but meanwhile decreased the selectivity between ABCB1 and ABCG2 transporters, while one triethylene glycol substitution did not affect the selectivity of the compound (**Table 1**). Another strategy to increase the water solubility is to introduce polar tertiary amine group into the molecule. Therefore we synthesised a series of amine substituted tetrahydroisoquinoline moiety.

The synthesis of mono-substituted tetrahydroisoquinoline moiety has been reported (**Scheme 3**) [8].



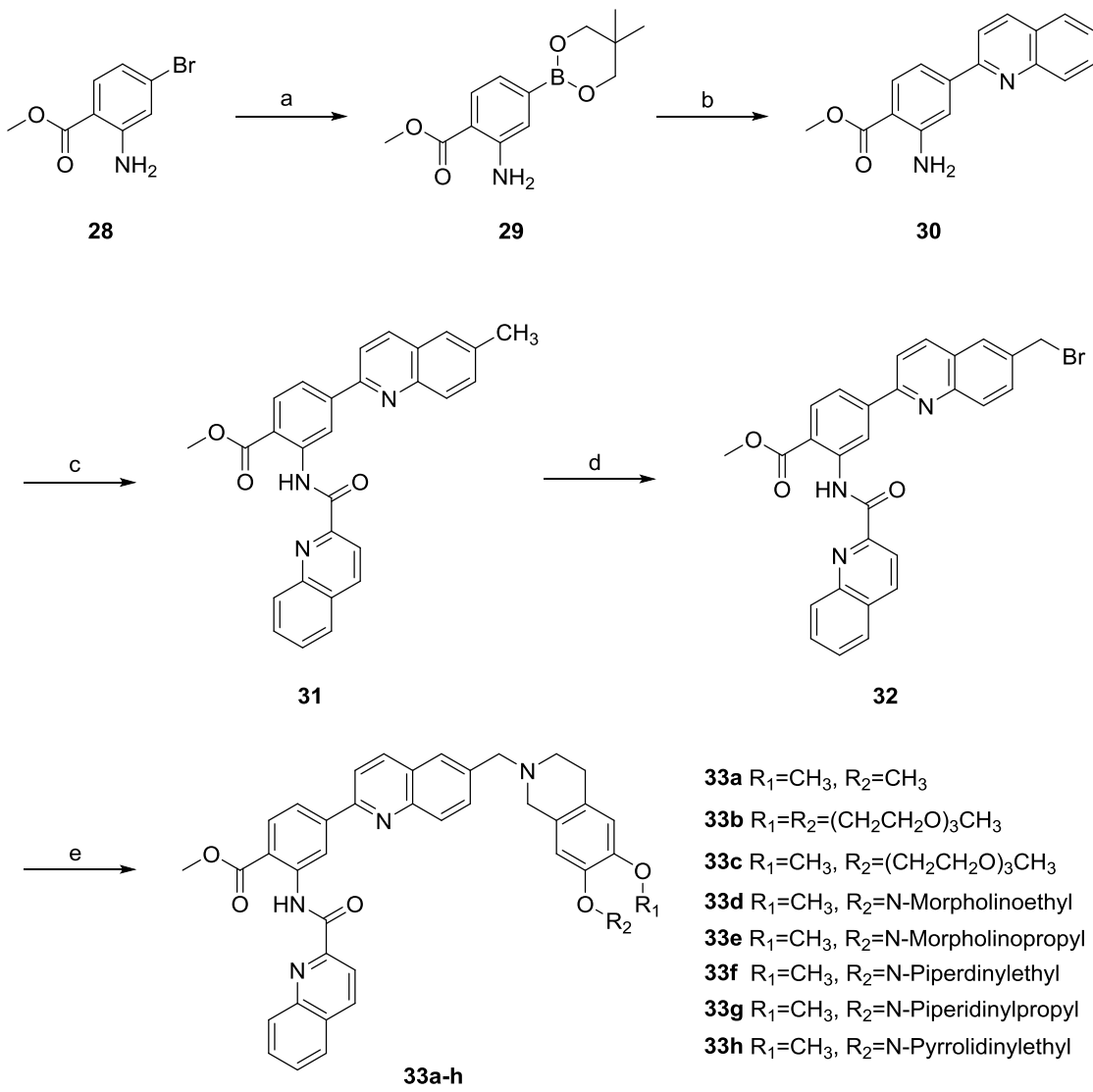
Scheme 2. Synthesis of tetrahydroisoquinoline moiety **9**. Reagents and conditions: (a) HBr/CH_3COOH, reflux; (b) Boc_2O, Et_3N, DCM, 0 ℃; (c) 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate, KOH, THF, reflux; (d) TFA, DCM, r.t.



Scheme 3. Synthesis of tetrahydroisoquinoline moiety **22-27**. Reagents and conditions: (a) 2,2-diethoxyethanamine, EtOH, reflux, 5 h; (b) Pt/H_2, 40bar, 3 ds; (c) 6N HCl, rt, 3 ds; (d) Pd/C, H_2, 40 bar, 1 d; (e) Boc_2O, Et_3N, DCM, overnight; (f) 2-(2-(2-methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate or aliphatic amines, reflux, overnight; (g) TFA, DCM, r.t.

Condensation of isovanillin **10** and aminoacetaldehyde diethyl acetal in ethanol gave imine **11** which was used directly in the next step without any workup. After reduction to amine **12**, it was treated with dilute hydrochloric acid and followed by hydrogenation with Pd/H_2 to yield the asymmetrically substituted tetrahydroisoquinoline with quantitative yield. The amine can be selectively protected with di-*tert*-butyl dicarbonate due to its higher reactivity than phenol. Followed by alkylation and deprotection, mono-substituted tetrahydroisoquinoline moieties **22-27** were obtained as triflates. [9]

Palladium catalysed borylation of arylbromide gave fluorescent boronic ester **29** in 90% yield. The Suzuki coupling of boronic ester **29** with 2-bromo-6-methyl quinoline **4** to 2-bromo-6-methyl quinolone **30** followed by the acylation with quinoline-2-carbonyl chloride gave compound **31** in good yield. NBS was added in three portions to give one-bromonated product **32**, which was used directly to the next step after work-up. The final compounds **33a-h** were generated by nucleophilic substitution reaction (**Scheme 4**).



Scheme 4. Synthesis of title compounds **33a-h**. Reagents and conditions: (a) $Pd(dppf)Cl_2$, KOAc, DMSO, 80 ℃, overnight; (b) 2-bromo-6-methyl quinoline **4**, $Pd(PPh_3)_4$, K_3PO_4, THF, 80 ℃, overnight ; (c) quinoline-2-carbonyl chloride, TEA, DCM, 40 ℃, overnight; (d) NBS, benzoyl peroxide, CCl_4, 80 ℃, 9 h; (e) tetrahydroisoquinolines **22-27**, DIPEA, CH_3CN, 80 ℃, overnight.

Biological evaluation

Inhibition of the ABCB1 and ABCG2 transporter

The synthesised compounds **33a-h** and the reference compounds fumitremorgin C, Ko143[10] and tariquidar were investigated for inhibition of ABCB1 and ABCG2 in a calcein-AM[11] and a

Hoechst 33342 microplate assay[4b] using ABCB1-overexpressing Kb-V1 and ABCG2-overexpressing MCF-7/Topo cells. The data are summarized in **Table 1**.

Table 1. Effect of **33a-h** and reference compounds on the transport activity of ABCG2 and ABCB1

Compound	**ABCG2[a]**		**ABCB1[b]**	
	IC_{50} [nm][c]	I_{max} [%][d]	IC_{50} [nm][c]	I_{max} [%][e]
FTC	731 ± 92	100	n.d.	
Ko143[f]	117 ± 53	103 ± 7	inactive[g]	
Tariquidar[f]	526 ± 85	69 ± 5	223 ± 8	103 ± 2
UR-ME22-1[f]	65 ± 8	63 ± 2	>29000[h]	
UR-COP78[i]	130 ± 29	88 ± 3	>50000	
UR-COP-25g[j]	59 ± 11	101 ± 5	7300 ± 910	14 ± 2
33a	602 ± 44	60 ± 0	inactive	
33b	536 ± 160	77 ± 1	2709 ± 158	41 ± 15
33c	1043 ± 53	107 ± 7	inactive	
33d	851 ± 93	81 ± 10	inactive	
33e	904 ± 45	58 ± 0	>10000	
33f	1167 ± 214	61 ± 5	>10000	
33g	1467 ± 568	70 ± 5	inactive	
33h	1820 ± 861	75 ± 5	>10000	

[a] Hoechst 33342 microplate assay using ABCG2-overexpressing MCF-7/Topo cells. [b] Calcein-AM microplate assay (unless otherwise indicated) using ABCB1-overexpressing Kb-V1 cells. [c] Mean values ± SEM from 2 to 3 independent experiments performed in triplicate or sextuplicate; n.d.: not determined. [d] Maximal inhibitory effect (I_{max}) relative to the response to FTC at a concentration of 10 μm (100% inhibition). [e] I_{max} expressed as percental inhibition relative to tariquidar at a concentration of 1 μm (100%). [f] Ref.[4b] [g] Inactive up to a concentration of 100 μm. [h] Data from flow cytometric calcein-AM assay.[11] [i] Ref.[3b]. [j] Ref.[5]

As shown in **Table 1**, all the quinoline analogues exhibit higher IC_{50} value but lower the maximal response compared with UR-COP-25g. The introduction of two triethylene glycol

chains at the tetrahydroisoquinoline core (compound **33b**) decreases the selectivity in ABCG2 transporter; whereas compound **33c** with one triethylene glycol chain maintains the good selectivity and showed higher maximal inhibitory effect than other compounds. The quinoline analogues turned out to be hardly soluble which was precipitated in the pharmacological assay leading to the big error of IC_{50} value. The poorer water solubility could be explained by the fact that the strong conjugation of the nitrogen atom decreasing the possibility to form H-bonds and the structure of the quinoline molecule is more planar than related analogues (**Figure 2**).

The modification of side chain at the tetrahydroisoquinoline core has slight effect on the ABCG2 activity. As shown in **Table 2**, the better water solubility tends to be accompanied with better ABCG2 inhibitory effect. Quinoline **33d** and **33h** bearing morpholine substitution have lower logP value showing a bit lower IC_{50} value than other amine substitution analogues (**33f**, **33g** and **33h**). Usually logP value of 5 is considered as an upper limit of desired lipophilicity for drug-like molecule, but all the compounds (UR-COP-25g and **33a-h**) went beyond the scope. The big molecular weight of the compounds is also the factor decreasing hydrophilicity of the compounds. Compound **33d** which bears ethylmorpholine has almost the same logP value as compound **33c** and **33d**. Interestingly, they also show the similar activity on ABCG2 transporter. This suggests that ethylmorpholine can be used as a substitution of triethylene glycol chains to improve the drug-like property of the compounds. None of the compounds show activity at the ABCC1 transporter. Since quinoline compounds are inferior to UR-COP-25g, no future investigation was performed on this series of compounds.

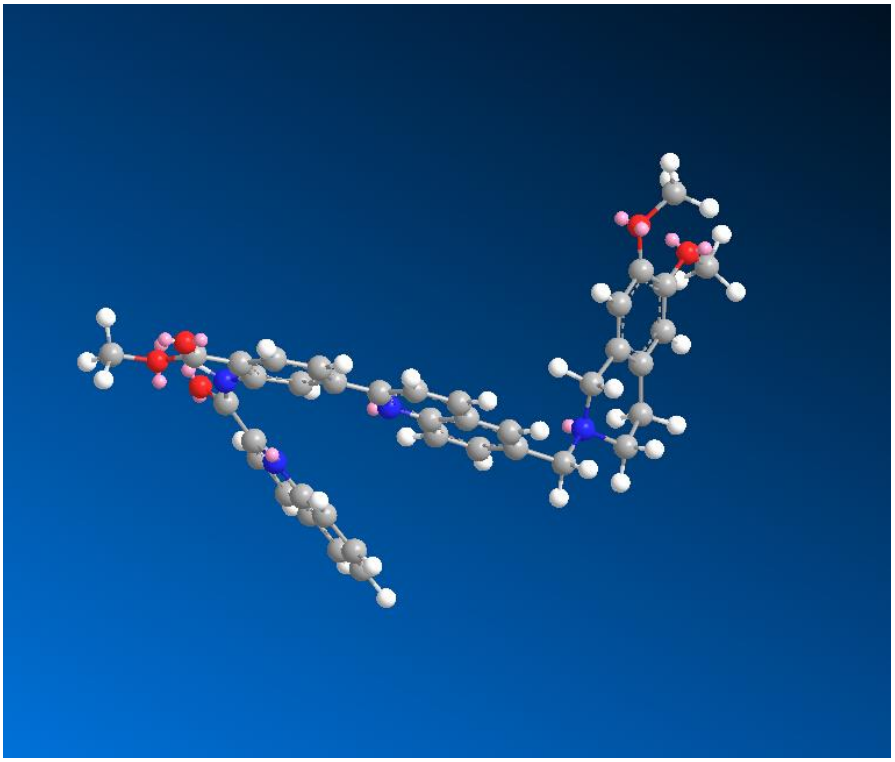


Figure 2. The structure of quinoline analogue **33a** (energy minimization by ChemBio 3D Ultra 13.0).

Table 2. Molecular weight and calculated logP value for indole analogue UR-COP-25g and quinoline analogues **33a-h**

Compound	Molecular Weight	logP[a]
UR-COP-25g	772.90	7.47
33a	638.72	7.84
33b	903.04	7.67
33c	770.88	7.71
33d	737.86	7.64
33e	751.88	7.70
33f	735.88	8.71
33g	751.90	8.77
33h	737.88	8.27

[a] The partition coefficient (log p) of the quinoline compounds **33a-h** and UR-COP-25g were calculated with Marvinsketch 5.10.1.

Conclusions

A new class of quinoline analogues targeting breast cancer resistance protein derived from tariquidar was synthesised and tested in ABCB1, ABCG2 and ABCC1 assay. All tested compounds show weak or no inhibitory activity over ABCB1 and ABCC1 transporter but good inhibitory activity towards ABCG2. However, these compounds have worse activities on ABCG2 than reference indole compound UR-COP-25g. The replacement of triethylene glycol chains with ethylmorpholine maintained the activity of the compounds and decreased molecular weight. In future, our work should be focused on solving the solubility problem via structure-activity study. Through optimization of the pharmacophore, the size of compounds can be minimized and the hydrophilicity may largely be enhanced.

Experimental

Chemistry

^{1}H, ^{13}C and 2D NMR spectra were obtained at 298 K using a Bruker AVANCE 300 spectrometer (operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C), Bruker AVANCE 400 spectrometer (operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) and Bruker AVANCE 600 spectrometer (operating at 600.25 MHz for ^{1}H and 150.93 MHz for ^{13}C) (Bruker, Karlsruhe, Germany). The spectra were obtained using chloroform-d (99.8%, Deutero GmbH) or methanol-d_4 (99.8%, Deutero GmbH) and referenced against non-deuterated (^{1}H)/deuterated (^{13}C) solvents. The shift values (δH and δC) are always given in ppm with *J* values in Hz. The melting points were measured using a Stanford Research Systems OptiMelt MPA 100. The high-resolution mass spectra were obtained using a Finnigan MAT SSQ 710A spectrometer at 70 eV (HREIMS, positive and negative mode) or an Agilent 6540 UHD (HRESIMS, positive and negative mode). Silica gel 60 M (40-63 µm, Merck) was used for the flash column chromatography. The starting materials and reagents were purchased from commercial suppliers and used without further purification. The solvents were p.a. grade for the reaction mixtures and industrial grade for the flash column chromatography. Analytical TLC was performed on silica gel coated alumina plates (MN TLC sheets ALUGRAM ® Xtra SIL G/UV254). The visualisation was performed using UV-light (254 and 366 nm). The logP values were calculated using Marvinsketch 5.10.1.

N-(5-Methylphenyl)cinnamamide **(2)**

E-3-Phenylpropenoyl chloride (1.00 g, 9.30 mmol) was stirred vigorously with p-toluidine (1.55 g, 9.30mmol) and K_2CO_3 (1.99 g, 14.4 mmol) in water (4.6 mL) and acetone (4.6 mL) at 0 ℃ for 2 h. The mixture was then poured into ice-water (10 mL). The precipitate gave N-(5-Methylphenyl)cinnamamide **2** as white powder. Yield: 2.21 g, 100%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.75 (d, J = 15.6Hz, 1H, COCH), 7.50 − 7.54 (m, 4H, ArH), 7.37 − 7.39 (m, 3H, ArH), 7.16 (d, J = 8.4Hz, 2H, ArH), 6.54 (d, J = 15.6Hz, 1H, ArCH), 2.33(s, 3H, CH_3). The spectroscopic data are in accordance with the literature values.[12]

6-Methylquinolin-2(1H)-one **(3)**

Compound **2** (1.00 g, 4.21 mmol) and $AlCl_3$ (2.660 g, 19.95 mmol) were heated to 125 ℃ in chlorobenzene (10 mL) for 24 h. The mixture was cooled to 50 ℃ and poured onto ice. The mixture was extracted with EtOAc. Evaporation and recrystallization (EtOH) gave 6-Methylquinolin-2(1H)-one **3** as pale yellow solid. Yield: 1.56 g, 37%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.18 (s, 1H, NH), 7.78 (d, J = 9.5 Hz, 1H, COCH), 7.36 (d, J = 4.6 Hz, 3H, ArH), 6.72 (d, J = 9.5 Hz, 1H, ArCH), 2.42 (s, 3H, CH_3). The spectroscopic data are in accordance with the literature values.[13]

2-Bromo-6-methylquinoline **(4)**

$POBr_3$ (7.20 g, 37.0 mmol) was heated with **3** (2.00 g, 12.6 mmol) at 140 ℃ for 3 h. The cooled mixture was poured into ice-water. The precipitate was collected and dried. Chromatography on silica gel (PE (50−70°C) : EtOAc = 25 : 1) gave 2-bromo-6-methylquinoline **4** as pale yellow solid. Yield: 1.40 g, 50%. ^{1}H NMR (300MHz, $CDCl_3$) δ 7.90 − 7.95(m, 2H, ArH), 7.55 − 7.57(m, 2H, ArH), 7.48(d, J = 8.7Hz, 1H, ArH), 2.53(s, 3H, CH_3).

