Graphical Abstract

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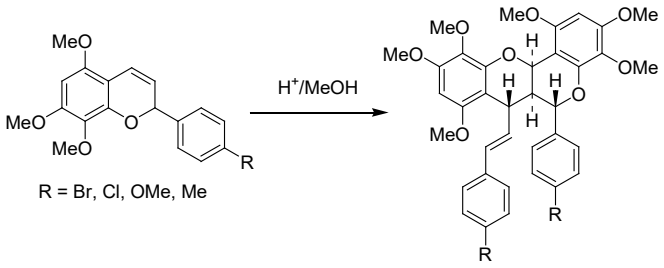
Ruth Devakaram[a], David StC Black[a], Katherine T. Andrews[b,c], Gillian M. Fisher[b,c], Rohan A. Davis[b] and Naresh Kumar[a*]

[a] *School of Chemistry, The University of New South Wales, UNSW, Sydney, NSW 2052, Australia*
[b] *Eskitis Institute for Cell and Molecular Therapies, Griffith University, Queensland 4111, Australia*
[c] *Queensland Institute of Medical Research, Tropical Parasitology Laboratory, 300 Herston Road, Herston, Brisbane 4006, Australia*



Synthesis and antimalarial evaluation of novel benzopyrano[4,3-*b*]benzopyran derivatives

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Dependensin
Dimerization
Flavenes
Acid-catalyzed
Benzopyrans

ABSTRACT

7-Methoxyflavenes and 5,7,8-trimethoxyflavenes were found to undergo stereoselective acid-catalyzed rearrangement to generate the benzopyrano[4,3-*b*]benzopyran ring system present in the natural product, dependensin. Dependensin and its analogues were subjected to antimalarial growth inhibition assays against *Plasmodium falciparum* and found to have IC_{50} values ranging between 1.9 and 3.9 μM.

1. Introduction

Flavonoids are a group of heterocyclic polyphenolic compounds widely distributed in the plant kingdom. They occur naturally in fruits, vegetables, nuts, seeds and flowers and form a significant part of our daily diet.[1] Flavonoids are known to display a diverse range of biological and pharmacological activities.[1] For example, baicalein, oroxylin A and wogonin, the three major flavonoids isolated from *Scutellaria baicalensis*, a traditional Chinese herb are well noted for their broad spectrum of biological activities, in particular for their antioxidant properties.[2]

Biflavonoids are comprised of two identical or non-identical flavonoid units, joined symmetrically or unsymmetrically through linkers of varying lengths. The large number of possibilities in the length, position and type of linkages, as well as in the number and nature of substituents, gives rise to an enormous structural diversity to the biflavonoids class.[3] Biflavonoids have received increasing recognition due to their wide spectrum of pharmacological properties, including anticancer, antiinflammatory, antimicrobial, antiviral and anticlotting activities.[3]

Despite their promising biological activities, the development of biflavonoids as therapeutic agents has been hampered by their low abundance in nature, tedious extraction and purification procedures, and limited biological data of the biflavonoids class. Therefore, it is highly desirable to develop efficient synthetic methodologies, which could generate not only the natural products themselves but also their synthetic analogues for pharmacological applications.

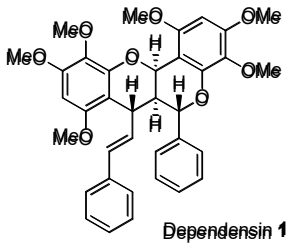
Dependensin **1** is a dimeric flavonoid isolated from the root bark of a Tanzanian medicinal plant, *Uvaria dependens*. The crude extract of this plant shows potent anti-malarial activity.[4] It is well-known that malaria constitutes a major health problem in tropical and sub-tropical regions of the world, with 200 to 350 million cases annually and mortality reaching 1 million, particularly among children in sub-Saharan Africa.[5] Malaria is caused by protozoan parasites of the phylum Apicomplexa, which are transmitted by bloodfeeding Anopheline mosquitoes. There are four main human malaria parasites, i.e. *Plasmodium ovale*, *P. malaria*, *P. vivax* and *P. falciparum*, with the latter two being responsible for the majority of malaria cases worldwide. *P. falciparum*, the most virulent of the four main species infecting humans, has become resistant to nearly all currently employed

* Corresponding author. Tel.: +61 2 9385 4698; fax: +61 2 9385 6141.
E-mail address: n.kumar@unsw.edu.au (N. Kumar).

antimalarial drugs used for prophylaxis and treatment.[6] Even artemisinin combination therapy (ACT), the current first treatment for drug-resistant *P. falciparum* malaria is now under threat.[7]

First cases of resistance against *P. falciparum* were reported in the 1970s against the popular antimalarials chloroquine and sufadoxine-pyrimethamine, barely 10 years after these drugs were introduced for the treatment of malaria. Chloroquine has also been the therapy of choice for the treatment of blood stage vivax malaria, but chloroquine resistance in *P. vivax* has developed since the nineties of the last century. Besides the increasing drug resistance in plasmodia strains world-wide, an effective drug employment is hampered by high costs and major side effects of other antimalarials like mefloquine or atovaquone.[6]

These developments emphasize the need for new strategies in combating the tropical disease. Hence, our research group is addressing the need to develop new drugs. Here, we present data on the development of efficient synthetic methodologies to dependensin and its analogues, as potential new lead antimalarial compounds.