1,2,3,4-Tetrahydroisoquinoline-6,7-diol hydrobromide **(6)**

The synthesis of compound **6** has already been described.[14] To a mixture of HBr (24 mL, 48% in H_2O) and CH_3COOH (96 mL) was added 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline (4.21

g, 21.8 mmol) and refluxed for 8 h. The solvent mixture was removed by distillation. The product was used directly in the next step without further purification. Yield: 5.36 g, 100%. ^{1}H NMR (400 MHz, CD_3OD) δ 2.95 (t, J = 6.3 Hz, 2H, CH_2), 3.31 (quint, J = 1.7 Hz, 1H, NH), 3.42 (d, J = 6.4 Hz, 2H, CH_2), 4.18 (s, 2H, CH_2), 6.59 (s, 1H, ArH), 6.62 (s, 1H, ArH).

tert-Butyl 6,7-dihydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate **(7)**

Compound **7** was prepared according to known procedures [14] from **6** (5.36 g, 21.8 mmol), di-*tert*butyldicarbonate (4.52 g, 20.7 mmol), and triethylamine (11.0 g, 109 mmol). Flash column chromatography (PE (50−70°C) : EtOAc = 1:1) of the sticky brown crude product yielded a slightly yellow solid. Yield: 4.51 g, 78 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.66 (t, J = 5.6 Hz, 2H), 3.58 (s, 2H), 4.41 (s, 2H), 6.55 (s, 1H), 6.58 (s, 1H).

tert-Butyl 6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinoline- 2(1H)-carboxylate **(8)**

Compound **7** (4.51 g, 17.0 mmol), potassium hydroxide (1.74 g, 31.0 mmol), and 2-(2-(2-Methoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (9.87 g, 31.0 mmol) were dissolved in tetrahydrofurane and refluxed overnight. The solvent was evaporated and the residue was taken up in ethyl acetate, washed with water and brine, and concentrated. Flash column chromatography (EtOAc) of the crude product yielded **8** as yellow oil. Yield: 5.30 g, 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 9 H, ^{t}Bu), 2.65 (t, J = 5.3 Hz, 2H), 3.30 (s, 6 H, OCH_3), 3.46 − 3.49 (m, 4 H, PEG), 3.51-3.56 (m, 2H), 3.56 − 3.61 (m, 8H, PEG), 3.65 − 3.68 (m, 4H, PEG), 3.77 (t, J = 5.1 Hz, 4H, PEG), 4.06 (t, J = 5.04 Hz, 4H, PEG), 4.39 (s, 2H), 6.57 (s, 1H), 6.60 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 28.4, 28.5, 40.6, 41.9, 44.9, 45.5, 59.0, 69.0, 69.1, 69.6, 70.5, 70.7, 70.8, 71.9, 79.7, 112.7, 115.1, 126.2, 127.6, 147.5, 147.5, 154.9.

6,7-Bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1,2,3,4-tetrahydroisoquinoline 2,2,2-trifluoroacetate **(9)**

Compound **8** (5.02 g, 9.0 mmol) was dissolved in DCM and trifluoroacetic acid (10.26 g, 90 mmol) and stirred overnight at room temperature. The solvent was evaporated to yield **9** as a gray solid, which was used without further purification. Yield: 5.14 g, 100%. Mp. 47 ℃ (45.4 – 48.0 ℃).^{1}H NMR (400 MHz, CD_3OD) δ 3.00 (t, J =6.2 Hz, 2H), 3.35 (s, 6H), 3.49 (t, J = 6.4 Hz, 2H), 3.52 − 3.56 (m, 4H, PEG), 3.63 − 3.68 (m, 8 H, PEG), 3.71 − 3.74 (m, 4H, PEG), 3.82-3.85 (m, 4H, PEG), 4.07 − 4.13 (m, 4H, PEG), 4.25 (s, 2H), 6.68 (s, 1H), 6.73 (s, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 25.6, 43.1, 45.6, 59.2, 70.2, 70.3, 70.9, 71.4, 71.7, 71.8, 72.9, 113.9, 115.8, 122.0, 125.7, 149.3, 150.0. HRMS (EI-MS) $[M+H]^+$ calcd. for $C_{23}H_{40}NO_8$ 458.2748, found 458.2757.

6-Methoxy-1,2,3,4-tetrahydroisoquinolin-7-ol hydrochloride **(14)**

Compound **14** was prepared according to literature-known procedures.[8a] The catalyst was removed by centrifugation and the crude product was recrystallized from ethanol. Yield: 4.38 g, 44%. ^{1}H NMR (400 MHz, MeOD) δ 3.02 (t, J = 6.2 Hz, 2H), 3.45 (t, J = 6.3 Hz, 2H), 3.84 (s, 3H), 4.20 (s, 2H), 6.61 (s, 1H), 6.77 (s, 1H).

tert-Butyl 7-hydroxy-6-methoxy-3,4-dihydroisoquinoline-2(1H)-carboxylate **(15)**

Compound **15** was prepared from **14** (4.38 g, 20.3 mmol) according to literature-known procedures.[8b] Flash column chromatography (PE (50−70°C) : EtOAc = 3:2) of the sticky brown crude product yielded **15** as colorless oil. Yield: 3.12 g, 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.73 (t, J = 5.4 Hz, 2H), 3.61 (s, 2H), 3.85 (s, 3H), 4.45 (s, 2H), 6.59 (s, 1H), 6.64 (s, 1H).

tert-Butyl 6-methoxy-7-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4- dihydroisoquinoline-2(1H)-carboxylate **(16)**

Compound **16** was prepared from **15** (0.20 g, 0.72 mmol) according to literature-known procedures.[8b] The crude product was purified with flash column chromatography (PE (50−70°C) : EtOAc = 3:2) to obtain a brownish oil. Yield: 0.26 g, 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.73 (t, J = 5.2 Hz, 2H), 3.37 (s, 3H), 3.53-3.56 (m, 2H), 3.59-3.69 (m,

6H), 3.71-3.75 (m, 2H), 3.82 (s, 3H), 3.86 (t, *J* = 5.2 Hz, 2H), 4.14 (t, *J* = 5.2 Hz, 2H), 4.46 (s, 2H), 6.60 (s, 1H), 6.64 (s, 1H).

tert-Butyl 6-methoxy-7-(2-morpholinoethoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate **(17)**

The method for preparation of Compound **17** was described in the literature.[8b] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.61 (s, 2H, ArH), 4.46 (s, 2H, $CONCH_2Ar$), 4.11 (t, *J* = 6.0 Hz, 2H, OCH_2Ar), 3.82 (s, 3H, OCH_3), 3.77 – 3.70 (m, 4H, OCH_2, OCH_2), 3.64 − 3.60(m, 2H, NCH_2), 2.83 (t, *J* = 6.0 Hz, 2H, CH_2Ar), 2.74 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.62 – 2.54 (m, 4H, NCH_2, NCH_2), 1.48 (s, 9H, ^{t}Bu). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.9, 148.1, 146.7, 127.2, 125.5, 112.0, 111.6, 79.7, 66.8(2C), 60.4, 57.5, 56.0, 54.0(2C), 45.6, 44.9, 29.7, 28.5(3C). HRMS (EI-MS) $[M+H]^+$ calcd for $C_{21}H_{32}N_2O_5$ 393.2384, found 393.2384.

tert-Butyl6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate **(18)**

The method for preparation of Compound **18** was described in the literature.[8b] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (d, *J* = 3.7 Hz, 2H), 4.39 (s, 2H), 3.74 (s, 3H), 3.64-3.61 (m, 4H), 3.55-3.51 (m,, 2H), 2.65 (t, *J* = 5.6 Hz, 2H), 2.36 – 2.35 (m, 6H), 1.98 – 1.86 (m, 2H), 1.40 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.8, 148.0, 147.0, 126.7, 125.2, 112.0, 111.1, 79.6, 67.4, 66.9(2C), 56.0, 55.7, 55.4, 53.7(2C), 43.0, 28.5(3C), 26.3. HRMS (EI-MS) (*m/z*) $[M+H]^+$ calcd for $C_{22}H_{34}N_2O_5$ 407.2540, found 407.2551.

tert-Butyl 6-methoxy-7-(2-(piperidin-1-yl)ethoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate **(19)**

The method for preparation of Compound **19** was described in the literature.[8b] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.62 (d, *J* = 3.4 Hz, 2H, ArH), 4.47 (s, 2H, $CONCH_2Ar$), 4.17 − 4.14 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.61 (t, *J* = 5.0Hz, 2H, $CONCH_2$), 2.85 (m, 2H, CH_2Ar), 2.74 (t, *J* = 5.7Hz, 2H, NCH_2), 2.57 (s, br, 4H, NCH_2, NCH_2), 1.74 – 1.55 (m, 6H, $CH_2CH_2CH_2$), 1.49 (s, 9H, ^{t}Bu). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.9, 148.0, 146.7, 127.3, 125.4, 112.0, 111.5, 79.8, 66.4, 57.5, 56.0(2C), 54.8(2C), 49.9, 45.5, 28.8, 28.5(3C), 25.4, 23.8. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{22}H_{34}N_2O_4$ 391.2591, found 391.2597.

tert-Butyl 6-methoxy-7-(3-(piperidin-1-yl)propoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate **(20)**

The method for preparation of Compound **20** was described in the literature.[8b] ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.61 (d, J = 6.5 Hz, 2H, ArH), 4.47 (s, 2H, $CONCH_2Ar$), 4.04 (t, J = 6.3 Hz, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 3.61 (s, br, 2H, $NHCH_2$), 2.73 (s, 2H, $ArCH_2$), 2.52 – 2.05 (m, 6H, NCH_2, NCH_2, NCH_2), 2.06(m, 2H, CH_2), 1.71 – 1.62 (m, 6H, $CH_2CH_2CH_2$), 1.48 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.9, 148.0, 147.0, 127.0, 125.8, 125.3, 112.0, 111.2, 79.7, 67.7, 60.4, 56.1, 55.8, 54.4(2C), 44.7, 29.7, 28.5(3C), 26.2, 25.4, 24.0. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{23}H_{36}N_2O_4$ 405.2748, found 405.2754.

tert-Butyl-6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate **(21)**

The method for preparation of Compound **21** was described in the literature.[8b] ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55 (d, J = 3.3 Hz, 2H, ArH), 4.40 (s, 2H, $CONCH_2Ar$), 4.07 (t, J = 6.2Hz, 2H, OCH_2), 3.75 (s, 3H, OCH_3), 3.59 – 3.55 (m, 2H, $CONCH_2$), 2.92 (t, J = 6.2 Hz, 2H, CH_2Ar), 2.69 − 2.64 (m, 6H, NCH_2, NCH_2, NCH_2), 1.74 − 1.79 (m, 4H, CH_2CH_2), 1.41 (s, 9H, *t*Bu). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.9, 146.5, 145.7, 126.2, 124.4, 110.9, 110.3, 78.8, 66.7, 61.5, 55.0, 53.7, 53.5(3C), 29.1, 27.5(3C), 22.4(2C). HRMS (EI-MS) $[M+H]^+$ calcd for $C_{21}H_{32}N_2O_4$ 377.2435, found 377.2432.

6-Methoxy-7-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-1,2,3,4-tetrahydroisoquinoline 2,2,2-trifluoroacetate **(22)**

Compound **22** has already been described.[8b] The boc-protected compound **16** (3.83 g, 9.0 mmol) was dissolved in DCM, then trifluoroacetic acid (2.61 g, 36.0 mmol) was added. The solution was stirred overnight and the solvent was removed by evaporation. The sticky brown oil **22** was used without purification. Yield: 3.95 g, 100%. ^{1}H NMR (400 MHz, MeOD) δ 3.03 (t, J = 6.3 Hz, 2 H), 3.35 (s, 3 H), 3.46 (t, J = 6.4 Hz, 2 H), 3.52 − 3.55 (m, 2 H), 3.62 − 3.66 (m, 4 H), 3.69 − 3.72 (m, 2 H), 3.81 (s, 3 H), 3.82 − 3.84 (m, 2 H), 4.09 − 4.12 (m, 2 H), 4.25 (s, 2 H), 6.78 (s, 1 H), 6.80 (s, 1 H).

4-(2-((6-Methoxy-1,2,3,4-tetrahydroisoquinolin-7-yl)oxy)ethyl)morpholine **(23)**

Compound **23** has already been described.[8b] ^{1}H NMR (300 MHz, MeOD) δ 6.65 (d, J = 9.3 Hz, 2H, ArH), 4.07 (t, J = 5.6 Hz, 2H, OCH_2), 3.90 – 3.80 (s, 2H, $HNCH_2Ar$), 3.77 (s, 3H, OCH_3), 3.73 – 3.64 (m, 4H, OCH_2, OCH_2), 3.02 (t, J = 6.0 Hz, 2H, $HNCH_2$), 2.82 – 2.69 (m, 4H, NCH_2, CH_2Ar), 2.63 – 2.54 (m, 4H, NCH_2, NCH_2). ^{13}C NMR (75 MHz, MeOD) δ 149.8, 148.0, 128.6, 128.2, 113.9, 113.4, 68.3, 67.7(2C), 58.8, 56.5, 55.3(2C), 48.1, 44.5, 29.0. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{16}H_{24}N_2O_3$ 293.1859, found 293.1860.

4-(3-((6-Methoxy-1,2,3,4-tetrahydroisoquinolin-7-yl)oxy)propyl)morpholine **(24)**

Compound **24** has already been described.[8b] ^{1}H NMR (300 MHz, MeOD) δ 6.63 (d, J = 12.7 Hz, 2H), 4.43 (s, NH, 1H), 3.97 (t, J = 6.2 Hz, 2H), 3.85 (s, 2H), 3.76 (s, 3H), 3.72 – 3.63 (m, 4H), , 3.03 (t, J = 6.0 Hz, 2H), 2.73 (t, J = 5.6 Hz, 2H), 2.56 – 2.40 (m, 6H), 2.01 – 1.86 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 149.7, 148.3, 127.9, 127.9, 113.9, 112.8, 68.5, 67.7, 67.7, 56.9, 56.6, 54.9, 48.0, 44.4, 28.9, 28.8, 27.4. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{17}H_{26}N_2O_3$ 307.2016, found 307.2020.

6-Methoxy-7-(2-(piperidin-1-yl)ethoxy)-1,2,3,4-tetrahydroisoquinoline **(25)**

Compound **25** has already been described.[8b] ^{1}H NMR (300 MHz, MeOD) δ 6.89 (d, J = 5.5 Hz, 2H, ArH), 4.31 (t, J = 4.8Hz, 2H, OCH_2), 4.27 (s, 2H, $NHCH_2Ar$), 3.87 (s, 3H, OCH_3), 3.70 (d, J = 12.4 Hz, 2H, $NHCH_2$), 3.54 (t, J = 5.1Hz, 2H, CH_2Ar), 3.47 (t, J = 6.4 Hz, 2H, NCH_2), 3.10 – 3.02 (m, 4H, NCH_2, NCH_2), 2.01 − 1.80 (m, 6H, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, MeOD) δ 151.0, 147.6, 127.2, 121.4, 114.5, 113.4, 65.2, 57.2, 56.5, 55.0(2C), 45.3, 42.8, 25.8, 24.2(2C), 22.6. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{17}H_{26}N_2O_2$ 291.2072, found 291.2068.

6-Methoxy-7-(3-(piperidin-1-yl)propoxy)-1,2,3,4-tetrahydroisoquinoline **(26)**

Compound **26** has already been described.[8b] ^{1}H NMR (600 MHz, MeOD) δ 6.85 (s, 1H, ArH), 6.80 (s, 1H, ArH), 4.25 (s, 2H, $NHCH_2Ar$), 4.10 (t, J = 5.6 Hz, 2H, OCH_2), 3.84 (s, 3H, OCH_3), 3.64 (d, J = 12.1 Hz, 2H, $NHCH_2$), 3.46 (t, J = 6.4 Hz, 2H, $ArCH_2$), 3.35 – 3.32 (m, 2H, NCH_2), 3.04 (t, J = 6.3 Hz, 2H, NCH_2), 2.96 (td, J_1 = 12.5, J_2 = 2.6 Hz, 2H, NCH_2), 2.29 – 2.18 (m, 2H, CH_2), 2.03 – 1.94 (m, 2H, CH_2), 1.84 – 1.73 (m, 2H, CH_2), 1.54 (qt, J_1 = 13.2, J_2=3.9 Hz, 2H,

CH_2). ^{13}C NMR (151 MHz, MeOD) δ 150.7, 148.4, 125.9, 121.1, 113.1, 112.6, 79.5, 68.1, 56.7, 56.4, 54.6, 45.4, 42.9, 25.7, 25.1, 24.4(2C), 22.7. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{18}H_{28}N_2O_2$ 305.2224, found 305.2224.

6-Methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-1,2,3,4-tetrahydroisoquinoline **(27)**

Compound **27** has already been described.[8b] ^1H NMR (300 MHz, MeOD) δ 6.68 (s, 1H), 6.64 (s, 1H), 4.08 (t, J = 5.8 Hz, 2H), 3.87 (s, 2H), 3.78 (s, 3H), 3.05 (t, J = 6.0 Hz, 2H), 2.91 (t, J = 5.8 Hz, 2H), 2.72 (m, 6H), 1.88 – 1.77 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ 149.8, 148.0, 128.4, 127.9, 113.9, 113.2, 69.2, 56.5, 56.0, 55.7(2C), 48.0, 44.4, 28.8, 24.3(2C). HRMS (EI-MS) $[M+H]^+$ calcd for $C_{16}H_{24}N_2O_2$ 277.1911, found 277.1915.

Methyl 2-amino-4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzoate **(29)**[15]

A mixture of Pd(dppf)Cl_2 (0.270 g, 0.331 mmol), KOAc (1.95 g, 19.870 mmol), bis(neopentyl glycolato)diboron (1.80 g, 7.968 mmol), and methyl 2-amino-4-bromobenzoate **37** (1.50 g, 6.551 mmol) was added to a flask under anhydrous conditions. After addition of anhydrous DMSO, the mixture was stirred at 80 ℃ for several hours and the reaction progress was checked by TLC. The reaction solution was cooled to room temperature and poured into ice-water. The mixture was extracted with ethyl acetate and the combined organic layers were washed with saturated brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography to give the corresponding aryl boronate as white solid. Yield: 1.55 g, 90%. ^1H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 8.0 Hz, 1H, ArH), 7.12 (s, ^1H, ArH), 7.04 (d, J = 8.0 Hz, 1H, ArH), 5.46 (s, br, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.76 (s, 4H, CH_2, CH_2), 1.02 (s, 6H, CH_3, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 148.5, 129.0(2C), 121.5, 120.2, 111.3, 71.3(2C), 50.5, 30.8, 20.8, 20.8. HRMS (EI-MS) [M•+] calcd for $C_{13}H_{18}BNO_4$ 262.1365, found 262.1361.

Methyl 2-amino-4-(6-methylquinolin-2-yl)benzoate **(30)**[16]

2-Bromo-6-methylquinoline **4** (1.38 g, 6.21 mmol), methyl 2-amino-4-(5, 5-dimethyl-1, 3, 2-dioxaborinan-2-yl) benzoate **29** (1.50 g, 5.70 mmol) and [Pd(PPh_3)$_4$] (0.72 g, 0.63 mmol) were placed into a Schlenk flask under a stream of nitrogen at room temperature. The mixture of

solids was stirred and degassed three times before it was dissolved in anhydrous and degassed THF (10 mL), aqueous K_3PO_4 (2 mol/L) was added and the reaction mixture was heated to 80 ℃ overnight. The resulting dark-brown reaction mixture was cooled to room temperature and diluted with water (10 mL). After extracted with CH_2Cl_2, washed with brine and concentrated *in vacuo*, the residue was purified by flash column chromatography over silica gel (PE (50−70°C) : EtOAc = 1:1) to give methyl 2-amino-4-(6-methylnaphthalen-2-yl)benzoate **30** as white solid. Yield: 1.09 g, 60%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.6 Hz, 1H, ArH), 8.10 (d, *J* = 8.4 Hz, 1H, ArH), 7.99 (d, *J* = 8.4 Hz, 1H, ArH), 7.82 (d, *J* = 8.6 Hz, 1H, ArH), 7.60 (s, 1H, ArH), 7.57 (d, *J* = 1.9 Hz, 1H, ArH), 7.55(d, *J* = 1.6Hz, 1H, ArH), 7.35 (dd, J_1 = 8.4Hz, J_2 = 1.7 Hz, 1H, ArH), 5.87 (s, br, 2H, NH_2), 3.91 (s, 3H, OCH_3), 2.56 (s, 3H, $ArCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) *δ* 167.4, 154.4, 149.7, 145.6, 143.7, 135.6, 135.2, 131.1, 130.8, 128.3, 126.5, 125.3, 118.1, 114.5, 114.3, 110.1, 50.6, 20.6. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{18}H_{16}N_2O_2$ 293.1285, found 293.1290.

Methyl 4-(6-methylquinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(31)**

Quinoline-2-carboxylic acid (1.0 equiv) was suspended in $SOCl_2$ (10−15 mL) and heated to reflux for 2 h. Excess $SOCl_2$ was removed under reduced pressure and the resulting quinoline-2-carbonyl chloride was obtained as yellow solid. Methyl 2-amino- 4-(6-methylnaphthalen-2-yl)benzoate **30** (1.0 equiv) and NEt_3 (1.2 equiv) were dissolved in CH_2Cl_2 and the freshly prepared quinoline-2-carbonyl chloride derived was added in small portions and stirred at room temperature for 30 min. Then, the solution was refluxed at 40 ℃ overnight, washed with 1N HCl and saturated aqueous solution of Na_2CO_3 (3×), dried over anhydrous Na_2SO_4 and concentrated to give the crude product which was purified by flash chromatography (PE (50−70°C) : EtOAc = 4:1) on silica gel to give **31** as white solid. ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 13.38 (s, 1H, NH), 9.79 (d, *J* = 1.6 Hz, 1H, ArH), 8.43 (d, J = 8.4 Hz, 1H, ArH), 8.40 (s, 1H, ArH), 8.38 (d, *J* = 3.9 Hz, 1H, ArH), 8.36 (s, 1H, ArH), 8.30 (d, *J* = 8.3 Hz, 1H, ArH), 8.23 (d, *J* = 8.5 Hz, 1H, ArH), 8.14 (d, *J* = 7.5 Hz, 1H, ArH), 8.05 (d, *J* = 8.6 Hz, 1H, ArH), 7.93 (d, *J* = 8.1 Hz, 1H, ArH), 7.86 – 7.82 (m, 1H, ArH), 7.69 – 7.65 (m, 1H, ArH), 7.64 (s, 1H, ArH), 7.62 – 7.59 (m, 1H, ArH), 4.11 (s, 3H, OCH_3), 2.57 (s, 3H, $ArCH_3$). ^{13}C NMR (151 MHz, $CDCl_3$) *δ* 167.9, 163.8, 154.9, 150.1, 146.7, 141.2, 137.7, 137.1(2C), 136.9(2C), 132.5(2C), 131.9, 130.3, 130.2, 129.4, 128.3, 127.7, 126.4, 122.2(2C), 119.5, 119.5, 118.9, 116.9, 52.5, 21.7. HRMS (EI-MS) $[M+H]^+$ calcd

for $C_{28}H_{21}N_3O_3$ 448.1656, found 448.1658.

Methyl 4-(6-(bromomethyl)quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(32)**[17]

A solution of methyl 4-(6-methylnaphthalen-2-yl)-2-(quinoline-2-carboxamido)benzoate **31** (1g, 2.23 mmol), 0.48 g (0.268 mmol) of NBS, and 0.03 g (0.12 mmol) of dibenzoyl peroxide in 50 mL of carbon tetrachloride was refluxed for 6 h. After the reaction mixture was filtered, it was washed with aqueous $NaHCO_3$ and brine. The solvent was evaporated to obtain compound **32** which was sufficiently pure to be used without further purification. Yield: 0.61 g, 52%, white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.36 (s, 1H, -CONH), 9.79 (d, *J* = 1.5 Hz, 1H, ArH), 8.41 (d, *J* = 8.4 Hz, 1H, ArH), 8.38 – 8.36 (m, 1H, ArH), 8.35 (d, *J* = 8.5 Hz, 1H, ArH), 8.27 (d, *J* = 8.3 Hz, 1H, ArH), 8.23 (d, *J* = 3.4 Hz, 1H, ArH), 8.22 (d, *J* = 3.5 Hz, 1H, ArH), 8.11 – 8.08 (m, 1H, ArH), 8.07 (d, *J* = 8.6 Hz, 1H, ArH), 7.91 (d, *J* = 7.8 Hz, 1H, ArH), 7.85 – 7.81 (m, 2H, ArH), 7.77 (dd, *J* = 8.7, 1.9 Hz, 1H, ArH), 7.66 (t, *J* = 7.3 Hz, 1H, ArH), 4.68 (s, 2H, CH_2Br), 4.10 (s, 3H, OCH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.8, 163.8, 156.4, 150.0, 147.7, 146.6, 144.5, 141.2, 137.7, 137.1, 136.2, 131.8, 130.9, 130.6, 130.3, 130.2, 129.4, 128.3, 127.7, 127.4, 127.3, 126.6, 122.1, 119.9, 119.5, 118.9, 117.0, 52.5. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{28}H_{20}BrN_3O_3$ 526.0761, found 526.0764.

General procedure for the preparation of compounds **33a-h**

Tetrahydroisoquinoline derivatives (1.0 equiv.), methyl 4-(6-(bromomethyl) naphthalen-2-yl)-2-(quinoline-2-carboxamido) benzoate **32** (1.0 equiv.) and diisopropylethylamine (2.0 equiv.) were dissolved in CH_3CN and the mixture was refluxed overnight. Flash column chromatography ($CHCl_3$:CH_3OH = 20:1) gave the corresponding products.

Methyl4-(6-((6,7-dimethoxy-3, 4-dihydroisoquinolin-2(1H)-yl)methyl)quinolin -2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33a)**

Compound **33a** according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33a** as pale yellow solid. Mp. 158 ℃ (decomposition), ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.4(s, 1H, -CONH), 9.82(d, *J* = 1.7

Hz, 1H, ArH), 8.44(d, *J* = 8.4Hz, 1H, ArH), 8. 39(d, *J* = 8.5Hz, 1H, ArH), 8.37(d, *J* = 8.5Hz,1H, ArH), 8.29 (d, *J* = 8.3 Hz, 1H, ArH), 8.27 (d, *J* = 8.6 Hz, 1H, ArH), 8.20 (d, *J* = 8.6 Hz, 1H, ArH), 8.11 (dd, J_1 = 8.3, J_2 = 1.8 Hz, 1H, ArH), 8.08 (d, *J* = 8.6 Hz, 1H, ArH), 7.93 (d, *J* = 8.0 Hz, 1H, ArH), 7.84 (ddd, J_1 = 8.3, J_2 = 6.9, J_3 = 1.3 Hz, 2H, ArH), 7.78 (d, *J* = 8.6Hz, 1H, ArH), 7.68 (ddd, J_1 = 8.0, J_2 = 6.9, J_3 = 1.1 Hz, 1H, ArH), 6.63(s,1H, ArH), 6.49(s, 1H, ArH), 4.11 (s,3H, OCH_3), 3.86-3.85(m, 2H, -NCH_2Ar), 3.9 (s, 3H, ArOCH_3), 3.8 (s, 3H, ArOCH_3), 3.70 (s, br, 2H, ArCH_2N-), 2.89-2.86 (m,4H, -NCH_2CH_2Ar). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.9, 163.8, 160.1, 154.7, 150.1, 147.9, 147.4, 146.7, 145.1, 141.2, 137.7(2C), 136.8, 131.8, 131.3, 130.3, 130.2, 129.4, 128.3, 127.7 (2C), 127.4, 126.4, 122.0, 119.6, 119.5 (2C), 118.9 (2C), 116.8, 111.4, 109.5, 55.9, 55.9, 53.4, 52.5 (2C), 48.5, 28.2. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{46}H_{47}N_5O_5$ 639.2602, found 639.2604.

Methyl4-(6-((6-methoxy-7-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)methyl)quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33b)**

Compound **33b** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33b** as pale yellow solid. Mp.102 ℃ (decomposition), ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.37 (s, 1H, -CONH), 9.81 (d, *J* = 1.7 Hz, 1H, ArH), 8.43 (d, *J* = 8.5 Hz, 1H, ArH), 8.38 (d, *J* = 8.1 Hz, 1H, ArH), 8.36 (d, *J* = 7.8 Hz, 1H, ArH), 8.28 (d, *J* = 8.4 Hz, 1H, ArH), 8.25 (d, *J* = 8.6 Hz, 1H, ArH), 8.19 (d, *J* = 8.6 Hz, 1H, ArH), 8.11 (dd, *J* = 8.4, 1.8 Hz, 1H, ArH), 8.07 (d, *J* = 8.6 Hz, 1H, ArH), 7.92 (dd, J_1 = 8.2, J_2=0.9 Hz, 1H, ArH), 7.84 (m, 3H, ArH), 7.67 (m, 1H, ArH), 6.62 (s, 1H, ArH), 6.55 (s, 1H, ArH), 4.12 − 4.09(m, 5H, ArOCH_3, ArOCH_2), 3.89(s, 2H, -NCH_2Ar), 3.85-3.82(m, 5H, PEG), 3.72 − 3.69(m, 2H, -NCH_2Ar), 3.66 − 3.61(m, 5H, PEG), 3.53 − 3.50 (m, 2H, PEG), 3.35(s, 3H, OCH_3), 2.87 − 2.83(m, 4H, -NCH_2CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9, 163.8, 155.7, 150.1, 148.3, 147.9, 146.6, 146.6, 145.1, 141.2, 137.7, 136.8, 131.8, 131.3, 130.5, 130.3, 130.2, 130.0, 129.4, 128.3, 127.7, 127.2, 127.1, 126.8, 122.0, 119.5(2C), 118.9, 116.8 , 112.4, 112.1, 71.9, 70.8, 70.6, 70.5, 69.6, 68.7, 62.4, 59.0, 56.0, 55.6, 53.4, 52.5, 50.8, 28.6. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{45}H_{46}N_4O_8$ 771.3394, found 771.3388.

Methyl4-(6-((6,7-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33c)**

Compound **33c** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **26** as pale yellow solid. Mp.108℃（decomposition）,^{1}H NMR (300 MHz, $CDCl_3$) δ 13.59 (s, 1H, -CONH), 9.81 (d, J = 1.7 Hz, 1H, ArH), 8.43(d, J = 8.5Hz, 1H, ArH), 8.38 (d, J = 8.4 Hz, 1H, ArH), 8.36 (d, J = 8.4Hz, 1H, ArH), 8.29 − 8.23 (dd, J_1 = 8.6 Hz, J_2 = 9.4 Hz, 2H, ArH), 8.19 (d, J = 8.6, 1H, ArH), 8.12 − 8.08 (dd, J_1 = 8.4, J_2=1.8 Hz, 1H, ArH)， 8.07 (d, J = 8.6Hz, 1H, ArH)， 7.93 (d, J = 8.1, 1H, ArH), 7.86 − 7.83 (dd, J_1 = 6.8Hz, J_2 = 1.4 Hz, 1H, ArH) , 7.85 − 7.80 (dd, J_1 = 8.6, J_2 = 1.7Hz, 2H, ArH), 7.69 − 7.61(m, 1H, ArH), 6.67(s, 1H, ArH), 6.54 (s, 1H, ArH), 4.14 − 4.05 (m, 7H, $ArOCH_3$, $ArOCH_2$), 3.88 (s, 2H, -NCH_2), 3.85 − 3.80 (dd, J_1 = 8.7Hz, J_2 = 5.3Hz, 2H, -NCH2Ar), 3.82 − 3.79(d, J = 8.7 Hz, 2H, PEG), 3.75-3.60(m, 14H, PEG), 3.66 − 3.50 (m, 4H, PEG), 3.37 (s, 3H, OCH_3)， 3.36 (s, 3H, OCH_3), 2.85(s, br, 4H, -NCH_2CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.9, 162.8, 154.7, 149.1, 146.8, 146.5, 146.2, 145.6, 144.1, 140.2, 136.7, 135.8, 130.8, 130.3, 129.3, 129.2, 129.0, 128.4, 127.3, 126.7, 126.4, 126.0(2C), 121.0, 118.5, 118.4, 117.9, 115.7, 114.1, 112.2, 70.9, 70.9, 69.8, 69.8, 69.7, 69.7, 69.5, 69.5, 68.7, 68.7, 68.0, 61.4, 58.0, 58.0, 54.6, 51.6, 49.8, 28.7, 28.5. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{51}H_{58}N_4O_{11}$ 903.4175, found 903.4173.

Methyl4-(6-((6-methoxy-7-(2-morpholinoethoxy)-3,4-dihydroisoquinolin-2(1H)-yl) methyl) quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33d)**

Compound **33d** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33d** as pale yellow solid. Mp. 158 ℃ (decomposition), ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.38 (s, 1H, -CONH), 9.81 (d, J = 1.7 Hz, 1H, ArH), 8.43 (d, J = 8.4 Hz, 1H, ArH), 8.39 (d, J = 8.5 Hz, 1H, ArH), 8.37 (d, J = 8.6 Hz, 1H, ArH), 8.29 (d, J = 8.3 Hz, 1H, ArH), 8.25 (d, J = 8.5 Hz, 1H, ArH), 8.20 (d, J = 8.6 Hz, 1H, ArH), 8.10 (dd, J = 8.3, 1.7 Hz, 1H, ArH), 8.07 (d, J = 8.6 Hz, 1H, ArH), 7.92 (d, J = 8.0 Hz, 1H, ArH), 7.87 (s, 1H, ArH), 7.84 (ddd, J_1 = 8.8Hz, J_2 = 5.8Hz, J_3 = 4.6Hz, 2H, ArH), 7.68 − 7.67 (m, 1H, ArH), 6.63 (s, 1H, ArH), 6.52 (s, 1H, ArH). 4.11 (s, 3H, OCH_3), 4.09 (t, J = 6.0 Hz, 2H, OCH_2), 3.91 (s, 2H, -NCH_2Ar), 3.82 (s, 3H, $ArOCH_3$), 3.75 – 3.71 (m, 4H, OCH_2,

OCH_2), 3.63 (s, 2H, -NCH_2Ar), 2.88 (d, *J* = 5.0 Hz, 2H, CH_2Ar), 2.87 – 2.80 (m, 4H, -NCH_2, -NCH_2), 2.60 (s, br, 4H, -NCH_2, -NCH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.9, 163.8, 155.8, 150.1, 148.3, 147.9, 146.6, 146.4, 145.1, 141.2, 137.7, 136.8, 131.8, 131.3, 130.3 , 130.2, 130.1, 129.4, 128.3, 127.7, 127.4, 127.2, 126.8, 126.3, 122.0, 119.5, 119.5, 118.9, 116.8, 112.1, 112.0, 66.7, 62.3, 57.4, 56.0, 55.5, 53.9, 52.5, 50.8, 29.7, 28.8, 28.5, 19.6, 19.2. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{44}H_{43}N_5O_6$ 738.3286, found 738.3293.

Methyl4-(6-((6-methoxy-7-(3-morpholinopropoxy)-3,4-dihydroisoquinolin-2(1H)-yl) methyl)-quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33e)**

Compound **33e** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33e** as pale yellow solid. Mp. 155 ℃ (decomposition), ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.38 (s, 1H, -CONH), 9.80 (s, 1H, ArH), 8.42 (d, *J* = 8.5 Hz, 1H, ArH), 8.37 (dd, J_1 = 9.4, J_2 = 5.4 Hz, 2H, ArH), 8.27 (dd, J_1 = 14.4, J_2 = 6.0 Hz, 2H, ArH), 8.19 (d, *J* = 8.8 Hz, 1H, ArH), 8.12 – 8.03 (m, 2H, ArH), 7.91 (d, *J* = 8.1 Hz, 1H, ArH), 7.82 (d, *J* = 8.2 Hz, 3H, ArH), 7.66 (t, *J* = 7.5 Hz, 1H, ArH), 6.65 (s, 1H, ArH), 6.61(s, 1H, ArH), 4.09 (s, 3H, OCH3), 3.99 (t, *J* = 6.6 Hz, 2H, OCH_2), 3.85 (d, *J* = 5.6 Hz, 2H, -NCH_2Ar), 3.82 (s, 3H, ArOCH_3), 3.76 – 3.63 (m, 4H, -OCH_2,-OCH_2), 3.59 (s, 2H,-NCH_2Ar), 2.84 (dd, J_1 = 10.7, J_2 = 4.4 Hz, 4H, -NCH_2CH_2Ar), 2.51 – 2.44 (m, 6H, -NCH_2, -NCH_2, -NCH_2), 2.00-1.95 (m, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 163.8, 155.7, 150.1, 148.0, 147.8, 147.0, 146.6, 146.6, 145.1, 141.2, 137.8, 137.3, 136.8, 131.8, 131.3, 130.3, 130.3, 130.0, 129.4, 128.3, 127.7, 127.5, 127.0, 126.6, 126.4, 122.0, 119.5, 118.9, 116.8, 112.0, 111.5, 67.4, 66.9, 62.6, 56.1, 55.8, 55.5, 53.7, 53.7, 52.5, 51.1, 28.7, 28.5, 26.3. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{45}H_{45}N_5O_6$ 752.3443, found 752.3448.

Methyl4-(6-((6-methoxy-7-(2-(piperidin-1-yl)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl) methyl)-quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33f)**

Compound **33f** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33f** as pale yellow solid. Mp. 163 ℃ (decomposition), ^{1}H NMR (600 MHz, $CDCl_3$) δ 13.39 (s, 1H, -CONH),

9.81 (d, *J* = 1.7 Hz, 1H, ArH), 8.43 (d, *J* = 8.4 Hz, 1H, ArH), 8.39 (d, *J* = 8.5 Hz, 1H, ArH), 8.37 (d, *J* = 8.4 Hz, 1H, ArH), 8.29 (d, *J* = 8.3 Hz, 1H, ArH), 8.26 (d, *J* = 8.5 Hz, 1H, ArH), 8.19 (d, *J* = 8.6 Hz, 1H, ArH), 8.12 (dd, J_1 = 8.3, J_2=1.7 Hz, 1H, ArH), 8.08 (d, *J* = 8.6 Hz, 1H, ArH), 7.93 (d, *J* = 7.9 Hz, 1H, ArH), 7.84 (dtd, J_1 = 8.3, J_2 = 6.8, J_3 = 1.6 Hz, 3H, ArH), 7.69 − 7.66 (m, 1H, ArH), 6.62 (s, 1H, ArH), 6.54 (s, 1H, ArH). 4.11 (s, 3H, OCH_3), 3.87 (s, 2H, -NCH_2Ar), 3.82 (s, 3H, ArOCH_3), 3.61 (s, 2H, -NCH_2Ar), 2.86 (t, *J* = 5.5Hz, 2H, -NCH2), 2.80 (t, *J* = 5.6 Hz, 2H, -NCH_2), 2.59 (s, br, 4H, -NCH_2, -NCH_2), 1.66 − 1.46(m, 6H, $CH_2CH_2CH_2$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.0, 163.8, 155.7, 150.2, 148.1, 147.8, 146.7, 145.2, 141.2, 137.7(2C), 137.4, 136.8, 131.8, 131.3, 130.3, 130.2, 130.0(2C), 129.4, 128.4, 127.7, 127.4, 126.9, 126.8(2C), 122.0 , 119.5, 119.5, 118.9, 116.8, 112.0, 62.6, 57.4, 56.0, 55.8, (2C), 54.7, 52.5(2C), 50.9, 29.7, 28.8(2C), 24.3. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{45}H_{45}N_5O_5$ 736.3493, found 736.3498.