Dependensin **1**

The unique heterocyclic ring system present in dependensin contains a dense array of functionality and stereochemistry, which includes two-fused benzopyran rings, four stereocentres and one *trans* double bond.[4]

Nkunya *et al.* proposed that the natural product, dependensin originated from the acid-catalyzed reaction of the corresponding flavene, 5,7,8-trimethoxyflav-3-ene, but were unable to verify it experimentally, obtaining a ring-opened compound instead.[4] However, the successful synthesis of dependensin *via* the acid-catalyzed reaction of 5,7,8-trimethoxyflavene has been recently reported by our research group.[7] We have also previously shown that the benzopyrano[4,3-*b*]benzopyran ring system present in dependensin could be synthesized *via* the acid-catalyzed dimerization reaction of 4′,7-diacetoxyflav-3-ene.[8] Similar acid-catalyzed reactions of the corresponding 4′,5-diacetoxy- and 4′,6-diacetoxyflavenes produced a complex mixture of products.[8]

Encouraged by these results, we targeted the acid-catalyzed reactions of 7-methoxyflavenes and 5,7,8-trimethoxyflavenes to generate novel dependensin analogues for biological applications. Interestingly, we found that the dimerization products obtained were dependent on the position of the methoxy group in the flavene nucleus. We have also recently reported the synthesis of biflavonoids containing a new ring system, a tetrahydrochromeno[2,3-*b*]chromene, generated *via* the acid-catalyzed reactions of 5-methoxy- and 6-methoxyflavenes.[9]

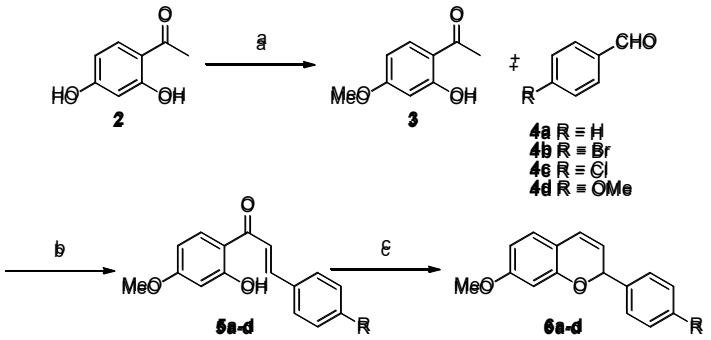
We report herein the facile one step methodology to the synthesis of a series of highly functionalized benzopyrano[4,3-*b*]benzopyrans from the acid-catalyzed reactions of 7-methoxyflavenes and 5,7,8-trimethoxyflavenes along with their antimalarial inhibition assay results.

2. Results and Discussion

2.1. Synthesis of 7-methoxyflavenes

Flavenes are direct precursors to the dimeric flavonoids and therefore, an efficient route to their synthesis was considered highly desirable for this study. The base catalyzed condensation of hydroxyacetophenones with aldehydes to generate chalcones has been commonly employed,[10] which has been followed by cyclization in the presence of strong acids to furnish flavanones in typically low yields.[10] Alternatively, intramolecular oxidative cyclization of chalcones with I_2/DMSO could yield the corresponding flavones in good yields.[11] However, in both cases, a further reduction of the flavones/flavanones to the corresponding flavanols followed by subsequent dehydration was required to yield the desired flavenes.[12]

Clark-Lewis and Jemison report the direct conversion of 2'-hydroxychalcones into the corresponding flav-3-enes by reduction with $NaBH_4$ in IPA.[13] This method of reductive cyclization could generate the desired flavenes in fewer steps as well as generate improved overall yields.



Scheme 1. Reagents and conditions: (a) CH_3I, K_2CO_3, acetone, reflux, 24 h, 92%; (b) NaOH, ethanol, 12 h, rt, 65-73%; (c) $NaBH_4$, IPA, 12 h, rt, 50-58%.

Hence, the hydroxy group in the commercially available 2′,4′-dihydroxyacetophenone **2** was masked by simple methylation by treatment with anhydrous K_2CO_3 and CH_3I in dry acetone to give 2′-hydroxy-4′-methoxyacetophenone **3** in 92% yield. Acetophenone **3** was condensed with various *para*-substituted aryl aldehydes **4a-d** in ethanolic NaOH solution to afford the corresponding chalcones **5a-d** in 65–73% yields. Finally, reductive cyclization of the chalcones using $NaBH_4$ in IPA as solvent yielded the desired flavenes **6a-d** in 50–58% yields (Scheme 1).

The ^{1}H NMR spectrum of 4′-bromo-7-methoxyflavene **6b** exhibited three doublets of doublets at δ 5.61 (J = 3.4, 9.8 Hz, 1H), δ 5.83 (J = 1.5, 3.4 Hz, 1H) and δ 6.50 (J = 1.5, 9.8 Hz, 1H) corresponding to H3, H2 and H4 respectively. The ^{1}H NMR spectra of the other flavenes were found to exhibit similar coupling patterns in ring A. Furthermore, the DEPT-135 and the broadband decoupled ^{13}C NMR spectra of flavenes **6a-d** indicated the absence of methylene (CH_2) groups, confirming that the flav-2-ene was not formed. The structure of 4′-bromo-7-methoxyflavene **6b** was confirmed by X-ray crystallography (Figure 1).[14]

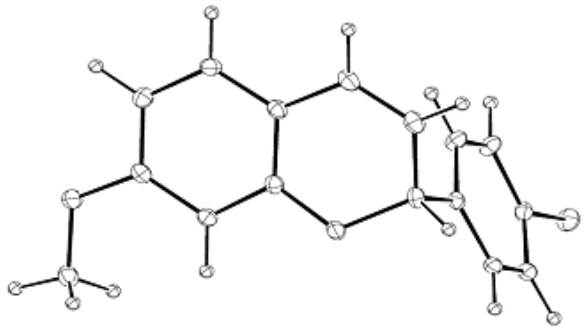
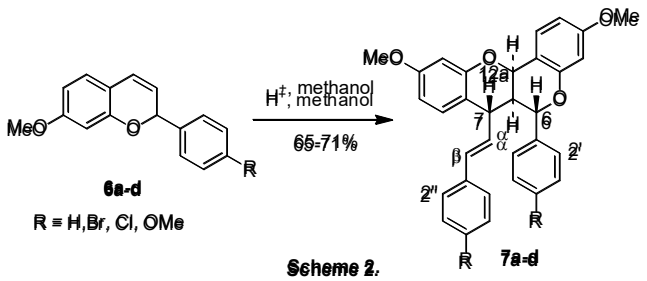


Figure 1. ORTEP diagram of compound **6b**.

2.1.1. Acid-catalyzed reactions of 7-methoxyflavenes

The 7-methoxyflavenes **6a-d** were subjected to the acid-catalyzed dimerization reaction, using HCl, TFA or glacial AcOH as catalysts in MeOH, giving bisflavonoids **7a-d** as analogues of the natural product dependensin (Scheme 2). The dimerization products obtained were the same irrespective of the catalyst utilized for these reactions. A similar acid-catalyzed dimerization reaction of 4′,7-diacetoxyflavene has been reported previously.[8]



Scheme 2.

These dimers **7a-d** contain four tertiary aliphatic protons, two of which are attached to oxygenated carbon atoms. Thus, the identification of protons H6, H7, H6a and H12a were of prime importance in assigning the structure of the product. In the dimeric flavonoid **7b**, a doublet of doublet of doublets at δ 2.43 (J = 2.1, 2.3, 10.9 Hz, 1H) correlated to H6a, a doublet of doublets at δ 3.13 (J = 2.1, 6.4 Hz, 1H) correlated to H7, and two doublets at δ 5.03 (J = 10.9 Hz, 1H) and δ 5.05 (J = 2.3 Hz, 1H) correlated to H6 and H12a respectively. The protons present on the *trans* double bond, represented as H_β and H_α, were identified as a doublet at δ 5.99 (J = 15.8 Hz, 1H) and a doublet of doublets at δ 6.22 (J = 6.4, 15.8 Hz, 1H) respectively. The dimeric flavonoids were obtained in moderate yields of 65–71% (Table 1).

Table 1. Dimerization products of 7-methoxyflavenes

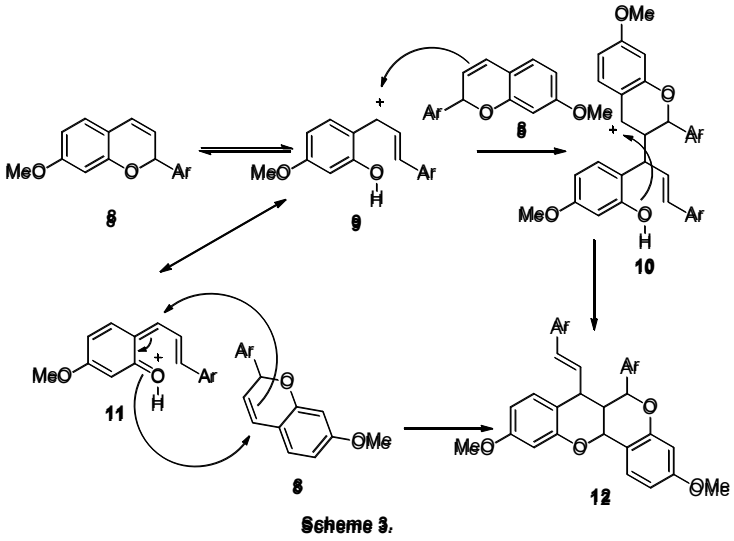
Flavene[a]	R	Benzopyrano benzopyran	Yields [%][b]
6a	H	**7a**	65
6b	Br	**7b**	71
6c	Cl	**7c**	69
6d	OMe	**7d**	66

[a] Catalysed by HCl

[b] Yields of isolated pure product

We have previously proposed a rationale for the observed acid-catalyzed dimerization reaction.[8] It is assumed that the flavene **8** protonates and ring opens resulting in the formation of a stable benzylic carbocation **9**. A second molecule of flavene **8** could possibly attack this benzylic carbocation **9** in such a way as to generate a second stable benzylic carbonium ion **10**, which upon cyclization, by loss of a proton yields the dimeric system **12** (Scheme 3).

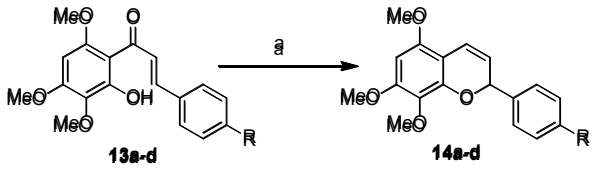
Alternatively, the low stability of flav-3-enes **8** containing the 2H-pyran ring may facilitate a Claisen-type rearrangement to occur, leading to the formation of reactive orthoquinone methide **11**. A Diels-Alder reaction of the intermediate orthoquinone methide **11** with the second molecule of flavene **8**, followed by ring closure could also possibly result in the formation of the dimer **12** (Scheme 3).



Scheme 3.

2.2. Synthesis of 5,7,8-trimethoxyflavenes

The synthesis of 5,7,8-trimethoxyflav-3-enes **14a-d** was undertaken with the aim of producing direct analogues of the natural product, dependensin. The 5,7,8-trimethoxyflavenes **14a-d** were synthesized from the corresponding chalcones **13a-d** in 56–63% yields by the method of reductive cyclization in a similar fashion as before (Scheme 4).



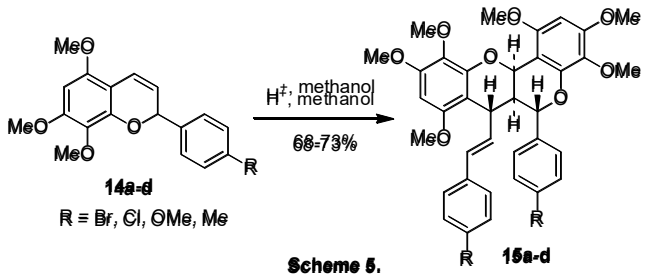
Scheme 4.

The chalcones **13a-d** were synthesized by base catalyzed Claisen-Schmidt condensation of 2′-hydroxy-3′,4′,6′-trimethoxyacetophenone with *para*-substituted benzaldehydes, **4b-e** (**4e** R = Me).

2.2.1. Acid-catalyzed reactions of 5,7,8-trimethoxyflavenes

The 5,7,8-trimethoxyflavenes were then subjected to acid-catalyzed reactions in MeOH as before, which furnished direct analogues of the natural product **15a-d** as desired (Scheme 5). The optimum yields were obtained with the use of TFA as

catalyst and it was observed that use of glacial acetic acid as catalyst resulted in longer reaction times.



Scheme 5.

The ^{1}H NMR spectra of these dimers were found to have coupling patterns similar to those resulting from the dimerization of 7-methoxyflavenes. The dimeric flavonoids were obtained in good yields of 68–73% (Table 2).