Methyl4-(6-((6-methoxy-7-(3-(piperidin-1-yl)propoxy)-3,4-dihydroisoquinolin-2(1H)-yl) methyl)-quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33g)**

Compound **33g** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33g** as pale yellow solid. Mp. 156℃ (decomposition)，^{1}H NMR (600 MHz, $CDCl_3$) δ 13.36 (s, 1H, -CONH), 9.79 (d, *J* = 1.6 Hz, 1H, ArH), 8.41 (d, *J* = 8.4 Hz, 1H, ArH), 8.36 (dd, J_1 = 8.2Hz, J_2 = 7.8Hz, 2H, ArH), 8.26 (d, *J* = 8.3 Hz, 1H, ArH), 8.23 (d, *J* = 8.5 Hz, 1H, ArH), 8.19 (d, *J* = 8.3 Hz, 1H, ArH), 8.09 (dd, J_1 = 8.3, J_2 = 1.7 Hz, 1H, ArH), 8.05 (d, *J* = 8.6 Hz, 1H, ArH), 7.90 (d, *J* = 7.6 Hz, 1H, ArH), 7.83 − 7.82 (m, 2H, ArH), 7.81 (d, *J* = 2.1Hz, 1H, ArH), 7.66 − 7.63 (m, 1H, ArH), 6.61 (s, 1H, ArH), 6.51 (s, 1H, ArH). 4.09 (s, 3H, OCH_3), 3.97 (t, *J* = 6.7 Hz, 2H, CH_2), 3.85 (s, 2H), 3.81 (s, 3H, ArOCH_3), 3.59 (s, 2H, -NCH_2Ar), 2.85 (t, *J* = 6.0 Hz, 2H, -NCH_2), 2.80 (t, *J* = 5.4 Hz, 2H, CH_2Ar), 2.48 (t, *J* = 7.2Hz, 2H, -NCH_2), 2.41 (s, br, 4H, -NCH_2, -NCH_2), 2.02 – 1.97 (m, 2H, OCH_2), 1.59 – 1.54 (m, 4H, CH_2CH_2), 1.41 (s, br, 2H, CH_2). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.9, 163.7, 155.6, 150.0, 148.0, 147.8, 146.6, 146.6, 145.0, 141.1, 137.7, 137.3, 136.7, 131.7, 131.2, 130.2, 130.2, 129.9, 129.3, 128.2, 127.6, 127.4, 126.9, 126.6, 126.4, 122.0, 119.4, 119.4, 118.8, 116.7, 112.0, 111.6, 67.7, 62.6, 56.0, 55.7, 55.7, 54.3(2C), 52.4, 51.0, 28.7, 26.4, 25.6(2C), 24.2. HRMS (EI-MS) $[M+H]^+$ calcd for $C_{46}H_{47}N_5O_5$ 750.3650, found 750.3659.

Methyl 4-(6-((6-methoxy-7-(2-(pyrrolidin-1-yl)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl) methyl)-quinolin-2-yl)-2-(quinoline-2-carbonylamino)benzoate **(33h)**

Compound **33h** was prepared according to the general procedure. The crude product was purified with flash column chromatography (EtOAc→$CHCl_3$: MeOH = 20:1) to obtain **33h** as pale yellow solid. Mp. 158 ℃ (decomposition), ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.40 (s, 1H, -CONH), 9.81 (d, J = 1.7 Hz, 1H, ArH), 8.44 (d, J = 8.6 Hz, 1H, ArH),8.40 (d, J = 8.6Hz, 1H, ArH),8.38 (d, J = 8.6Hz, 1H, ArH), 8.30 (d, J = 6.4 Hz, 1H, ArH), 8.27 (d, J = 6.6 Hz, 1H, ArH), 8.20 (d, J = 8.6 Hz, 1H, ArH), 8.11 (dd, J_1 = 8.4, J_2 = 1.7 Hz, 2H, ArH), 8.08 (d, J = 8.7 Hz, 1H, ArH), 7.93 (d, J = 8.1Hz, ArH), 7.85 (m, 3H, ArH), 6.63 (s, 1H, ArH), 6.57 (s, 1H, ArH), 4.43 (s, br, 2H, $ArOCH_2$), 4.12 (s, 3H, OCH_3), 3.90 (d, J = 2.1 Hz, 2H, -NCH_2Ar), 3.82 (s, 3H, OCH_3), 3.62 (s, 2H, -NCH_2Ar), 3.43 (s, 2H, -NCH_2), 2.87(s, 2H, CH_2Ar), 2.82 (s, 2H, -NCH_2), 2.15 (s, br, 4H, CH_2, CH_2), 1.34 – 1.20 (m, 4H, CH_2CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 163.8, 155.7, 150.1, 148.1, 147.8, 146.6, 145.4, 145.1, 141.2, 137.8, 137.2, 136.9, 131.8, 131.3, 130.3, 130.2, 130.0, 129.4, 128.3, 127.8, 127.7, 127.4, 127.1, 126.8, 122.1, 119.5, 119.5, 118.9, 116.8, 112.6, 112.0, 65.9, 62.5, 55.9, 55.6, 54.3(2C), 54.2, 52.6, 50.8, 28.8, 23.3(2C). HRMS (EI-MS) $[M+H]^+$ calcd for $C_{44}H_{43}N_5O_5$ 738.3286, found 738.3293.

Assay protocol for the determination of ABCB1 and ABCG2 inhibition

Drugs and Chemicals Used for Assays

Topotecan and vinblastine were purchased from Sigma (Munich, Germany), diluted in 70% ethanol to a concentration of 0.1 mM and stored at 4 ℃. Hoechst 33342 (Invitrogen, Karlsruhe, Germany) was dissolved in sterile water at a concentration of 0.8 mM. Calcein-AM (4 mM in anhydrous DMSO) and pluronic F127 were obtained from Biotium (Hayward, CA, USA). Fumitremorgin C (FTC; Merck, Darmstadt, Germany) was dissolved in DMSO and diluted to a concentration of 1 mM. Tariquidar was synthesised in our laboratory according to the literature[18] with slight modifications.[19] The test compounds were dissolved in DMSO at a concentration of 10 mM. All stock solutions were stored at −20 ℃. PBS (phosphate buffered saline) was made of 8.0 g/L NaCl, 1.0 g/L $Na_2HPO_4 \cdot 2H_2O$, 0.20 g/L KCl, 0.20 g/L KH_2PO_4 and 0.15 g/L $NaH_2PO_4 \cdot H_2O$ and adjusted to pH 7.4. A solution of 4% (m/m) paraformaldeyde (PFA) in PBS was made by stirring 2 g of PFA per 50 g total solution while heating on a magnetic

stirrer for approximately 30 min. If not otherwise stated, chemicals (p.a. quality) were obtained from Merck (Darmstadt, Germany). Purified water (Milli-Q system, Millipore, Eschborn, Germany) was used throughout.

Cell lines and Culture Conditions

MCF-7/Topo cells, an ABCG2 overexpressing subclone of MCF-7 cells (ATCC® HTB-22™, American Type Culture Collection, Rockville, MD, USA), were obtained as described[19] and cultured in water-saturated atmosphere (95% air, 5% CO_2) at 37 ℃ in 75 cm^2 culture flasks from Nunc (Wiesbaden, Germany) in Eagle's Minimum Essential Medium (EMEM; Sigma, Munich, Germany) containing L-glutamine, 2.2 g/L $NaHCO_3$ and 110 mg/L sodium pyruvate supplemented with 10% fetal calf serum (FCS; Biochrom, Berlin, Germany) and 550 nM topotecan to induce overexpression of the ABCG2 transporter. Human Kb-V1 cells, an ABCB1 overexpressing subclone of Kb cells (ATCC® CCL-17™), were obtained and cultured as described.[19] All cells were routinely monitored for *mycoplasma* contamination by PCR (Venor® GeM, Minerva Biolabs, Berlin, Germany) and only *mycoplasma* negative cultures were used.

Modulation of ABCB1 (p-gp): Determination in the Calcein-AM microplate assay

The assay was performed as described.[11]

Modulation of ABCG2: Determination in the Hoechst 33342 microplate assay

MCF-7/Topo cells were seeded into 96-well plates at a density of 20000 cells/well (total volume 100 µL) and allowed to attach to the surface of the microplates overnight in a water-satured atmosphere (95% air, 5% CO_2) at 37 ℃. The next day, the culture medium was removed, and the cells were incubated with loading suspension: EMEM (supplemented as described above) and 8 µM Hoechst 33342 in combination with the test compound at increasing concentrations (10 nM - 100 µM) for 2 h (37 ℃, 5% CO_2). FTC at a final concentration of 10 µM served as reference compound; under these conditions the response was defined as 100% inhibition of Hoechst 33342 efflux. The supernatants were drained and the cells were fixed for 20 min under light protection using 100 µL per well of a 4% PFA solution. Finally, MCF-7/Topo cells were washed twice with 250 µL of PBS per well to remove residual dye. Afterwards, cells were overlaid with

100 µL of PBS and the fluorescence intensities were determined using a GENios Pro microplate reader (TECAN Deutschland GmbH, Crailsheim, Germany). Measurement mode: fluorescence top; excitation filter: 340/35 nm; emission filter: 485/20 nm; number of reads: 10; integration time: 40 µs; lag time: 0 µs; mirror selection: automatic; plate definition file: GRE96ft.pdf; multiple reads per well (circle, 3x3); time between move and flash: 50 ms. On each plate, the optimal gain was calculated by determination of the fluorescence intensity in the presence of the reference compound fumitremorgin C. By analogy with the protocol for the calcein-AM assay, the obtained fluorescence values were normalized with respect to the number of cells per well (crystal violet staining). All values were corrected by subtracting the fluorescence intensity in the absence of ABCG2 modulator (DMSO control value), and the maximal response was referred to the signal caused by 10 µM of the reference compound FTC (100%). IC_{50} values were calculated using SIGMA PLOT 11.0, "Four parameter logistic curve" fitting. Errors were expressed as standard error of the mean (SEM).

Assay protocol for the determination of ABCC1 inhibition

Drugs and chemicals used for assays

Calcein-AM (4 mM in anhydrous DMSO) and pluronic F127 were obtained from Biotium (Hayward, CA, USA). Bovine serum albumin (BSA) was purchased from Serva (Heidelberg, Germany. Reversan (Tocris Bioscience, Bristol, UK) was dissolved in DMSO and diluted to a concentration of 3 mM.

The test compounds were dissolved in DMSO at a concentration of 10 mM if possible, depending on the solubility of the compounds. All stock solutions were stored at −20 ℃. Loading buffer was made of 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2 \cdot 6H_2O$, 1.5 mM $CaCl_2 \cdot 2H_2O$, 25 mM HEPES, 10 mM glucose, pH 7.4.

PBS (phosphate buffered saline) was made of 8.0 g/L NaCl, 1.0 g/L $Na_2HPO_4 \cdot 2H_2O$, 0.20 g/L KCl, 0.20 g/L KH_2PO_4 and 0.15 g/L $NaH_2PO_4 \cdot H_2O$. The pH value was adjusted to 7.3 − 7.4. A solution of 4% (m/m) paraformaldeyde (PFA) in PBS was made by stirring 2 g of PFA per 50 g total solution while heating on a magnetic stirrer for approximately 30 min. If not otherwise

stated, chemicals (p.a. quality) were obtained from Merck (Darmstadt, Germany). Purified water (Milli-Q system, Millipore, Eschborn, Germany) was used throughout.

Cell line and culture conditions

MDCKII-MRP1 cells: MDCKII cells (Madin-Darby Canine Kidney cells, strain II; an epithelial cell line; ATCC® CRL-2936), transfected with the gene encoding human ABCC1, were a kind gift from Prof. Dr. P. Borst (Netherland Cancer Institute, Amsterdam, NL). The cells were cultured in Dulbecco's Minimum Essential Medium (DMEM; Sigma, Munich, Germany) supplemented with 10% fetal calf serum (FCS; Biochrom, Berlin, Germany), 3.7 g/L of sodium hydrogen carbonate and 110 mg/L of sodium pyruvate.

Calcein-AM (MRP1) standard protocol

MDCKII-MRP1 cells were seeded into flat-bottomed 96-well plates at a density of 20000-25000 cells per well. On the following day, cells were washed with loading buffer in order to remove unspecific serum esterases. Afterwards, cells were incubated with loading suspension (loading buffer, 5 mg/mL BSA, 1.25 µL/mL pluronic F127 (20% in DMSO)) containing 0.5 µM calcein-AM and the test compound at increasing concentrations (10 nM – 100 µM) for 60 min (37 ℃ / 5% CO_2). In general, test compounds were investigated in triplicate, controls in sextuplicate, respectively. Reversan served as positive control at a final concentration of 30 µM corresponding to 100% ABCC1 inhibition.

Subsequently, the loading suspension was discarded, and the cells were fixed with 4% PFA solution in PBS for 20 min. After three washing cycles (loading buffer), fixed cells were overlaid with loading buffer and relative fluorescence intensities were determined at 535/25 nm at a GENios Pro microplate reader (TECAN Deutschland GmbH, Crailsheim, Germany) after excitation at 485/20 nm.

TECAN instrument settings: Measurement mode: fluorescence top; number of reads: 10; integration time: 40 µs; lag time: 0 µs; mirror selection: Dichroic 3 (e.g. Fl.); plate definition file: GRE96ft.pdf; multiple reads per well (Circle): 3x3; time between move and flash: 100 ms.

The following cell quantification procedure was performed by analogy with the protocol for the Hoechst 33342 assay. All values were corrected by subtraction of the fluorescence intensity in the absence of ABCC1 modulators (DMSO control value) and the maximal response was referred to the signal caused by 30 µM of the reference compound reversan (100%). IC_{50} values were calculated using SIGMA PLOT 11.0, "Four parameter logistic curve" fitting. Errors were expressed as standard error of the mean (SEM).

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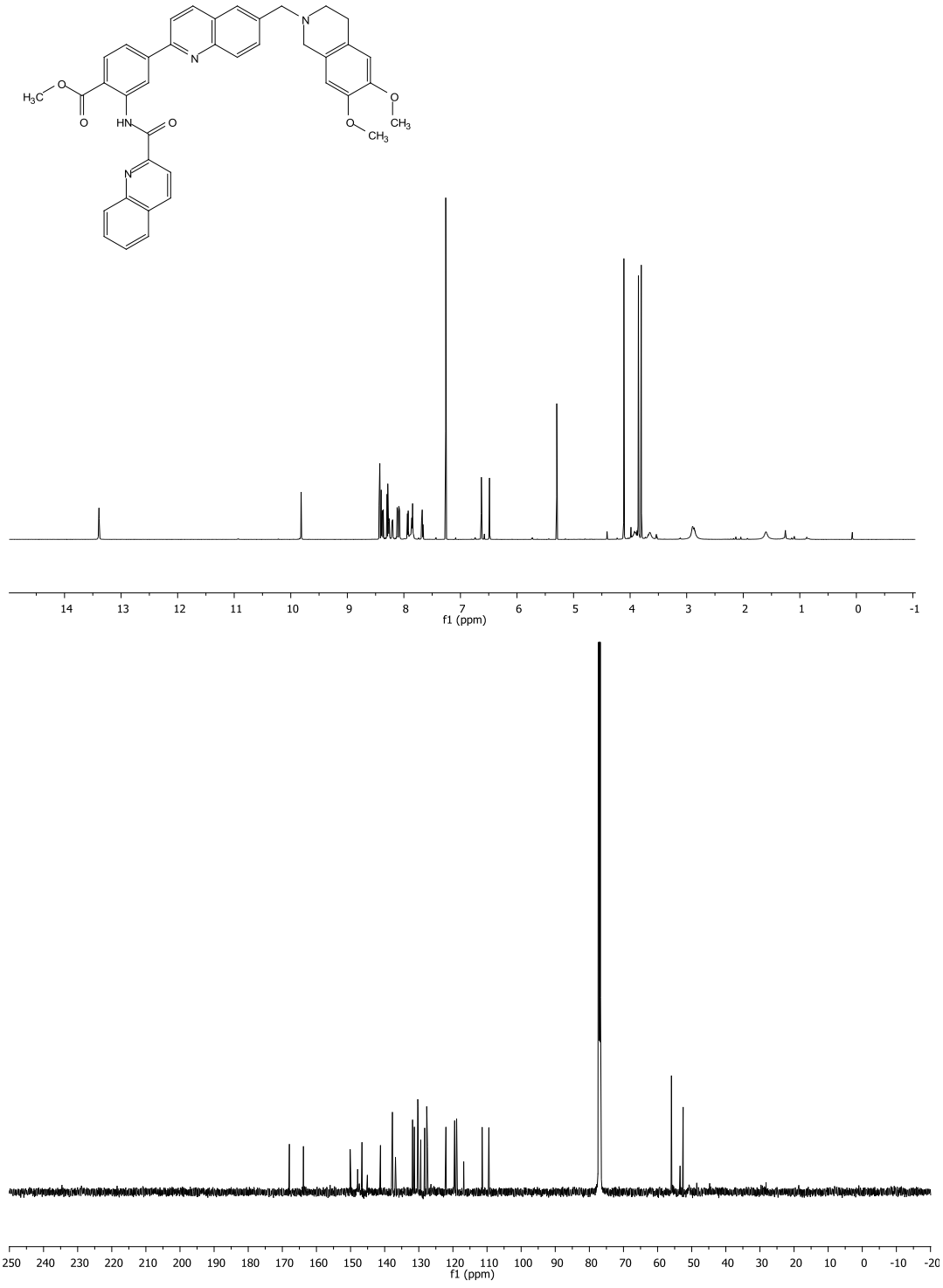
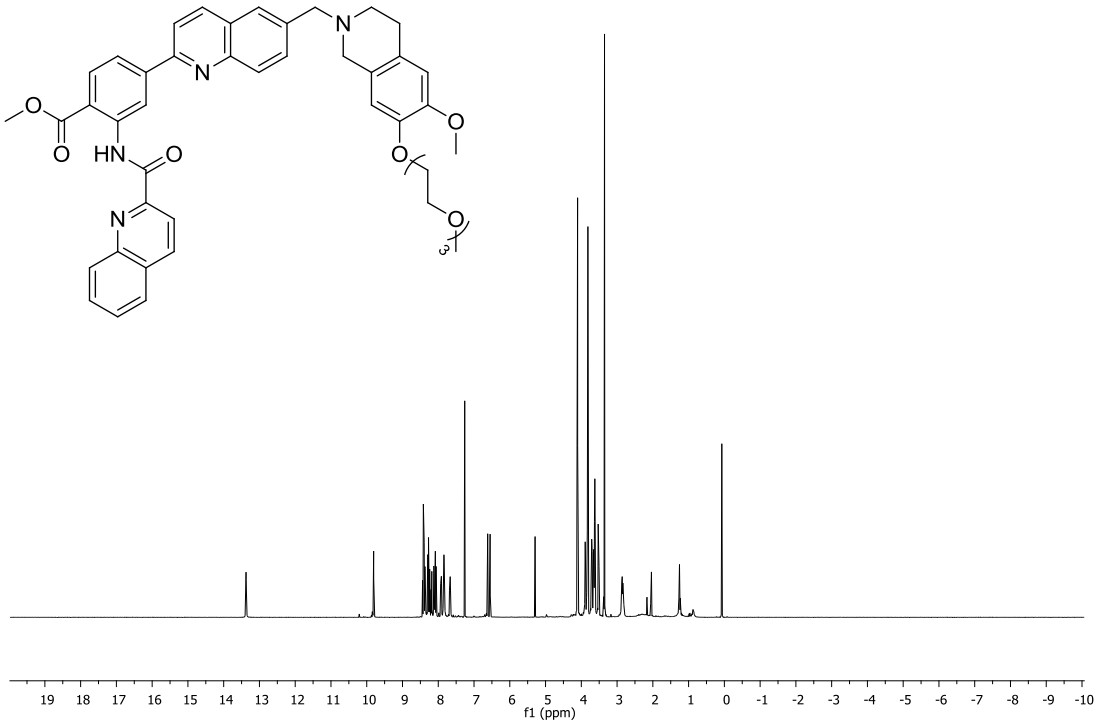
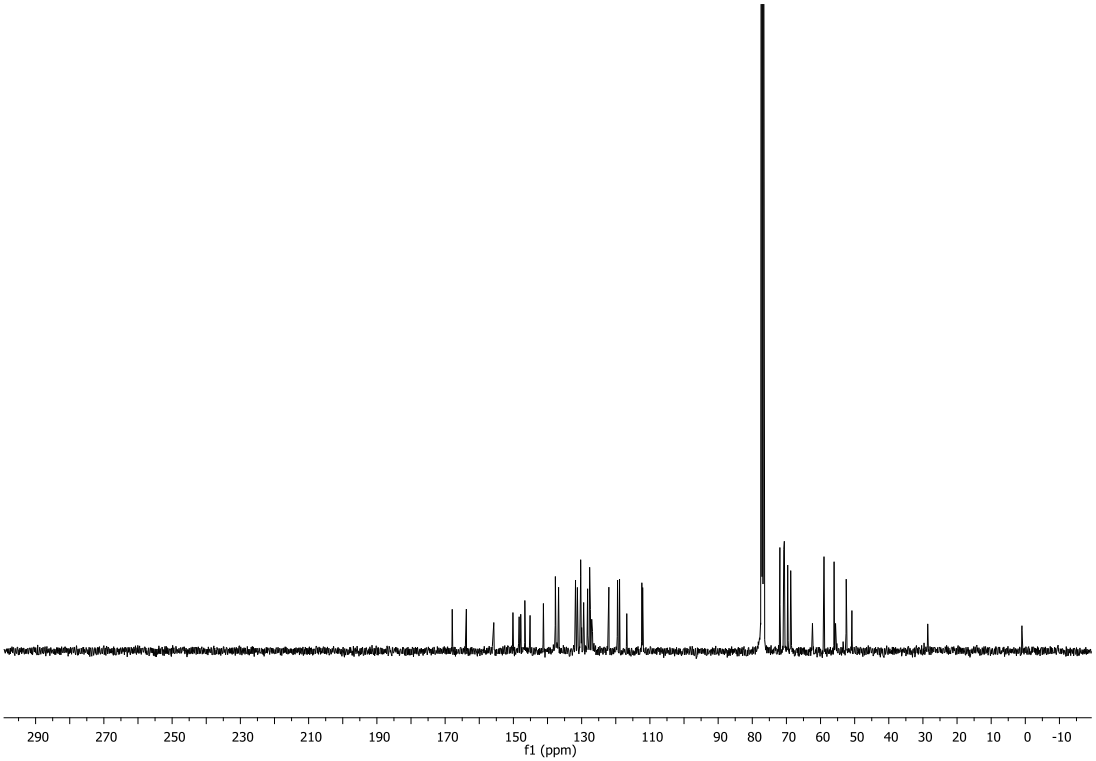
^{1}H and ^{13}C NMR spectra of selected final compounds