Table 2. Dimerization products of 5,7,8-trimethoxyflavenes

Flavene[a]	R	Benzopyrano benzopyran	Yields [%][b]
14a	Br	**15a**	68
14b	Cl	**15b**	70
14c	OMe	**15c**	71
14d	Me	**15d**	73

[a] Catalysed by TFA

[b]Yields of isolated pure product

The X-ray crystal structure[15] of compound **15c** confirmed the stereochemistry of the product, which was found to be identical to that of the parent dependensin as reported in the literature.[3]

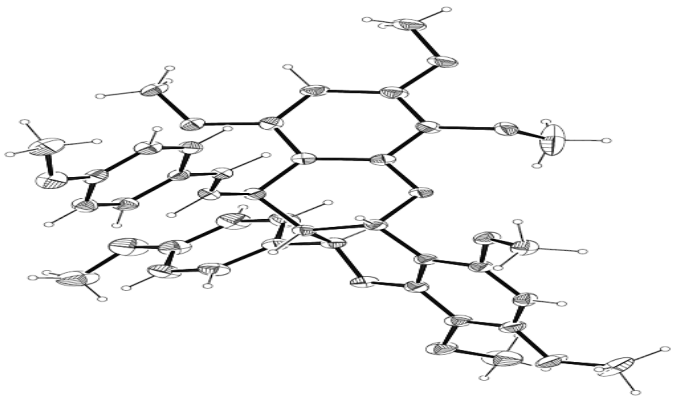


Figure 3. ORTEP diagram of compound **15c.**

Importantly, the acid-catalyzed reactions of 7-methoxyflavenes and 5,7,8-trimethoxyflavenes can give rise to different stereoisomers, owing to the presence of four stereocentres in their structures. Interestingly, we observed the formation of a single isomer in all cases, evident from the coupling constants of the aliphatic protons. Hence, these acid-catalyzed reactions were highly stereoselective; one of the reasons may be attributed to the exceptionally high stability of the carbocation intermediates generated during the course of their rearrangement.

The identity and purity (>98%) of all compounds were confirmed by ^{1}H and ^{13}C NMR spectroscopy (1D and 2D NMR spectroscopy), IR spectroscopy, mass spectrometry, thin layer chromatography and elemental analyses.

2.3. Biological activity of dependensin analogues

This paper is the first report of antimalarial activity for the natural product, dependensin **1** and it analogues **15a-d**. These novel benzopyrano[4,3-*b*]benzopyran derivatives were screened for antimalarial activity against a chloroquine sensitive *P. falciparum* line (3D7) using standard *in vitro* growth inhibition assays. The antimalarial drug, chloroquine was used as the positive growth inhibition control. Table 3 gives the IC_{50} values of the tested compounds.

Table 3. Antimalarial activities for compound **1**, **15a-d**

compound	*P. falciparum* 3D7 IC_{50} (±SD) (μM)	NFF[a] cytotoxicity % Inhibition (±SD) at 50μM
1	3.9 (±1.5)	46.9 (±0.3)
15a	2.9 (±1.7)	46.3 (±5.4)
15b	3.3 (±1.2)	98.4 (±0.1)
15c	3.2 (±0.6)	43.2 (±3.8)
15d	1.9 (±0.50	47.2 (±0.6)
chloroquine	0.02 (±0.01)	nd

[a]NFF, neonatal foreskin fibroblast cells; nd, not determined

Among the compounds screened for antimalarial activity, compound **15d**, which bears methyl substituent at C4′ and C4′′, was found to be the most active with an IC_{50} value of 1.9 μM. However, all analogues showed similar antimalarial activity thus identifying that C4′ and C4′′ substituents have minimal effect on the ability of these molecules to inhibit malaria parasite growth. Likewise, all compounds showed similar cytotoxicity against NFF cells which are commonly used as a normal cell control (Table 3). All compounds, except **15b**, have around 10 fold selectivity for malaria parasites versus the NFF cells. The synthesis of further dimeric analogues that incorporate substituent variation at other positions around the tetracyclic dependensin skeleton are required in order to determine whether this structure class has the potential to provide antimalarial lead molecules.

3. Conclusion

A facile simple one-step methodology to the synthesis of a series of novel benzopyrano[4,3-*b*]benzopyrans has been developed *via* the acid-catalyzed dimerization reactions of 7-methoxyflavenes and 5,7,8-trimethoxyflavenes. Moderate antimalarial activity coupled with synthetic tractability of several synthetic bisflavonoids suggests that this structure class may warrant further investigation as potential antimalarial agents.

4. Experimental Section

4.1. Materials and methods

All reagents and solvents were obtained from commercial sources and purified if necessary. Melting points were measured using a Mel-Temp melting point apparatus and are uncorrected. Microanalyses were performed on a Carlo Erba Elemental Analyser EA 1108 at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. ^{1}H and ^{13}C NMR spectra were obtained on Bruker DPX300 (300 MHz). Mass spectra were recorded on either a Bruker FT-ICR MS (EI) or a Micromass ZQ2000 (ESI). Infrared spectra were recorded with a Thermo Nicolet 370 FTIR Spectrometer using KBr discs. Column chromatography was carried out using Merck 230–400 mesh ASTM silica gel, and preparative thin layer chromatography was performed using Merck silica gel 7730 60GF^{254}.

4.1.1. 2′-Hydroxy-4′-methoxyacetophenone (3):

To a solution of 2′,4′-dihydroxyacetophenone **2** (5.0 g, 32.86 mmol) in acetone (75 mL), was added K_2CO_3 (9.1 g, 65.72 mmol). The reaction mixture was cooled to 0 °C and MeI (2.1 mL, 32.86 mmol) was added to it slowly. The reaction mixture was then refluxed for 24 h. The solvent was evaporated, and the residue was acidified using 2M HCl to pH 3. The mixture was extracted with EtOAc (3 x 100 mL), washed with brine (100 mL), dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was recrystallized from EtOAc/light petroleum (2:8) to afford the title compound as off-white crystals (5.0 g, 92%). M.p. 53-55 °C, *lit*.[16] 52-54 °C.

4.1.2. General procedure for the synthesis of chalcones (5a-d):

To a solution of the appropriate acetophenone **3** (1.0 equiv) in EtOH (50 mL) was added the corresponding aldehydes **4a-d** (1.0 equiv). This was followed by slow addition of crushed NaOH pellets (2.5 equiv). The reaction mixture was stirred for 12 h at r.t., poured into ice (250 g) and acidified using conc. HCl to pH 3. The solid so obtained was filtered and air-dried. Recrystallization from EtOH afforded the desired chalcones as yellow/orange crystals.

In cases when the chalcones did not precipitate out on acidification, the aqueous layer was extracted with DCM (3 x 100 mL). The organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue so obtained was then recrystallized from EtOH.

4.1.2.1. 2′-Hydroxy-4′-methoxychalcone (5a):

Yellow crystals, Yield: 72%; M.p. 107-108 °C, *lit*.[17] 104–105 °C.

4.1.2.2. 4-Bromo-2′-hydroxy-4′-methoxychalcone (5b):

Yellow crystals, Yield: 65%; M.p. 138–140 °C, *lit*.[18] 138–140 °C.

4.1.2.3. 4-Chloro-2′-hydroxy-4′-methoxychalcone (5c):

Yellow crystals, Yield: 73%; M.p. 100–102 °C, *lit*.[19] 109–110 °C.

4.1.2.4. 2′-Hydroxy-4,4′-dimethoxychalcone (5d):

Yellow crystals, Yield: 71%; M.p. 114–116 °C, *lit*.[20] 116–118 °C.

4.1.3. General procedure for the synthesis of flavenes (6a-d, 14a-d):

To a solution of the appropriate chalcone (1.0 equiv) in IPA (30 mL) at 50 °C was slowly added $NaBH_4$ (3.0 equiv) in small portions over 15 minutes. The reaction mixture was cooled to r.t. and left to stir overnight. The solvent was evaporated partially, ice (50 g) was added and the resulting solution was acidified using 10% AcOH to pH 5. The solution was extracted with DCM (2 x 150 mL), and the organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Purification of the residue by column chromatography over silica gel using DCM/light petroleum (20:80) eluted the desired flavene and further elution using DCM/light petroleum (40:60) gave the unreacted chalcone. The flavenes so obtained were either low melting white solids or yellow sticky oily residues. As the flavenes were found to be relatively unstable, they were immediately used in the next subsequent step of dimerization. Otherwise, they were dissolved in MeOH and stored at room temperature to avoid decomposition.

4.1.3.1. 7-Methoxyflav-3-ene (6a):

White solid, Yield: 58%; M.p. 103–105 °C, *lit*.[21] 102–103 °C.

4.1.3.2. 4′-Bromo-7-methoxyflav-3-ene (6b):

Yellow sticky residue, Yield: 53%; UV (MeOH): λ_{max} 202 (ε 46842 $cm^{-1}M^{-1}$), 230 (50861), 306 (11829) nm; IR (KBr): ν_{max} 3448, 3022, 2975, 2934, 2843, 1614, 1503, 1444, 1308, 1275, 1198, 1154, 1110, 1026, 968, 812, 708 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 3H, CH_3O), 5.61 (dd, *J* = 3.4, 9.8 Hz, 1H, H3), 5.83 (dd, *J* = 1.5, 3.4 Hz, 1H, H2), 6.37 (d, *J* = 2.3 Hz, 1H, H8), 6.43 (dd, *J* = 2.3, 8.3 Hz, 1H, H6), 6.50 (dd, *J* = 1.5, 9.8 Hz, 1H, H4), 6.92 (d, *J* = 8.3 Hz, 1H, H5), 7.32 (d, *J* = 8.7 Hz, 2H, H2′, H6′), 7.49 (d, *J* = 8.7 Hz, 2H, H3′, H5′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 55.2 (CH_3O), 76.4 (C2), 101.8 (C8), 107.1 (C6), 114.4 (C4a), 121.3 (C4), 122.3 (C4′), 124.0 (C3), 127.3 (C5), 128.7 (C2′, C6′), 131.7 (C3′, C5′), 139.8 (C1′), 154.0 (C8a), 160.9 (C7); (TOF-ESI) *m/z* Calcd. for $C_{16}H_{13}BrO_2Na$ $(M + Na)^+$ 339.00 (Br^{79}). Found 339.04 (Br^{79}); Anal. Calcd. for $C_{16}H_{13}BrO_2$: C, 60.59; H, 4.13. Found: C, 60.87; H, 4.35.

4.1.3.3. 4′-Chloro-7-methoxyflav-3-ene (6c):

White solid, Yield: 50%; M.p. 76-78 °C; UV (MeOH): λ_{max} 203 (ε 26199 $cm^{-1}M^{-1}$), 230 (27838), 306 (6703) nm; IR (KBr): ν_{max} 3442, 3022, 2978, 2933, 2842, 1614, 1503, 1444, 1313, 1275, 1252, 1198, 1154, 1110, 1026, 969, 812, 705 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 3H, CH_3O), 5.63 (dd, *J* = 3.8, 9.8 Hz, 1H, H3), 5.85 (dd, *J* = 1.9, 3.8 Hz, 1H, H2), 6.37 (d, *J* = 2.3 Hz, 1H, H8), 6.43 (dd, *J* = 2.3, 8.3 Hz, 1H, H6), 6.51 (dd, *J* = 1.9, 9.8 Hz, 1H, H4), 6.92 (d, *J* = 8.3 Hz, 1H, H5), 7.31-7.39 (m, 4H, H2′, H3′, H5′, H6′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 55.2 (CH_3O), 76.3 (C2), 101.8 (C8), 107.1 (C6), 114.4 (C4a), 121.1 (C4), 124.0 (C3), 127.3 (C5), 128.4 (C2′, C6′), 128.7 (C3′, C5′), 134.1 (C4′), 139.3 (C1′), 154.0 (C8a), 160.9 (C7); MS (TOF-ESI) *m/z* Calcd. for $C_{16}H_{13}ClO_2$ $(M + 1)^+$ 273.07. Found 273.08; Anal. Calcd. for $C_{16}H_{13}ClO_2.1/10H_2O$: C, 70.00; H, 4.85. Found: C, 70.09; H, 4.47.

4.1.3.4. 4′,7-Dimethoxyflav-3-ene (6d):

White solid, Yield: 54%; M.p. 79–81 °C, *lit*.[22] 81–82 °C.