^{1}H and ^{13}C NMR spectra for methyl 4-(6-((6, 7-dimethoxy-3, 4-dihydroisoquinolin-2(1H)-yl) methyl) quinolin-2-yl)-2-(quinoline-2-carboxamido) benzoate (**33a**) (600 MHz, $CDCl_3$)

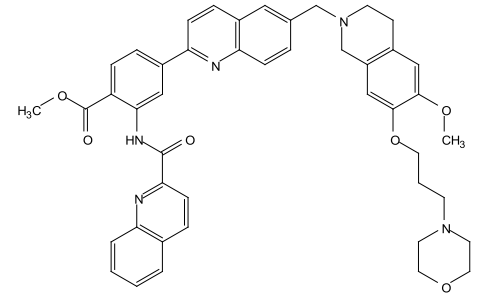


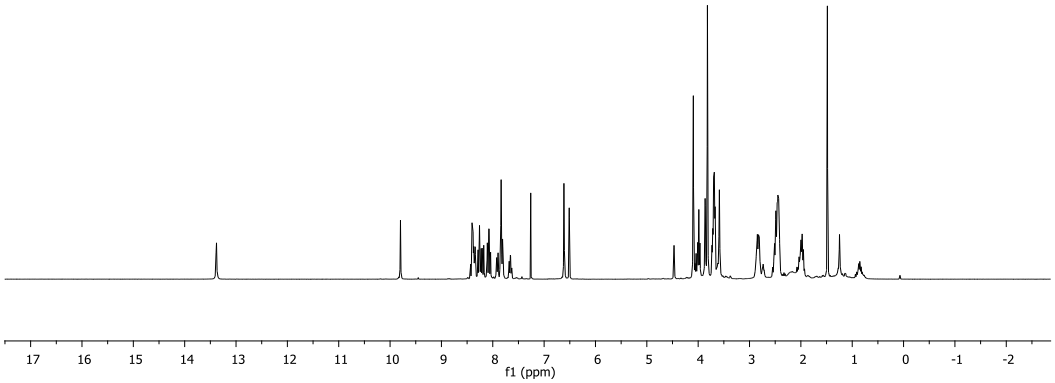
^{1}H and ^{13}C NMR spectra for methyl 4-(6-((6-methoxy-7-(2-(2-(2-methoxyethoxy) ethoxy)-3, 4-dihydroisoquinolin-2(1H)-yl) methyl) quinolin-2-yl)-2-(quinoline-2-carboxamido) benzoate (**33c**) (300 MHz, $CDCl_3$)

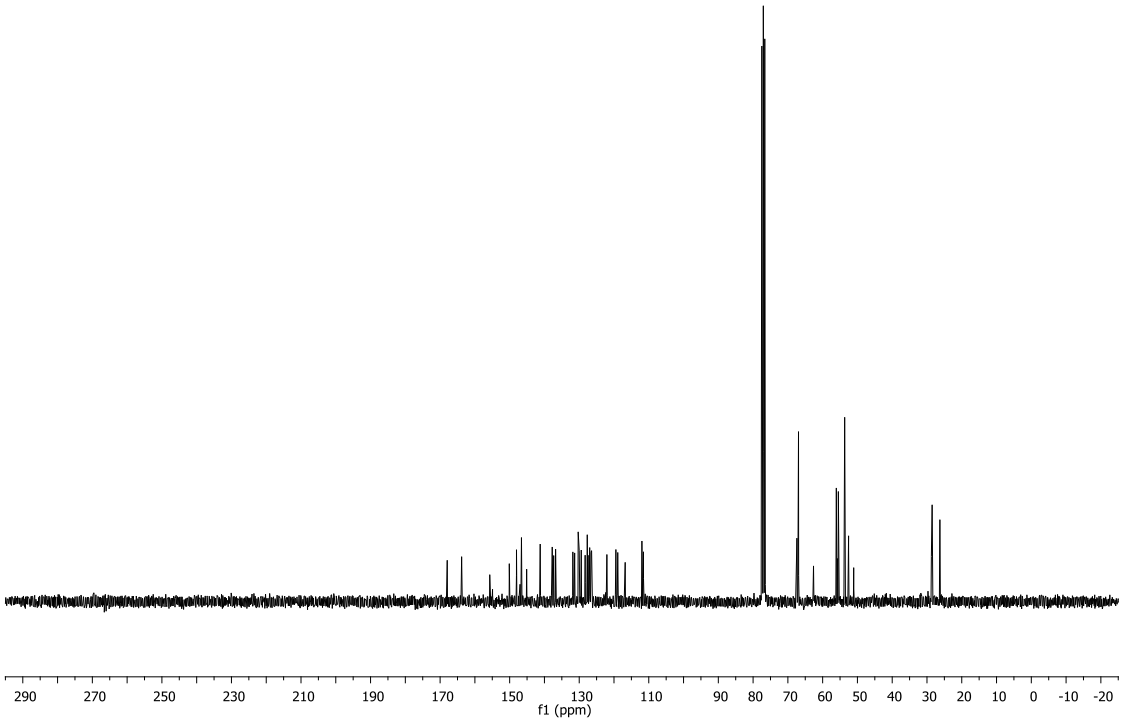




^{1}H and ^{13}C NMR spectra for methyl 4-(6-((6-methoxy-7-(3-morpholinopropoxy)-3, 4-dihydroisoquinolin-2(1H)-yl) methyl) quinolin-2-yl)-2-(quinoline-2-carboxamido) benzoate (**33e**) (300 MHz, $CDCl_3$)

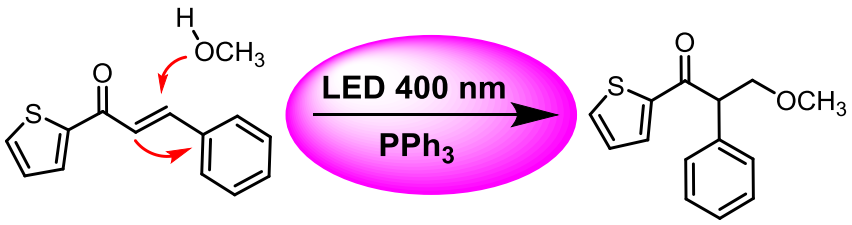






Chapter 5

Triphenylphosphine mediated photo-rearrangement and methanol addition of aryl chalcones to 1-propanones



Abstract

Aryl chalcones rearrange and add methanol giving substituted propane-1-ones upon UV-A irradiation in the presence of PPh_3. We propose two possible mechanisms for this photo-rearrangement. The reaction involves either the formation of a phosphine-carbonyl intermediate, nucleophilic addition of MeOH and 1, 2 aryl migration or the formation of ylide and carbene intermediates. Intermediates trapped from the reaction mixture support the first mechanistic hypothesis.

Keywords

Photo-rearrangement, triphenylphosphine, chalcone, methanol

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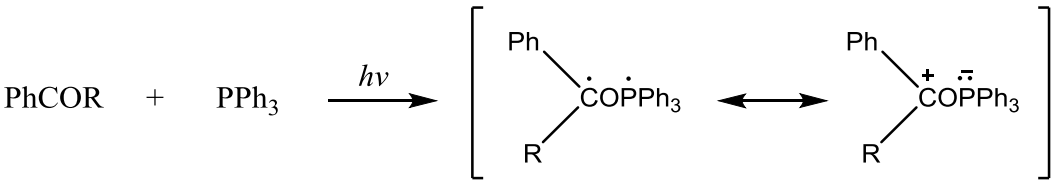
Author contributions:
Q.Sun carried out all the photoreactions and wrote the manuscript. C. J. Yao synthesized compounds **1k**-**1m** given in **Table 2**.

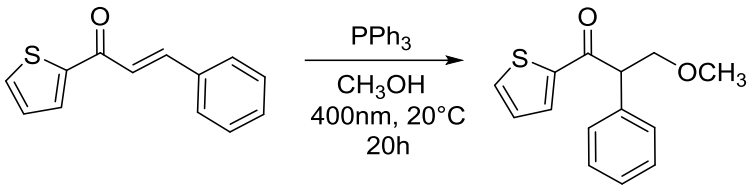
Introduction

The photo chemistry of chalcones has always attracted the interest of organic chemists and in recent years particular the reactions of aryl enones under visible light photoredox catalysis were studied.[1] Typical reaction conditions use a ruthenium complex as visible light absorbing photoredox catalyst and a tertiary amine as sacrificial electron donor to initiate a photoinduced electron transfer reducing the enone to the corresponding radical anion, which undergoes e.g. inter- or intramolecular [2 + 2] cycloaddition[1d, 1g] or a reductive coupling.[1i] The photochemistry of aryl ketones in the presence of PPh_3 was studied already more than 40 years ago,[2] but investigations focused on the photogeneration of ylides [3] and Norrish type II reactions.[2d] Pandey et al. described in 1997 a photocatalytic system for the reductive cyclizations of enones, where DCA were employed as photoredox catalyst and PPh_3 as sacrificial electron donor.[4] In addition to its role as electron donor similar to tertiary amines, PPh_3 has some unique properties: It is sterically more hindered; it is no hydrogen atom donor and an efficient quencher of the carbonyl triplet state (**Scheme 1**). Therefore the photochemical behavior of α, β-unsaturated ketones in the presence of PPh_3 caused our interest.



Scheme 1. Photolysis of aryl ketones in the presence of PPh_3.

We investigated the photoreaction of a variety of chalcone derivatives in the presence of PPh_3 applying different solvents, catalysts and light sources. The reaction of chalcone **1a** with 10 mol % of DCA and PPh_3 (1 equiv.) in MeOH after 20 h irradiation at 400 nm gave an unexpected rearrangement and methanol addition product **2a** (**Scheme 2**) instead of the expected cyclization or Michael addition product. Further studies showed that the reaction proceeds without addition of a photosensitizer, but not in the dark indicating a direct photochemical process. Similar rearrangements have been performed using hypervalent iodine [5] or thallium reagents through oxidative processes.[6] However, since PPh_3 is not an oxidative reagent, we propose a different mechanism and developed a convenient experimental procedure for the interesting rearrangement.

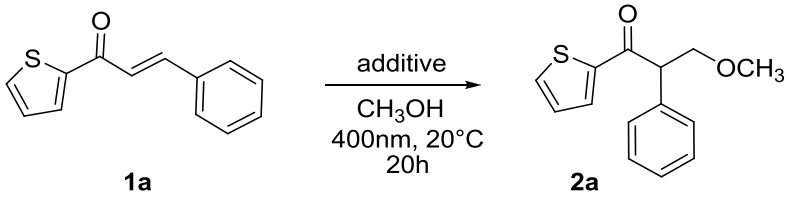


Scheme 2. Photo-rearrangement and methanol addition of 2-thienyl chalcone **1a**.

Results and Discussion

Initially the required amount of PPh_3 for the photoreaction was investigated (**Table 1**). The desired product was obtained in 84% yield with 0.5 equiv. of PPh_3. Decreasing the amount of PPh_3 to 0.25 equiv. does not reduce the product yield, but with catalytic amounts of less than 10 mol% PPh_3, the yield of the reaction dropped to 28%. During work 30 to 40% of the PPh_3 could be recycled by column chromatography. Triphenylphosphineoxide was isolated as a byproduct. The results indicate that PPh_3 acts as a catalyst, but decomposes during the reaction yielding PPh_3=O. Using PPh_3=O, $PHPh_2$ and DIPEA instead of PPh_3 did not yield the desired product **2a**, but the formation of small amounts of [2 + 2] cycloaddition product was observed. Control experiments without PPh_3, without light or under reflux conditions gave no product revealing that PPh_3 and light are essential (Entries 8, 9 and 10). The solvent MeOH was replaced by EtOH, i-PrOH or CF_3CH_2OH, but no product formation was detected in these solvents by GC-MS analysis of the reaction mixture. Besides, the reaction was also carried out with photosensitizers, e.g., $Ru(bpy)_3Cl_2 \cdot 6H_2O$ and Eosin Y, at 450nm and 530nm respectively. The formation of [2 + 2] cycloaddition product and a reductive coupling product was observed when $Ru(bpy)_3Cl_2 \cdot 6H_2O$ was used in the reaction, while no reaction occurred, when it was irradiated with Eosin Y at 530nm.

Table 1: Investigation of different reaction conditions for the photo-rearrangement/addition reaction of **1a**.



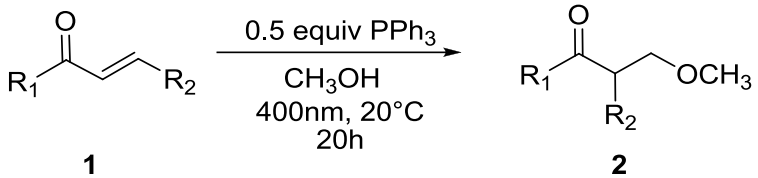
1a **2a**

Entry	Conditions	Yield[%][a][b]
1	PPh_3 (1.0 equiv)	56
2	PPh_3 (0.5 equiv)	84
3	PPh_3 (0.25 equiv)	73
4	PPh_3 (0.10 equiv)	28
5	PPh_3=O (1.0 equiv)	0
6	$PHPh_2$ (1.0 equiv)	0
7	DIPEA (1.0 equiv)	0
8	no PPh_3	0
9	PPh_3 (1.0 equiv), no light	0
10	PPh_3 (1.0 equiv), no light, reflux	0

[a] Isolated yield. [b] The reactions were carried out in 1.0 mL of CH_3OH under N_2 atmosphere.

Next, we investigated the substrate scope of aryl chalcones for the photoreaction and the results are summarized in **Table 2**. Phenyl and naphthyl chalcones rearrange using the described reaction conditions. The X-ray structure analysis of compound **2n** confirmed its structure (**Figure 1**) as assigned from spectroscopic data. Chalcones bearing moderate electron withdrawing, neutral and electron donating substitutes reacted smoothly affording the corresponding products in moderate to good yields. Strong electron withdrawing and donating substitutes like -OCH_3, -NO_2 and -CN on either aromatic ring inhibit the rearrangement reaction; products of the [2 + 2] cycloaddition were observed in these cases.

Table 2: Scope of aryl chalcones in the photo-rearrangement/addition reaction.



1 **2**

Entry	Aryl-chalcone	R_1	R_2	product	Yield [%][a],[b]
1	**1a**	2-thienyl	Ph	**2a**	84
2	**1b**	2-thienyl	4-F-C_6H_4	**2b**	42
3	**1c**	2-thienyl	4-Br-C_6H_4	**2c**	55
4	**1d**	2-thienyl	4-Cl-C_6H_4	**2d**	37
5	**1e**	2-thienyl	3-Br-C_6H_4	**2e**	32
6	**1f**	2-thienyl	4-Me-C_6H_4	**2f**	79
7	**1g**	2-thienyl	4-MeO-C_6H_4	**2g**	trace
8	**1h**	2-thienyl	4-CN-C_6H_4	**2h**	-
9	**1i**	Ph	Ph	**2i**	78
10	**1j**	Ph	4-Me-C_6H_4	**2j**	60
11	**1k**	4-MeO-C_6H_4	4-Br-C_6H_4	**2k**	-
12	**1l**	4-MeO-C_6H_4	Ph	**2l**	trace
13	**1m**	2-NO_2-C_6H_4	Ph	**2m**	-
14	**1n**	2-naphthyl	Ph	**2n**	48

[a] Isolated yield. [b] The reactions were carried out in 1.0 mL of CH_3OH under N_2 atmosphere.