4.1.3.5. 4′-Bromo-5,7,8-trimethoxyflav-3-ene (14a):

Yellow sticky residue, Yield: 56%; UV (MeOH): λ_{max} 204 (ε 61481 $cm^{-1}M^{-1}$), 223 (49023), 296 (15338) nm; IR (KBr): ν_{max} 3438, 3010, 2933, 2840, 1609, 1561, 1504, 1464, 1437, 1347, 1239, 1206, 1138, 1116, 1070, 1010, 811 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66, 3.82 and 3.86 (3s, 9H, 3 x CH_3O), 5.66 (dd, *J* = 3.8, 10.2 Hz, 1H, H3), 5.84 (dd, *J* = 1.5, 3.8 Hz, 1H, H2), 6.06 (s, 1H, H6), 6.82 (dd, *J* = 1.5, 10.2 Hz, 1H, H4), 7.34 (d, *J* = 8.3 Hz, 2H, H2′, H6′), 7.46 (d, *J* = 8.3 Hz, 2H, H3′, H5′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 55.8, 56.0 and 61.2 (3 x CH_3O), 75.7 (C2), 89.3 (C6), 105.1 (C4a), 119.1 (C4), 122.2 (C4′), 126.8 (C3), 128.8 (C2′, C6′), 130.4 (C3′, C5′), 139.3 (C8), 146.2 (C8a), 146.5 (C1′), 151.7 (C5), 153.2 (C7); MS (TOF-ESI) *m/z* Calcd. for $C_{18}H_{17}BrO_4$ $(M + 1)^+$ 377.04 (Br^{79}). Found 376.96 (Br^{79}); Anal. Calcd. for $C_{18}H_{17}BrO_4$: C, 57.31; H, 4.54. Found: C, 57.53; H, 4.83.

4.1.3.6. 4′-Chloro-5,7,8-trimethoxyflav-3-ene (14b):

Yellow sticky residue, Yield: 58%; UV (MeOH): λ_{max} 204 (ε 103221 $cm^{-1}M^{-1}$), 221 (74779), 297 (18053) nm; IR (KBr): ν_{max} 3431, 3015, 2934, 2838, 1610, 1505, 1465, 1435, 1351, 1239, 1214, 1136, 1116, 1066, 1031, 809 cm^{-1}; ^{1}H NMR (300 MHz,

$CDCl_3$): δ 3.80, 3.83 and 3.86 (3s, 9H, 3 x CH_3O), 5.66 (dd, *J* = 3.0, 9.8 Hz, 1H, H3), 5.85 (dd, *J* = 1.5, 3.0 Hz, 1H, H2), 6.05 (s, 1H, H6), 6.85 (dd, *J* = 1.5, 9.8 Hz, 1H, H4), 7.30 (d, *J* = 8.6 Hz, 2H, H2′, H6′), 7.40 (d, *J* = 8.6 Hz, 2H, H3′, H5′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 55.8, 56.0 and 61.1 (3 x CH_3O), 75.7 (C2), 89.3 (C6), 105.1 (C4a), 119.0 (C4), 128.4 (C2′, C6′), 128.5 (C3), 128.6 (C3′, C5′), 134.0 (C4′), 138.8 (C8), 138.9 (C1′), 146.5 (C8a), 151.2 (C5), 153.7 (C7); MS (TOF-ESI) *m/z* Calcd. for $C_{18}H_{17}ClO_4Na$ $(M + Na)^+$ 355.07. Found 355.01; Anal. Calcd. for $C_{18}H_{17}ClO_4.H_2O$: C, 61.63; H, 5.46. Found: C, 61.80; H, 5.42.

4.1.3.7. 5,7,8,4′-Tetramethoxyflav-3-ene (14c):

Yellow sticky residue; Yield: 63%; UV (MeOH): λ_{max} 204 (ε 25961 $cm^{-1}M^{-1}$), 232 (20630), 274 (8299) nm; IR (KBr): ν_{max} 3434, 3008, 2935, 2839, 1607, 1511, 1464, 1439, 1347, 1246, 1211, 1135, 1114, 1064, 1032, 811 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.61, 3.78, 3.82 and 3.84 (4s, 12H, 4 x CH_3O), 5.68 (dd, *J* = 3.8, 10.2 Hz, 1H, H3), 5.84 (dd, *J* = 1.5, 3.8 Hz, 1H, H2), 6.06 (s, 1H, H6), 6.85 (dd, *J* = 1.5, 10.2 Hz, 1H, H4), 6.87 (d, *J* = 8.3 Hz, 2H, H3′, H5′), 7.38 (d, *J* = 8.3 Hz, 2H, H2′, H6′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 55.2, 55.8, 56.0 and 61.1 (4 x CH_3O), 76.2 (C2), 89.1 (C6), 105.3 (C4a), 113.6 (C3′, C5′), 119.7 (C4), 127.4 (C3), 128.7 (C2′, C6′), 131.7 (C1′), 139.3 (C8), 146.7 (C8a), 151.1 (C5), 153.5 (C7), 159.5 (C4′); MS (TOF-ESI) *m/z* Calcd. for $C_{19}H_{20}O_5Na$ $(M + Na)^+$ 351.12. Found 351.04; Anal. Calcd. for $C_{19}H_{20}O_5.1/2H_2O$: C, 67.64; H, 6.27. Found: C, 67.59; H, 6.17.

4.1.3.8. 4′-Methyl-5,7,8-trimethoxyflav-3-ene (14d):

Yellow sticky residue, Yield: 63%; UV (MeOH): λ_{max} 205 (ε 15503 $cm^{-1}M^{-1}$), 257 (5150) nm; IR (KBr): ν_{max} 3363, 3003, 2926, 2840, 1606, 1510, 1460, 1425, 1352, 1240, 1214, 1139, 1113, 1068, 1032, 795 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.64 (s, 3H, CH_3), 3.81, 3.83 and 3.87 (3s, 9H, 3 x CH_3O), 5.69 (dd, *J* = 3.8, 9.8 Hz, 1H, H3), 5.87 (dd, *J* = 1.5, 3.8 Hz, 1H, H2), 6.05 (s, 1H, H6), 6.83 (dd, *J* = 1.5, 9.8 Hz, 1H, H4), 7.14 (d, *J* = 8.3 Hz, 2H, H3′, H5′), 7.35 (d, *J* = 8.3 Hz, 2H, H2′, H6′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 21.3 (CH_3), 55.8, 56.0 and 61.0 (3 x CH_3O), 76.5 (C2), 89.3 (C6), 105.4 (C4a), 120.5 (C4), 126.8 (C3), 127.1 (C2′, C6′), 129.1 (C3′, C5′), 137.4 (C4′), 137.9 (C1′), 139.8 (C8), 146.8 (C8a), 151.1 (C5), 153.5 (C7); MS (TOF-ESI) *m/z* Calcd. for $C_{19}H_{20}O_4Na$ $(M + Na)^+$ 335.13. Found 335.06; Anal. Calcd. for $C_{19}H_{20}O_4.3/4MeOH$: C, 70.52; H, 6.89. Found: C, 70.82; H, 7.12.

4.1.4. General procedure for acid-catalyzed dimerization reactions (7a-d, 15a-d):

To a solution of the appropriate flavene in MeOH (20 mL) was added 10 drops of acid (HCl, TFA or AcOH) and the solution was heated at 60-70 °C for 12 h. The solvent was partially removed under reduced pressure and EtOAc (25 mL) was added. The organic layer was washed with saturated $NaHCO_3$ solution (20 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Purification of the crude product by column chromatography over silica gel using DCM/light petroleum (50:50) gave the desired dimer. The dimers were recrystallized twice from absolute EtOH to yield analytically pure products.

*4.1.4.1. 6a,12a-Dihydro-3,10-dimethoxy-6-phenyl-7-[(1**E**)-2-(phenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (7a):*

White solid; Yield: 65%; M.p. 110-112 °C; UV (MeOH): λ_{max} 207 (ε 39892 $cm^{-1}M^{-1}$), 285 (3618) nm; IR (KBr): ν_{max} 3421, 3026, 2954, 2910, 2834, 1610, 1587, 1504, 1443, 1268, 1198, 1160, 1130, 1112, 1033, 1010, 958, 834 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (ddd, *J* = 2.1, 2.3, 10.6 Hz, 1H, H6a), 3.17 (dd, *J* = 2.1, 6.4 Hz, 1H, H7), 3.78 and 3.79 (2s, 6H, 2 x CH_3O), 5.04 (d, *J* = 10.6 Hz, 1H, H6), 5.08 (d, *J* = 2.3 Hz, 1H, H12a), 6.04 (d, *J* = 15.8 Hz, 1H, H_β), 6.24 (dd, *J* = 6.4, 15.8 Hz, 1H, H_α), 6.50-6.52 (m, 3H, H4, H9, H11), 6.59 (dd, *J* = 2.3, 8.3 Hz, 1H, H2), 6.85 (d, *J* = 9.0 Hz, 1H, H8), 7.30-7.42 (m, 11H, H1, H2′, H3′, H4′, H5′, H6′, H2″, H3″, H4″, H5″, H6″); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 37.9 (C7), 41.4 (C6a), 55.2 (CH_3O), 55.3 (CH_3O), 67.1 (C12a), 76.8 (C6), 101.2 (C4), 101.8 (C11), 108.1 (C9), 108.3 (C2), 112.1 (C7a), 113.5 (C12b), 126.1 (C4′), 127.3 (C4″), 127.4 (C2′, C6′), 127.4 (C2″, C6″), 128.0 (C_β), 128.4 (C3″, C5″), 128.7 (C3′, C5′), 131.0 (C1), 131.8 (C8), 133.2 (C_α), 136.7 (C1″), 138.6 (C1′), 153.5 (C4a), 155.7 (C11a), 159.8 (C10), 161.5 (C3); HRMS (ESI) *m/z* Calcd. for $C_{32}H_{28}O_4Na$ $(M + Na)^+$ 499.1888. Found 499.1847; Anal. Calcd. for $C_{32}H_{28}O_4.MeOH$: C, 77.93; H, 6.34. Found: C, 77.77; H, 6.22.

*4.1.4.2. 6a,12a-Dihydro-3,10-dimethoxy-6-(4′-bromophenyl)-7-[(1**E**)-2-(4″-bromophenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (7b):*

White solid, Yield: 71%; M.p. 128-130 °C; UV (MeOH): λ_{max} 206 (ε 84733 $cm^{-1}M^{-1}$), 267 (29112) nm; IR (KBr): ν_{max} 3431, 3018, 2953, 2911, 2833, 1610, 1587, 1504, 1443, 1267, 1197, 1160, 1130, 1112, 1034, 1009, 965, 826, 807 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (ddd, *J* = 2.1, 2.3, 10.9 Hz, 1H, H6a), 3.13 (dd, *J* = 2.1, 6.4 Hz, 1H, H7), 3.78 and 3.79 (2s, 6H, 2 x CH_3O), 5.03 (d, *J* = 10.9 Hz, 1H, H6), 5.05 (d, *J* = 2.3 Hz, 1H, H12a), 5.99 (d, *J* = 15.8 Hz, 1H, H_β), 6.22 (dd, *J* = 6.4, 15.8 Hz, 1H, H_α), 6.52 (dd, *J* = 2.3, 8.3 Hz, 1H, H9), 6.55 (d, *J* = 2.3 Hz, 2H, H4, H11), 6.61 (dd, *J* = 2.3, 8.3 Hz, 1H, H2), 6.84 (d, *J* = 8.3 Hz, 1H, H8), 7.12 (d, *J* = 8.6 Hz, 2H, H2′, H6′), 7.21 (d, *J* = 8.3 Hz, 2H, H3″, H5″), 7.32 (d, *J* = 8.3 Hz, 1H, H1), 7.38 (d, *J* = 8.3 Hz, 2H, H2″, H6″), 7.55 (d, *J* = 8.6 Hz, 2H, H3′, H5′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 38.0 (C7), 41.3 (C6a), 55.2 and 55.3 (2 x CH_3O), 66.9 (C12a), 75.9 (C6), 101.2 (C4), 101.4 (C11), 108.2 (C9), 108.5 (C2), 111.5 (C7a), 113.4 (C12b), 121.2 (C4′), 122.7 (C4″), 127.7 (C2″, C6″), 129.0 (C2′, C6′), 130.8 (C_β), 130.9 (C8), 131.2 (C1), 131.5 (C3′, C5′), 131.9 (C3″, C5″), 133.8 (C_α), 135.5 (C1″), 137.6 (C1′), 153.4 (C4a), 155.4 (C11a), 160.0 (C10), 161.6 (C3); MS (TOF-ESI) *m/z* Calcd. for $C_{32}H_{26}Br_2O_4$ $(M + 1)^+$ 633.03 (Br^{79}). Found 632.98 (Br^{79}); Anal. Calcd. for $C_{32}H_{26}Br_2O_4$: C, 60.59; H, 4.13. Found: C, 60.37; H, 4.16.