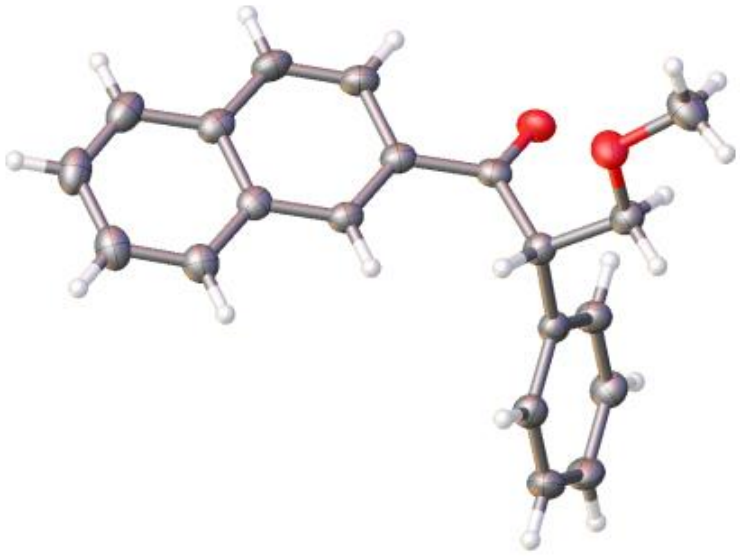
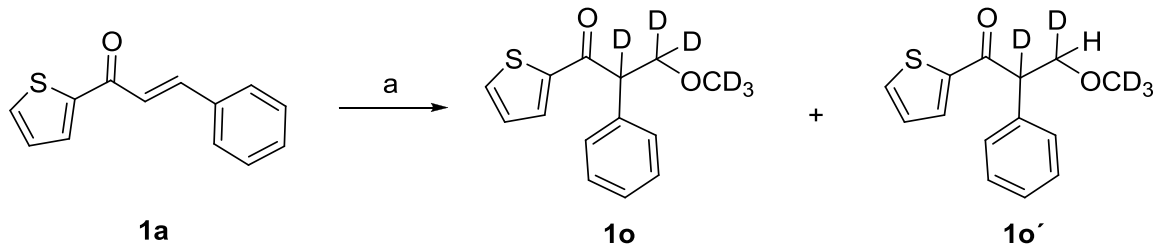


Figure 1. Structure of compound **2n** obtained from the photo-rearrangement/addition reaction of aryl chalcone **1n**, in the solid state.

Several reactions were performed to investigate the mechanism of the photo-rearrangement/addition reaction. The presence of the persistent radical TEMPO did not affect the reaction and no radical trapping products were identified indicating the absence of a radical mechanism. Based on Fox's earlier mechanistic proposal,[3] a phosphonium ylide could be formed through a carbene intermediate upon irradiation. However, our attempts to trap the phosphonium ylide by reaction with benzaldeyde or the carbene with styrene and cyclohexene were without success.

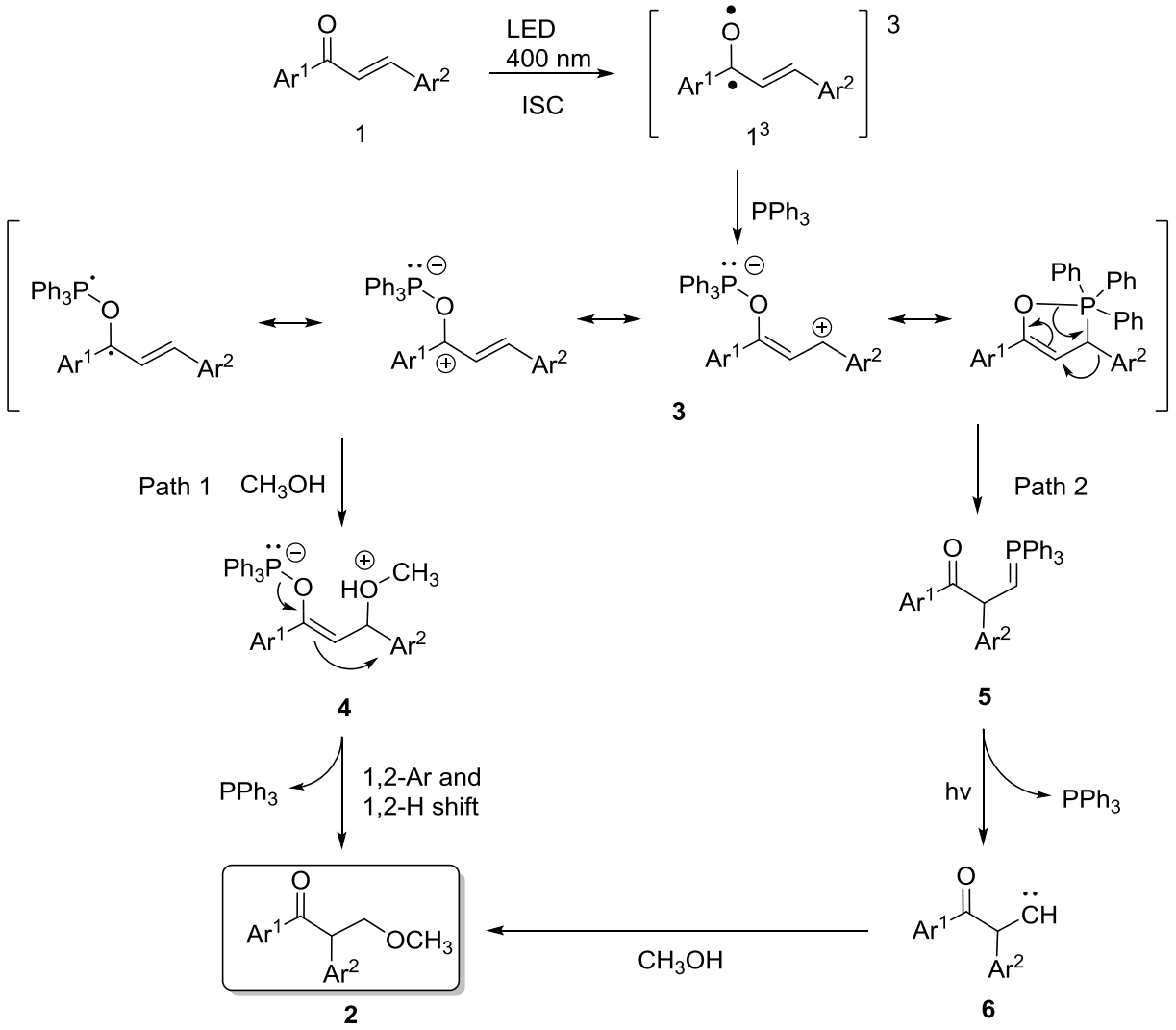


Scheme 3. Photo-rearrangement/addition reaction of **1a** in CD_3OD leads to deuterium incorporation. Reaction conditions: a) 0.5 equiv PPh_3, CD_3OD, 20 ℃, 20 h.

Several reactions were performed to investigate the mechanism of the photo-rearrangement/addition reaction. Initially, the reaction was performed in deuterated methanol giving products 1o and 1o' in a ratio of approximately 5:1 (**Scheme 3**). The more

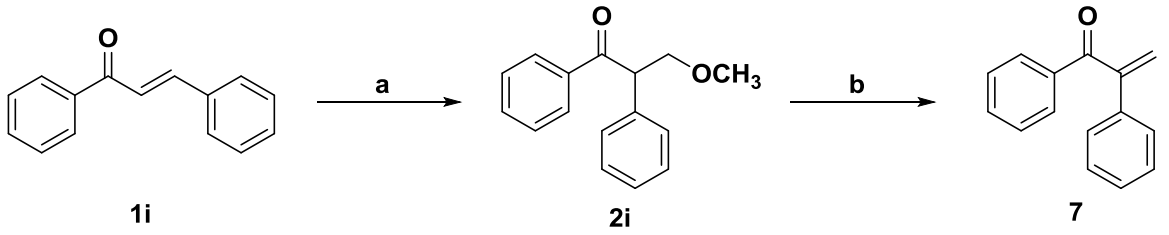
acidic α-hydrogen atom is fully deuterated, while the less reactive β-hydrogen atoms are not completely exchanged by deuterium.

The presence of the persistent radical 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) did not affect the reaction and no radical trapping products were identified indicating the absence of a radical mechanism. On the basis of related reports,[2d, 3, 7] we propose two possible mechanisms shown in **Scheme** 4. The photochemical excitation of the α,β-unsaturated ketone **1** and ISC gives its triplet state 1^3, which is captured by PPh_3 forming **3** through the intervention of an exciplex. The electronic structure of compound **3** is described by four resonance structures. In pathway 1, compound **3** is nucleophilic attacked by the solvent MeOH giving intermediate 4. 1,2-Aryl shift and 1,2-hydrogen shift provide the product **2** of a rearrangement addition reaction sequence. In pathway 2, 1,2-aryl migration occurres giving phosphonium ylide **5**. It is known that ylides can be photochemically cleaved to carbenes.[7] Carbene **6** is formed upon irradiation and quickly trapped by MeOH to give product **2**. Our attempts to identify the intermediate presence of a phosphonium ylide by reaction with benzaldehyde or of the carbene with styrene, cyclohexene and other alcohols were without success. We also did not observe any products arising from the carbene intermediate as described in previous reports.[2d, 3] Intermediate **3** tends to react *via* O-P bond cleavage to the products.[8] The extendedπsystem of the α,β-unsaturated ketone may stabilize the carbonyl group. Therefore, the mechanistic hypothesis of pathway 1 may be more likely, but we cannot rule out a reaction along pathway 2 from our experimental results.



Scheme 4. Suggested mechanistic hypothesis of the photo-rearrangement/addition reaction of aryl chalcones in the presence of PPh_3

To demonstrate a synthetic application, we used the photoreaction product **2i** for the synthesis of 2-substituted enone **7** (**Scheme 5**). Functionalized terminal enones are useful compounds in organic synthesis. They are highly reactive and can undergo conjugate addition reactions with nucleophiles yielding a variety of bioactive products. The reaction of chalcone **1i** with PPh_3 under standard photoreaction conditions provided the corresponding product **2i**, which was then further converted into 1,2-diphenylprop-2-en-1-one **7** by heating to 160−170 ℃ with 1% $NaOCH_3$ in toluene.[9]



Scheme 5. Synthesis of the 2-substituted terminal enone **7** using the PPh_3 mediated photo-rearrangement/addition reaction of compound **1i** and subsequent elimination of methanol. Reaction conditions: a) 0.5 equiv PPh_3, CH_3OH, 20 ℃, 20 h, 78%. b) 1% NaOMe, $PhCH_3$, 160−170 ℃, 1.5 h, 72%.

Conclusions

In conclusion, we have reported the rearrangement and methanol addition reaction of aryl chalcones mediated by PPh_3 under UV-A irradiation. The reaction proceeds smoothly at room temperature without sensitizers using 400 nm emitting LEDs. The rearrangement product can be further converted into 2-substituted terminal enones, which are interesting molecular structures with potential biologically activity. On the basis of previous reports and our experiments, we proposed a mechanism involving the triplet state of the arly chalcone quenched by PPh_3. Nucleophilic addition of MeOH to this intermediate, 1,2-aryl and 1,2-hydrogen atom migration afford diaryl-1-propanones.

Experimental

General Information

^{1}H, ^{13}C NMR spectra were obtained at 298 K using a Bruker AVANCE 300 spectrometer (operating at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C), Bruker AVANCE 400 spectrometer (operating at 400.13 MHz for 1H and 100.62 MHz for 13C). The spectra were obtained using chloroform-d (99.8%, Deutero GmbH) and referenced against non-deuterated (^{1}H) / deuterated (^{13}C) solvents. The shift values (δ H and δ C) are always given in ppm with *J* values in Hz. The melting points were measured using a Stanford Research Systems OptiMelt MPA 100. The highresolution mass spectra were obtained using a Finnigan MAT SSQ 710A spectrometer at 70 eV (HREIMS, positive and negative mode) or an Agilent 6540 UHD (HRESIMS, positive and

negative mode). Automated flash chromatography was performed on a Biotage® IsoleraTM Spektra One device. Silica gel 60 M (40-63 μm, Merck) was used for the flash column chromatography. The starting materials and reagents were purchased from commercial suppliers and used without further purification. The solvents were p.a. grade for the reaction mixtures and industrial grade for the flash column chromatography. Analytical TLC was performed on silica gel coated alumina plates (MN TLC sheets ALUGRAM® Xtra SIL G/UV254). The visualization was performed using UV-light (254 and 366 nm). UV–Vis analyses were performed with Varian Cary 50 UV/Vis spectrophotometer and Agilent 8453 UV-Vis Spectrometer. For UV measurements 10 mm Hellma fluorescence quartz cuvettes (117.100F-QS) with a screw cap with PTFE-coated silicon septum were used. Chalcone **1i** was purchased from sigma-aldrich.

Irradiation Source:

Philips LUXEON® Rebel (purple, max = 400 ±10 nm, 1000 mA, 1.2 W)

General procedure for preparation of α,β-unsaturated ketones 1a-1h and 1j-1n.

To a stirred solution of acetophenone (10 mmol) in methanol (5 mL) was added dropwise a solution of sodium hydroxide (13 mmol) in methanol (10 mL). Fifteen minutes later, the resulting mixture was further treated with substituted benzaldehydes (10 mmol) and stirred at room temperature. When the reaction was complete (disappearance of acetophenone, monitored by TLC), 40 mL water was added. The solid products were filtered off, washed with water (3 × 25 mL), cold methanol (3 × 25 mL) and dried to give corresponding α,β-unsaturated ketones.[10]

(E)-3-Phenyl-1-(thiophen-2-yl)prop-2-en-1-one **(1a)**

Pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.81 (m, 2H), 7.69 (dd, J = 4.9, 0.9 Hz, 1H), 7.67 -7.67 (m, 2H), 7.47 – 7.39 (m, 4H), 7.20 (dd, J = 4.9, 3.9 Hz, 1H). The spectroscopy is in accordance with literature.[11]

(E)-3-(4-Fluorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **(1b)**

Pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 – 7.77 (m, 2H), 7.70 (dd, J = 4.9, 1.0 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.35 (d, J = 15.6 Hz, 1H), 7.23 – 7.16 (m, 1H), 7.16 – 7.07 (m, 2H). The spectroscopy is in accordance with literature.[12]

(E)-3-(4-Bromophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **(1c)**

Pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (dd, J = 3.8, 1.0 Hz, 1H), 7.78 (d, J = 15.6 Hz, 1H), 7.70 (dd, J = 4.9, 1.0 Hz, 1H), 7.59 – 7.48 (m, 4H), 7.45 – 7.35 (m, 1H), 7.20 (dd, J = 4.9, 3.9 Hz, 1H). The spectroscopy is in accordance with literature.[13]

(E)-3-(4-Chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **(1d)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (dd, J = 3.8, 0.9 Hz, 1H), 7.80 (d, J = 15.6 Hz, 1H), 7.70 (dt, J = 9.3, 4.7 Hz, 1H), 7.59 (dd, J = 8.8, 2.2 Hz, 2H), 7.44 – 7.34 (m, 3H), 7.20 (dd, J = 4.9, 3.9 Hz, 1H). The spectroscopy is in accordance with literature.[14]

(E)-3-(3-Bromophenyl)-1-(thiophen-2-yl)prop-2-en-1-one **(1e)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 3.8, 1.0 Hz, 1H), 7.78 (dd, J = 11.1, 8.9 Hz, 2H), 7.71 (dd, J = 4.9, 1.0 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.41 (d, J = 15.5 Hz, 1H), 7.29 (dd, J = 13.2, 5.3 Hz, 1H), 7.21 (dd, J = 4.9, 3.9 Hz, 1H). The spectroscopy is in accordance with literature.[12]

(E)-1-(Thiophen-2-yl)-3-(p-tolyl)prop-2-en-1-one **(1f)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 – 7.79 (m, 2H), 7.68 (dd, J = 4.9, 1.0 Hz, 1H), 7.55 (d, J = 8.1 Hz, 2H), 7.43 – 7.34 (m, 1H), 7.23 (d, J = 8.0 Hz, 2H), 7.19 (dd, J = 4.9, 3.8 Hz, 1H), 2.40 (s, 3H). The spectroscopy is in accordance with literature.[12]

(E)-3-(4-Methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one **(1g)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 – 7.75 (m, 2H), 7.67 (dd, J = 4.9, 1.0 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.37 – 7.28 (m, 1H), 7.18 (dd, J = 4.9, 3.8 Hz, 1H), 6.99 – 6.90 (m, 2H). The spectroscopy is in accordance with literature.[12]

(E)-4-(3-Oxo-3-(thiophen-2-yl)prop-1-en-1-yl)benzonitrile **(1h)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, J = 3.8, 1.0 Hz, 1H), 7.82 (d, J = 15.6 Hz, 1H), 7.76 – 7.69 (m, 5H), 7.48 (d, J = 15.6 Hz, 1H), 7.22 (dd, J = 4.9, 3.9 Hz, 1H). The spectroscopy is in accordance with literature.[15]

(E)-1-Phenyl-3-(p-tolyl)prop-2-en-1-one **(1j)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (dd, J = 5.3, 3.3 Hz, 2H), 7.80 (d, J = 15.7 Hz, 1H), 7.62 – 7.45 (m, 6H), 7.23 (d, J = 8.0 Hz, 2H). The spectroscopy is in accordance with literature.[16]

(E)-3-(4-Bromophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one **(1k)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 – 7.95 (m, 2H), 7.73 (d, J = 15.7 Hz, 1H), 7.59 – 7.45 (m, 5H), 7.04 – 6.93 (m, 2H), 3.90 (s, 3H). The spectroscopy is in accordance with literature.[17]

(E)-1-(4-Methoxyphenyl)-3-phenylprop-2-en-1-one **(1l)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 – 8.00 (m, 2H), 7.87 – 7.74 (m, 1H), 7.70 – 7.61 (m, 2H), 7.60 – 7.50 (m, 1H), 7.43-7.41 (m, 3H), 7.04 – 6.94 (m, 2H), 3.89 (s, 3H). The spectroscopy is in accordance with literature.[18]

(E)-1-(2-Nitrophenyl)-3-phenylprop-2-en-1-one **(1m)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 – 8.18 (dd, J = 0.1, 8.2 Hz,1H), 7.81 – 7.75 (m, 1H), 7.70 – 7.64 (m, 1H), 7.54 – 7.50 (m, 3H), 7.41 – 7.36 (m, 3H), 7.26 (d, J = 16.3 Hz, 1H), 7.02 (d, J = 16.3 Hz, 1H). The spectroscopy is in accordance with literature.[19]

(E)-1-(Naphthalen-2-yl)-3-phenylprop-2-en-1-one **(1n)**

White powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.11 (dd, J = 8.6, 1.7 Hz, 1H), 8.04 – 7.98 (m, 1H), 7.98 – 7.85 (m, 3H), 7.75 – 7.67 (m, 3H), 7.60 (pd, J = 6.9, 1.5 Hz, 2H), 7.48 – 7.42 (m, 3H). The spectroscopy is in accordance with literature.[20]

General procedure for the photo-rearrangement/addition reaction.

In a 5 mL snap vial equipped with magnetic stirring bar the PPh_3 (0.5 equiv, 0.125mmol) and aryl chalcone derivatives (1.0 equiv, 0.25mmol) were added in 1 mL of CH_3OH, and the resulting reaction mixture was degassed by three "pump-freeze-thaw" cycles via a syringe needle. The vial was irradiated through the vial's plane bottom side using 400 nm purple LEDs with cooling device maintaining a temperature around 20 ℃. After 20h of irradiation, the solvent was removed and purified by flash column chromatography using petrol ether (50−70°C)/ethyl acetate (99:1 to 99:5) as eluent.

3-Methoxy-2-phenyl-1-(thiophen-2-yl)propan-1-one **(2a)**

Colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, J = 3.8, 1.1 Hz, 1H), 7.61 – 7.57 (m, 1H), 7.39 – 7.34 (m, 2H), 7.32 (ddd, J = 7.6, 4.5, 1.2 Hz, 2H), 7.06 (dd, J = 4.9, 3.9 Hz, 1H), 4.71 (dd, J = 8.9, 5.3 Hz, 1H), 4.18 (t, J = 9.0 Hz, 1H), 3.64 (dd, J = 9.1, 5.3 Hz, 1H), 3.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.1, 144.1, 136.3, 134.0, 132.7, 129.0, 128.3, 128.1, 127.7, 74.4, 59.2, 55.3. HRMS (EI-MS) calcd for $C_{14}H_{14}O_2S$ $[M+H]^+$ 247.0787 found 247.0788.

2-(4-Fluorophenyl)-3-methoxy-1-(thiophen-2-yl)propan-1-one **(2b)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (dd, J = 3.8, 1.1 Hz, 1H), 7.63 – 7.59 (m, 1H), 7.37 – 7.30 (m, 2H), 7.07 (dt, J = 7.3, 3.6 Hz, 1H), 7.04 – 6.96 (m, 2H), 4.73 – 4.66 (m, 1H), 4.13 (t, J = 8.9 Hz, 1H), 3.62 (dd, J = 9.1, 5.5 Hz, 1H), 3.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.1, 143.8, 134.3, 132.7, 129.9, 129.8, 128.2, 116.1, 115.8, 74.3, 59.2, 54.3. HRMS (EI-MS) calcd for $C_{14}H_{13}FO_2S$ $[M+H]^+$ 265.0693 found 265.0696.

2-(4-Bromophenyl)-3-methoxy-1-(thiophen-2-yl)propan-1-one **(2c)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (dd, J = 3.8, 1.1 Hz, 1H), 7.60 (dd, J = 4.9, 1.1 Hz, 1H), 7.46 – 7.40 (m, 2H), 7.27 – 7.22 (m, 2H), 7.06 (dd, J = 4.9, 3.9 Hz, 1H), 4.67 (dd, J = 8.5, 5.7 Hz, 1H), 4.20 – 4.03 (m, 1H), 3.62 (dd, J = 9.1, 5.7 Hz, 1H), 3.34 (s, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 190.7, 143.7, 135.4, 134.5, 132.8, 132.1, 130.0, 128.3, 121.8, 74.1, 59.3, 54.5. HRMS (EI-MS) calcd for $C_{14}H_{13}BrO_2S$ $[M+H]^+$ 324.9892 found 324.9894.

2-(4-Chlorophenyl)-3-methoxy-1-(thiophen-2-yl)propan-1-one **(2d)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 – 7.69 (m, 1H), 7.65 – 7.54 (m, 1H), 7.29 (d, J = 2.1 Hz, 3H), 7.12 – 6.98 (m, 1H), 4.68 (dd, J = 9.1, 5.7 Hz, 1H), 4.19 – 4.03 (m, 1H), 3.62 (dd, J = 9.1, 5.7 Hz, 1H), 3.34 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.8, 143.7, 134.8, 134.4, 133.7, 132.8, 129.6, 129.2, 128.3, 74.2, 59.3, 54.5. HRMS (EI-MS) calcd for $C_{14}H_{13}ClO_2S$ $[M+H]^+$ calcd for 281.0398 found 281.0398.

2-(3-Bromophenyl)-3-methoxy-1-(thiophen-2-yl)propan-1-one **(2e)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (dd, J = 3.8, 1.0 Hz, 1H), 7.62 (dd, J = 4.9, 1.0 Hz, 1H), 7.53 (dd, J = 6.4, 4.7 Hz, 1H), 7.41 – 7.34 (m, 1H), 7.34 – 7.28 (m, 1H), 7.23 – 7.16 (m, 1H), 7.11 – 7.04 (m, 1H), 4.67 (dd, J = 8.6, 5.6 Hz, 1H), 4.17 – 4.08 (m, 1H), 3.63 (dd, J = 9.1, 5.6 Hz, 1H), 3.34 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.5, 143.8, 138.5, 134.6, 132.9, 131.3, 130.9, 130.5, 128.3, 127.0, 123.0, 74.2, 59.3, 54.7. HRMS (EI-MS) calcd for $C_{14}H_{13}BrO_2S$ $[M+H]^+$ calcd for 324.9892 found 324.9891.

3-Methoxy-1-(thiophen-2-yl)-2-(p-tolyl)propan-1-one **(2f)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (dd, J = 3.8, 0.9 Hz, 1H), 7.54 (dd, J = 4.9, 0.9 Hz, 1H), 7.28 (s, 1H), 7.12 (d, J = 8.0 Hz, 2H), 7.03 (dd, J = 4.9, 3.9 Hz, 1H), 4.71 (dd, J = 8.9, 5.3 Hz, 1H), 4.22 – 4.13 (m, 1H), 3.62 (dd, J = 9.1, 5.3 Hz, 1H), 3.35 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3, 144.1, 137.4, 134.0, 133.3, 132.7, 129.7, 128.2, 74.4, 59.2, 54.8, 21.1. HRMS (EI-MS) calcd for $C_{15}H_{16}O_2S$ $[M+H]^+$ 261.0944 found 261.0946.

3-Methoxy-1,2-diphenylpropan-1-one **(2i)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 – 7.95 (m, 2H), 7.50 – 7.45 (m, 1H), 7.43 – 7.37 (m, 2H), 7.32 (dt, J = 8.7, 1.7 Hz, 4H), 7.25 – 7.21 (m, 1H), 4.90 (dd, J = 8.7, 5.3 Hz, 1H), 4.25 – 4.14 (m, 1H), 3.65 (dd, J = 9.1, 5.3 Hz, 1H), 3.36 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.3, 136.7, 136.3, 133.1, 129.0, 128.8, 128.6, 128.4, 127.6, 74.7, 59.2, 53.8. HRMS (EI-MS) calcd for $C_{16}H_{16}O_2$ $[M+H]^+$ calcd for 241.1223 found 241.1228.

3-Methoxy-1-phenyl-2-(p-tolyl)propan-1-one **(2j)**

Colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 – 7.94 (m, 2H), 7.49 – 7.45 (m, 1H), 7.41-7.29 (m, 2H), 7.23 (d, J = 8.1 Hz, 2H), 7.12 (d, J = 7.9 Hz, 2H), 4.87 (dd, J = 8.7, 5.3 Hz, 1H), 4.18 (t, J = 8.9 Hz, 1H), 3.63 (dt, J = 11.1, 5.5 Hz, 1H), 3.36 (s, 3H), 2.29 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.4, 137.3, 136.7, 133.3, 133.0, 129.8, 128.8, 128.5, 128.2, 74.7, 59.2, 53.4, 21.1. HRMS (EI-MS) calcd for $C_{17}H_{18}O_2$ $[M+H]^+$ 255.1380, found 255.1377.

3-Methoxy-1-(naphthalen-2-yl)-2-phenylpropan-1-one **(2n)**

Colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.04 (dd, J = 8.7, 1.8 Hz, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.82 (dd, J = 12.2, 6.8 Hz, 2H), 7.54 (ddd, J = 9.2, 5.1, 1.4 Hz, 2H), 7.41-7.38 (m, 2H), 7.34 – 7.28 (m, 2H), 7.25 – 7.21 (m, 1H), 5.06 (dd, J = 8.7, 5.3 Hz, 1H), 4.25 (t, J = 8.9 Hz, 1H), 3.71 (dd, J = 9.1, 5.3 Hz, 1H), 3.38 (s, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 198.3, 136.5, 135.5, 134.1, 132.4, 130.6, 129.7, 129.1, 128.5, 128.4, 127.7, 127.6, 126.7, 124.4, 74.8, 59.2, 53.8. HRMS (EI-MS) calcd for $C_{20}H_{18}O_2$ $[M+H]^+$ 291.1380 found 291.1381.

1,2-Diphenylprop-2-en-1-one **(7)**

White solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 – 7.86 (m, 2H), 7.60 – 7.32 (m, 8H), 6.08 (s, 1H), 5.65 (s, 1H). The NMR spectra is in accordance with literature.[21]

^{1}H NMR data for [2 + 2] cycloaddition products

(3,4-Diphenylcyclobutane-1, 2-diyl)bis (thiophen-2-yl methanone).

Pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (dd, J = 4.9, 1.0 Hz, 2H), 7.43 (dd, J = 3.8, 1.0 Hz, 2H), 7.32 (d, J = 4.4 Hz, 4H), 6.96 (dd, J = 4.9, 3.9 Hz, 1H), 4.49 – 4.37 (m, 2H), 4.05 – 3.92 (m,2). The spectroscopy is in accordance with literature.[22]

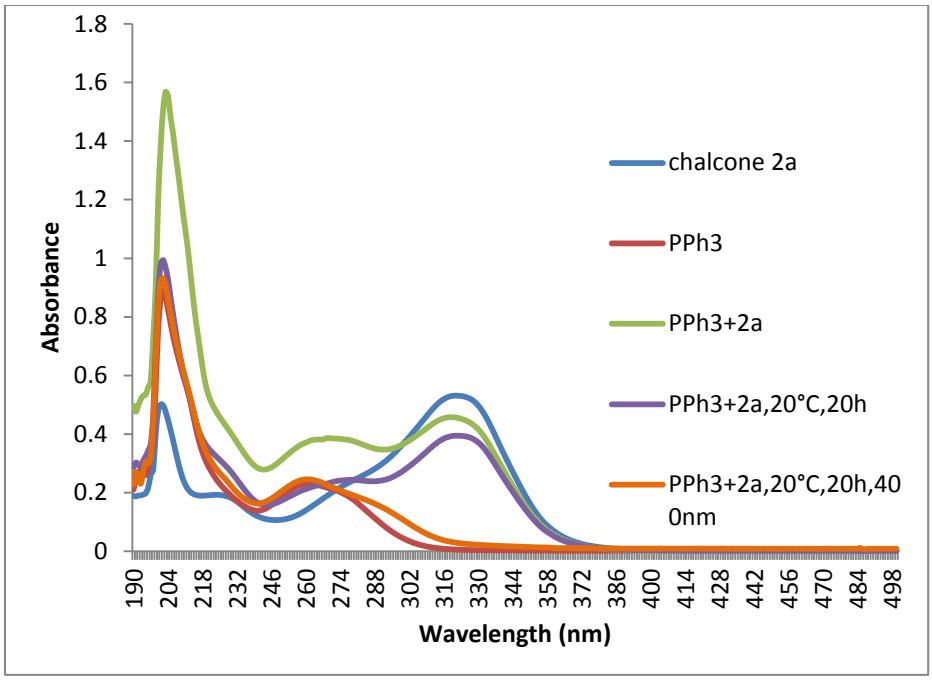
(3,4-Diphenylcyclobutane-1,2-diyl)bis((4-methoxyphenyl)methanone).

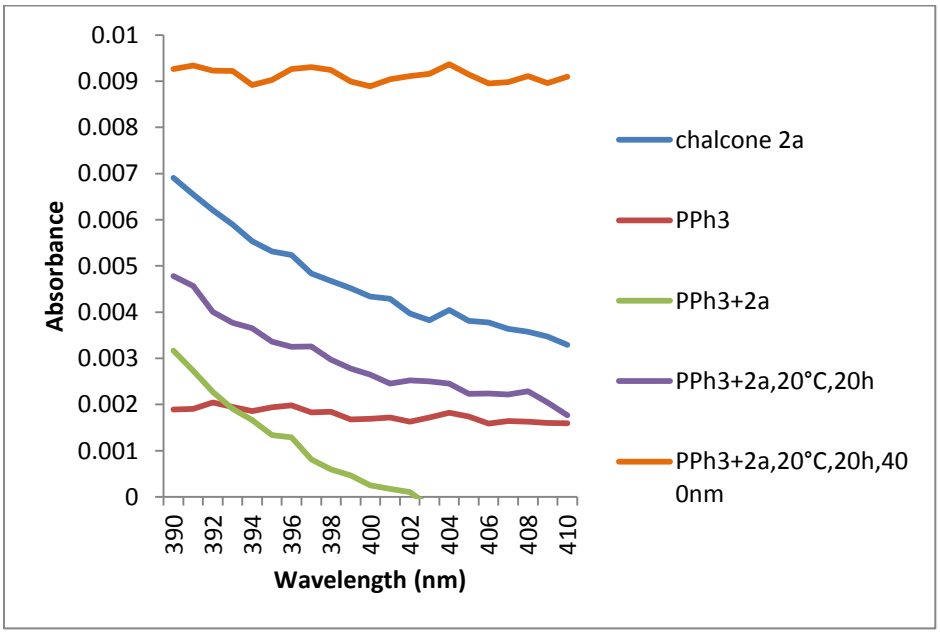
White solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 – 7.77 (m, 4H), 7.31 – 7.29 (m, 8H), 7.25 – 7.21 (m, 2H), 6.82 – 6.74 (m, 4H), 4.59 – 4.49 (m, 2H), 4.02 – 3.93 (m, 2H), 3.79 (s, 6H). The spectroscopy is in accordance with literature.[23]

UV spectra of chalcone 2a and PPh_3 before and after irradiation

The absorbance in the range of 390-410 nm is very weak. Molar absorptivity at 390 nm was in the range of 100-500 cm^{-1}. After 20h of irradiation, the reaction mixture turnes to light yellow color and showed stronger absorption at 390 nm. The peak of chalcone **2a** at 324 nm dramatically decreases demonstrating the consumption of chalcone **2a**.

UV–visible spectra of the compounds were determined in MeOH solution (conc. 2×10^{-5}M).





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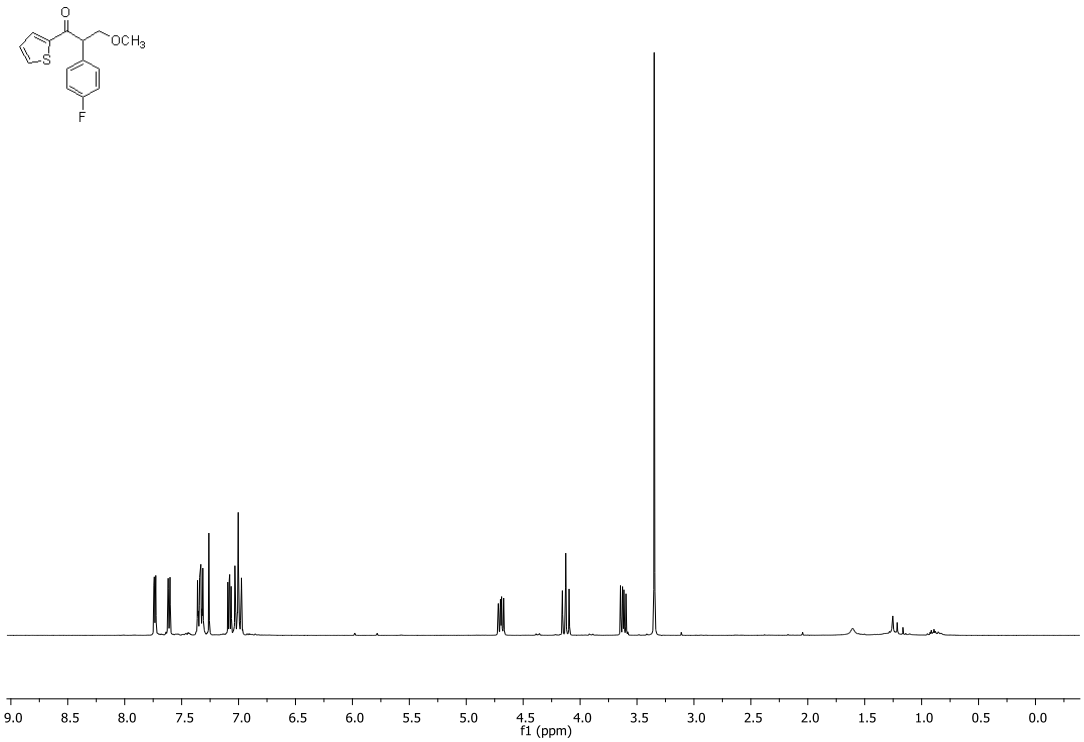
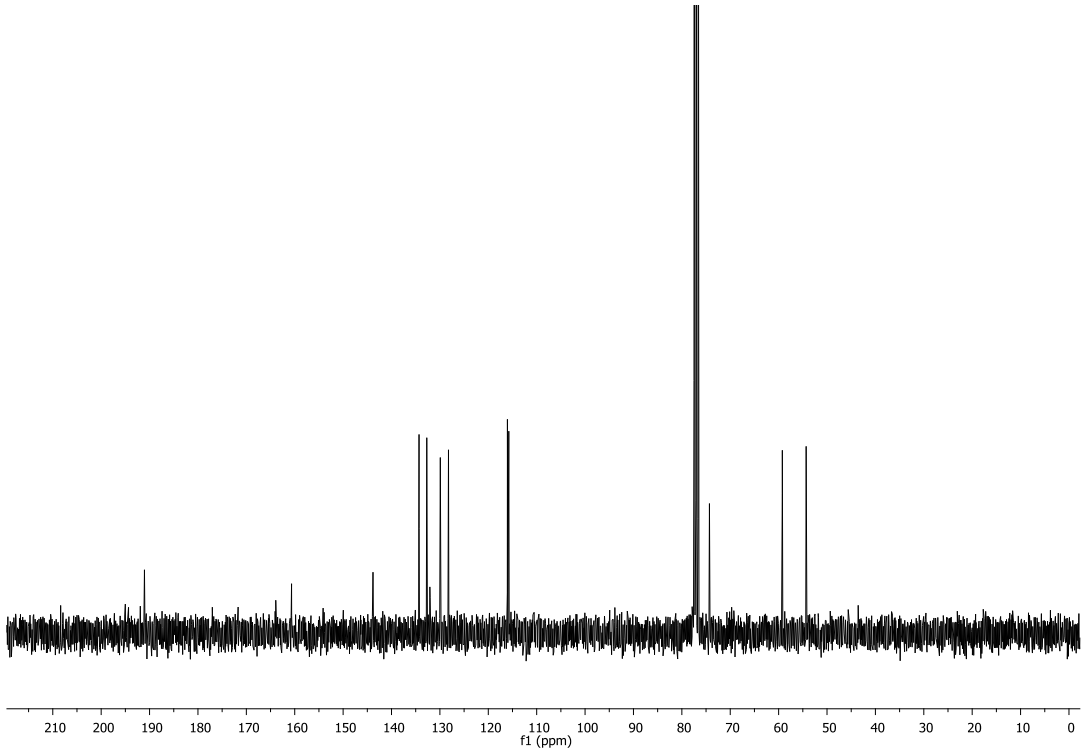
^{1}H and ^{13}C NMR spectra of selected compounds

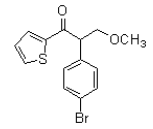
^{1}H and ^{13}C NMR spectra for 2-(4-fluorophenyl)-3-methoxy-1-(thiophen-2-yl)propan-1-one **(2b)**

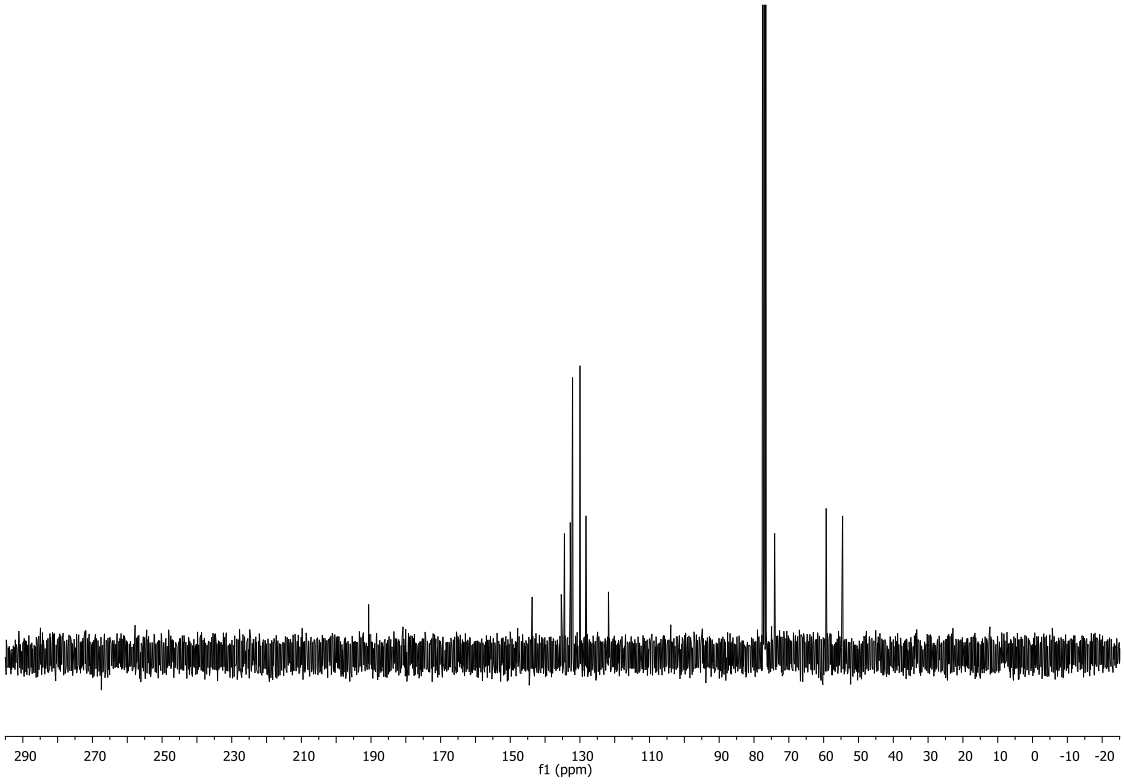
(300MHz, $CDCl_3$)



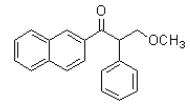


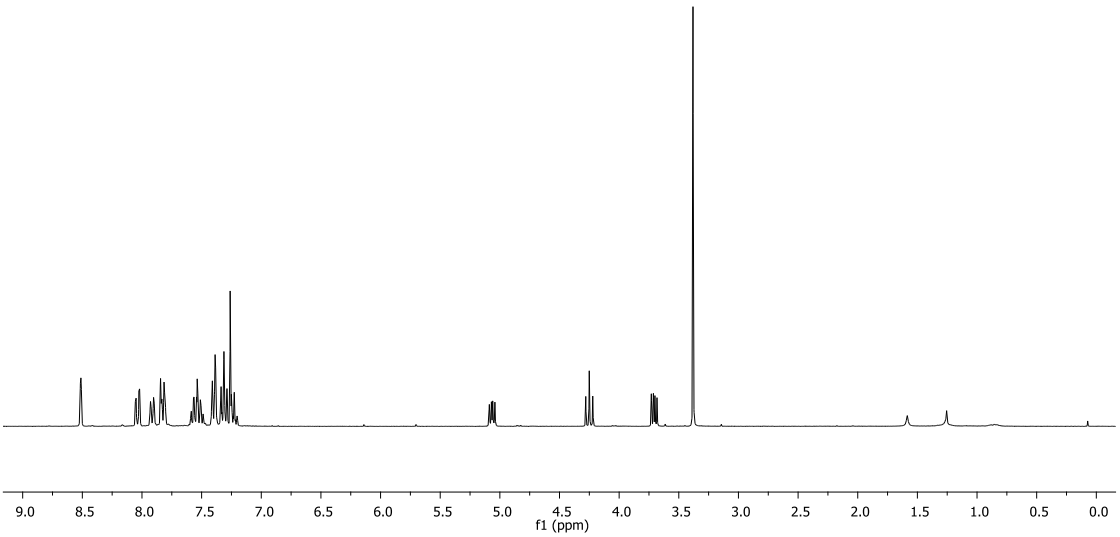
^{1}H and ^{13}C NMR spectra for 2-(4-bromophenyl)-3-methoxy-1-(thiophen-2-yl)propan-1-one **(2c)** (300MHz, $CDCl_3$)

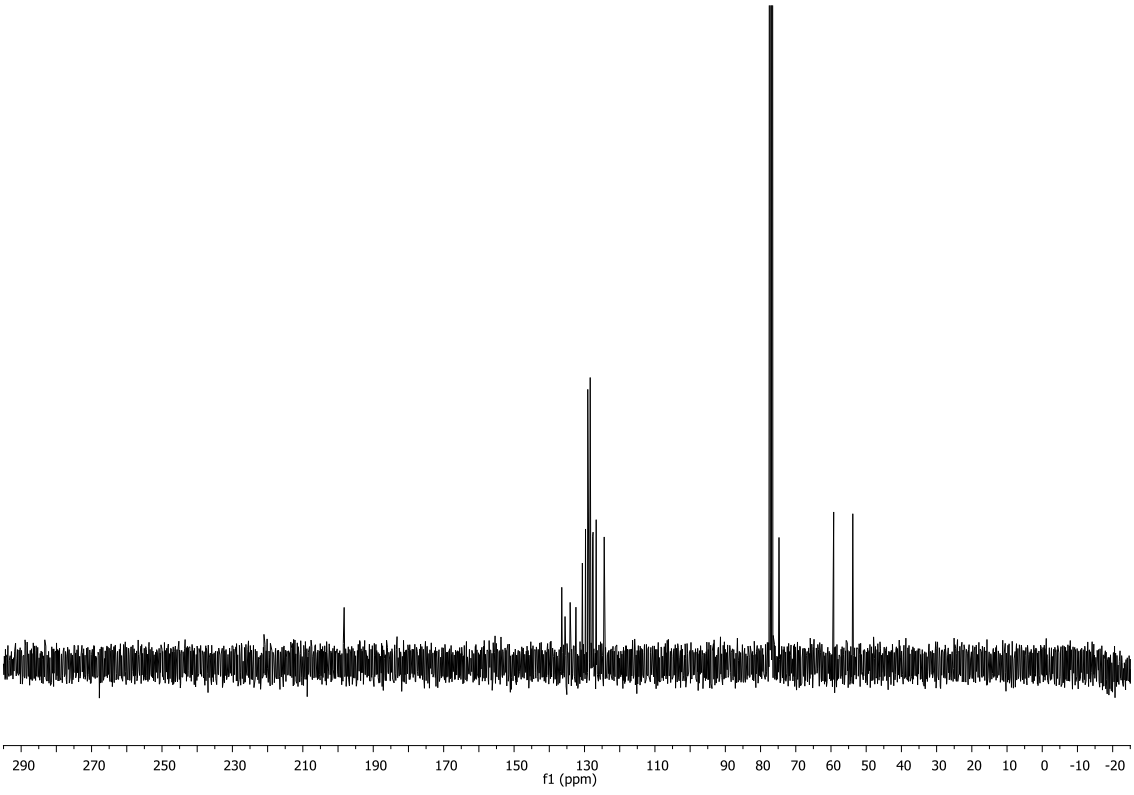




^{1}H and ^{13}C NMR spectra for 3-methoxy-1-(naphthalen-2-yl)-2-phenylpropan-1-one **(2n)**
(300MHz, $CDCl_3$)







Abbreviation

ABCB1	ATP-binding cassette sub-family B member 1	MS	Mass spectrometry
ABCG2	ATP-binding cassette sub-family G member 2	equiv	Equivalent
ABCC1	ATP-binding cassette, sub-family C member 1	ES	Electrospray
CDCl3	Deuterated chloroform	ESI	Electrospray ionization
DCM	Dichloromethane	Et_2O	Diethyl ether
DMF	Dimethylformamide	EtOAc	Ethyl acetate
DMSO	Dimethyl sulfoxide	EtOH	Ethanol
DMSO-d6	Deuterated dimethyl sulfoxide	eV	Electron volts
GC	Gas chromatography	MeOD	Deuterated methanol
HR-MS	High resolution mass spectrometry	MeOH	Methanol
ISC	Inter system crossing	MHz	Mega hertz
min	Minute	Mp	Melting point
mL	Milli liter	NMR	Nuclear magnetic resonance
mmol	Milli mole	PE	petroleum ether
ppm	Parts per million	SCE	Saturated calomel electrode
TEMPO	(2,2,6,6-Tetramethyl-piperidin-1-yl)oxyl	TLC	Thin layer chromatography
TMS	Tetramethylsilane	UV	Ultra violet
PEG	Polyethylene glycol	DIPEA	Diisopropylethylamin
conc.	concentrated	Pd/C	Palladium on charcoal

r.t	Room temperature	THF	Tetrahydrofurane
SEM	Standard error of the mean	TFA	Trifluroacetic acid

Summary

The first part of the thesis (chapter 1 and 2) deals with natural or natural-like phenols with anti-angiogenic property. Chapter 1 reviews recent reports of anti-angiogenic natural phenolic compounds specifically addressing their chemistry, synthesis and possible structure modifications. Thirteen representatives of eight different natural or natural-like phenolic subclasses with significant anti-angiogenic activity are presented. Whenever available, structure-activity relationship is also discussed.

In chapter 2, we describe a synthetic approach for natural and natural-like acylphloroglucinols with anti-proliferative, anti-oxidative and tube-formation inhibitory activity. Two series of mono- and bicyclic acylphloroglucinols derived from secondary metabolites in the genus *Hypericum* (Hypericaceae) were synthesised and tested *in vitro* for anti-proliferative and tube-formation inhibitory activity in human microvascular endothelial cells (HMEC-1). In addition, their anti-oxidative activity was determined via an ORAC-assay. Our experiments show simpler acylphloroglucinols containing simpler substitution patterns than hyperforin can show good anti-proliferative effects and remarkable tube formation inhibition.

The second part of the thesis (chapter 3 and 4) reports the synthesis and bioactive evaluation of compounds aiming to selectively inhibit ABCC1 and ABCG2 transporter, respectively. In chapter 3, a series of flavonoids were synthesised and characterized in cellular assays for modulation of the ABC transporters-ABCC1 (MDCKII-MRP1 cells), ABCB1 (Kb-V1 cells) and ABCG2 (MCF-7/Topo cells). The most potent ABCC1 modulators identified among these flavonoid-type compounds were comparable to reversan regarding their potency, but superior in terms of selectivity over ABCB1 and ABCG2.

In chapter 4, we report synthesis of quinoline analogues targeting breast cancer resistance protein derived from tariquidar. All tested compounds show weak or no inhibitory activity over ABCB1 and ABCC1 transporter but good inhibitory activity towards ABCG2. However, compared with our reference indole compounds, the replacement of anilide core by quinoline moiety gave less potent compounds. The introduction of amine groups on the tetrahydroisoquinoline moiety can increase the water solubility to some extent, but the poor water solubility is still the main problem for this series of compounds.

In chapter 5, photo-rearrangement and methanol addition of aryl chalcones to 1-propanones in the presence of triphenylphosphine is reported. We propose two possible mechanistic hypotheses for the rearrangement/addition reaction. To further demonstrate the applicability of the reaction, we applied it to synthesis of 2-substituted terminal enones.

Zusammenfassung

Der erste Teil der vorliegenden Arbeit (Kapitel 1 und 2) beschäftigt sich mit natürlichen oder naturstoffverwandten Phenolen mit antiangiogenetischen Eigenschaften. Kapitel 1 gibt einen Überblick über phenolgruppenenthaltende Naturstoffe mit Angiogenese hemmender Wirkung, dabei wird auf ihre Chemie, die Synthese sowie mögliche strukturelle Modifikationen eingegangen. Dreizehn Vertreter von acht verschiedenen Unterklassen natürlicher oder von natürlichen Strukturen abgeleitete Phenole mit hoher antiangiogenetischer Aktivität werden vorgestellt und falls möglich anhand ihrer Struktur-Wirkungsbeziehung diskutiert.

In Kapitel 2 beschreiben wir eine Syntheseroute für natürliche und naturstoffverwandte Acylphloroglucine mit antiproliferativer, antioxidativer und „Tube Formation“ hemmender Aktivität. Zwei Serien von natürlichen und naturverwandten mono und bizyklischen Acylphloroglucinen, abgeleitet von Sekundärmetaboliten der Gattung Hypericum (Hypericaceae), wurden synthetisiert und *in vitro* auf ihre antiproliferative Wirkung und ihre Angiogenesehemmung (Tube Formation Assay) in humanen mikrovaskulären Endothelzellen (HMEC-1) getestet. Zusätzlich wurde ihre antioxidative Wirkung mittels eines ORAC Assays bestimmt. Unsere Experimente zeigen, dass einfachere Acylphloroglucine mit einfacherem Substitutionsmuster als Hyperforin gute antiproliferative Wirkung und bemerkenswerte Angiogenesehemmung zeigen können.

Der zweite Teil der Arbeit (Kapitel 3 und 4) beschäftigt sich mit der Synthese von Wirkstoffen zur selektiven Inhibierung des ABCC1 beziehungsweise ABCG2 Transporters, sowie der Bewertung ihrer biologischen Aktivität. In Kapitel 3 wird die Synthese einer Reihe von Flavonoiden dargestellt und mittels zellbasierter Assays auf ihre Wirkung auf die ABC Transporter ABCC1 (MDCKII-MRP1 Zellen), ABCB1 (Kb-V1 Zellen and ABCG2 (MCF-7/Topo Zellen) untersucht. Die wirksamsten ABCC1 Inhibitoren der Serie zeigen eine vergleichbare Aktivität wie Reversan, sind diesem jedoch in Hinblick auf die Selektivität gegenüber der verwandet Transporter ABCB1 und ABCG2 überlegen.

In Kapitel 4 wird die Synthese eines von Tariquidar abgeleiteten Chinolinderivates zur Inhibierung des Breast Cancer Resistance Proteins beschrieben. Sämtliche untersuchte Substanzen zeigten keine oder nur sehr schwache Hemmung der ABCB1 und ABCC1 Transporter, besaßen jedoch eine hohe Aktivität gegenüber ABCG2. Allerding zeigte sich im

Vergleich zu den Indolverbindungen, dass der Austausch der Anilideinheit durch ein Chinolin zu einer verringerten Aktivität der Verbindungen führte. Durch Einführen einer Aminogruppe am Tetrahydroisochinolin kann die Wasserlöslichkeit der Verbindung geringfügig verbessert werden, jedoch bleibt die geringe Wasserlöslichkeit dieser Substanzklasse nach wie vor problematisch.

Kapitel 5 beschreibt die photochemische Umlagerung von Arylchalkonen zu 1-Propanonen in der Gegenwart von Triphenylphosphin und unter Angriff von Methanol. Für den Mechanismus dieser Umlagerungs-Additions-Reaktion werden zwei unterschiedliche Hypothesen aufgestellt und erläutert. Um den synthetischen Nutzen dieser neu entdeckten Reaktion aufzuzeigen, haben wir sie auf die Darstellung eines 2-substituierten Enones angewendet.

Curriculum Vitae

Personal data

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Date of birth: September.29th.1985

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University education

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Ph.D. in Medicinal Chemistry

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Research area:

- Syntheses of natural-like acylphloroglucinols for pharmacological testing
- Syntheses of ABC modulators
- PPh_3-mediated photo rearrangement of chalcone analogues

09/2008-07/2011

Master of Science in Medicinal Chemistry

West China School of Pharmacy, Sichuan University, China

Advisor: Prof.Dr.Taiping Hou

Master thesis:

- Synthesis, bioactive and molecular docking study on neonicotinoid analogues
- Iron trichloride-assisted selective synthesis of vascular protective agent probucol monosuccinate

09/2004-07/2008

Bachlor of Science in Pharmacy

West China School of Pharmacy, Sichuan University, China

Advisor: Prof.Dr.Taiping Hou

Bachlor thesis:

- Anti-fungal study on metabolites isolated from *Ligularia Virgaurea*

Skills

- Detailed knowledge of spectroscopical analytics in theory and practice (NMR, IR, UV-Vis and MS), chromatographic methods and cyclic voltammetry
- Good knowledge of chemistry software, MS Office and Endnote

Teaching experience

- Supervised one undergraduate and four master students during their research projects at the University of Regensburg
- Assisted to supervise master students lab course in 2012

Publication

- **Qiu Sun**, Sebastian Schmidt, Martina Tremmel, Jörg Heilmann, Burkhard König. Synthesis of Natural-like Acylphloroglucinols with Anti-proliferative, Anti-oxidative and Tube-formation Inhibitory Activity, *Eur. J. Med. Chem*, 2014, 85, 621-628.
- **Qiu Sun**, Jörg Heilmann, Burkhard König. Natural phenolic metabolites with anti-angiogenic properties – a review from the chemical point of view. *Beilstein J. Org. Chem*, 2015, 11, 249-264.
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- Jose Obreque-Balboa, **Qiu Sun,** Bernhardt Günther, Burkhard König and Armin Buschauer.Synthesis and biological evaluation of flavonoid derivatives as novel and highly selective ABCC1 modulators. (joint first author, manuscript in preparation)
- Chang-jiang Yao, **Qiu Sun**, Rastogi Namrata, Burkhard König. Intermolecular Formyloxy-Arylation of Alkenes by Photoredox Meerwein Reaction. *ACS Catal.* 2015. **DOI:** 10.1021/acscatal.5b00314.
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- Tai-ping Hou, Yun Teng, **Qiu Sun**, Zhi-yi Yu. A New Fungitoxic Metabolite from *Spiraea alpina Pall.* Fitoterapia, 2009, 80, 237-240.

Conference

- 6th Summer School Medicinal Chemistry, University of Regensburg, September 26-28, 2012
- Advances in Drug Discovery – Chemistry and Biology, Prague, Czech Republic, September 1-5, 2014

- 7th Summer School Medicinal Chemistry, University of Regensburg, September 17-19, 2014

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