*4.1.4.3. 6a,12a-Dihydro-3,10-dimethoxy-6-(4′-chlorophenyl)-7-[(1**E**)-2-(4″-chlorophenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (7c):*

White solid, Yield: 69%; M.p. 131-133 °C; UV (MeOH): λ_{max} 206 (ε 44459 $cm^{-1}M^{-1}$), 261 (10880) nm; IR (KBr): ν_{max} 3454, 3021, 2958, 2910, 2835, 1610, 1587, 1504, 1443, 1267, 1198, 1160, 1130, 1111, 1034, 1013, 959, 836, 803 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (ddd, *J* = 2.1, 2.3, 10.6 Hz, 1H, H6a), 3.12 (dd, *J* = 2.1, 6.4 Hz, 1H, H7), 3.76 and 3.78 (2s, 6H, 2 x CH_3O), 5.03 (d, *J* = 10.6 Hz, 1H, H6), 5.06 (d, *J* = 2.3 Hz, 1H, H12a), 6.00 (d, *J* = 15.8 Hz, 1H, H_β), 6.20 (dd, *J* = 6.4, 15.8 Hz, 1H, H_α), 6.49-6.54 (m, 3H, H4, H9, H11), 6.60 (dd, *J* = 2.3, 8.3 Hz, 1H, H2), 6.83 (d, *J* = 8.3 Hz, 1H, H8), 7.19 (d, *J* = 8.3 Hz, 2H, H2′, H6′), 7.26 (d, *J* = 8.3 Hz, 2H, H3′, H5′), 7.30 (d, *J* = 8.3 Hz, 1H, H1), 7.32 (d, *J* = 8.6 Hz, 2H, H3″, H5″), 7.40 (d, *J* = 8.6 Hz, 2H, H2″, H6″); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 37.9 (C7), 41.4 (C6a), 55.2 and 55.3 (2 x CH_3O), 66.9 (C12a), 76.1 (C6), 101.2 (C11), 101.3 (C4), 108.2 (C9), 108.5 (C2), 111.5 (C7a), 113.4 (C12b), 127.4 (C3′, C5′), 128.5 (C2′, C6′), 128.6 (C3″, C5″), 128.9 (C_β), 130.9 (C2″, C6″), 131.1 (C4′), 133.0 (C4″), 133.7 (C1), 133.9 (C_α), 134.5 (C8), 135.1 (C1″), 137.1 (C1′), 153.4 (C4a), 155.5 (C11a), 160.0 (C3), 161.6 (C10); HRMS (ESI) *m/z*

Calcd. for $C_{32}H_{26}Cl_2O_4Na$ $(M + Na)^+$ 567.1108. Found 567.1090; Anal. Calcd. for $C_{32}H_{26}Cl_2O_4.1/4MeOH$: C, 69.99; H, 4.92. Found: C, 70.26; H, 5.22.

*4.1.4.4. 6a,12a-Dihydro-3,10-dimethoxy-6-(4′-methoxyphenyl)-7-[(1**E**)-2-(4″-methoxyphenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (7d):*

White solid, Yield: 66%; M.p. 113-115 °C; UV (MeOH): λ_{max} 204 (ε 137728 $cm^{-1}M^{-1}$), 272 (29148) nm; IR (KBr): ν_{max} 3454, 3010, 2928, 2919, 2843, 1608, 1594, 1511, 1469, 1443, 1410, 1250, 1178, 1088, 1033, 834, 777 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.48 (ddd, *J* = 2.1, 2.3, 10.9 Hz, 1H, H6a), 3.15 (dd, *J* = 2.1, 6.0 Hz, 1H, H7), 3.78 (s, 6H, 2 x CH_3O), 3.85 (s, 6H, 2 x CH_3O), 5.01 (d, *J* = 10.9 Hz, 1H, H6), 5.09 (d, *J* = 2.3 Hz, 1H, H12a), 5.99 (d, *J* = 15.8 Hz, 1H, H_β), 6.11 (dd, *J* = 6.0, 15.8 Hz, 1H, H_α), 6.49-6.53 (m, 3H, H4, H9, H11), 6.59 (dd, *J* = 2.3, 8.3 Hz, 1H, H2), 6.79-6.86 (m, 3H, H8, H3′, H5′), 6.95 (d, *J* = 8.7 Hz, 2H, H3″, H5″), 7.21 (d, *J* = 8.6 Hz, 2H, H2′, H6′), 7.26 (d, *J* = 8.7 Hz, 2H, H2″, H6″), 7.32 (d, *J* = 8.3 Hz, 1H, H1); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 37.9 (C7), 41.5 (C6a), 55.2 (2 x CH_3O), 55.3 (2 x CH_3O), 67.2 (C12a), 76.5 (C6), 101.1 (C4), 101.2 (C11), 108.0 (C9), 108.2 (C2), 112.3 (C7a), 113.6 (C12b), 113.8 (C3′, C5′), 114.1 (C3″, C5″), 127.3 (C2′, C6′), 128.6 (C_β), 128.7 (C2″, C6″), 129.5 (C1″), 130.6 (C1′), 131.1 (C1), 131.2 (C8), 133.0 (C_α), 153.5 (C4a), 155.8 (C11a), 159.0 (C4′), 159.5 (C4″), 159.7 (C10), 159.8 (C3); MS (TOF-ESI) *m/z* Calcd. for $C_{34}H_{32}O_6$ $(M + 1)^+$ 537.23. Found 537.22; Anal. Calcd. for $C_{34}H_{32}O_6$: C, 76.10; H, 6.01. Found: C, 76.26; H, 6.29.

*4.1.4.5. 6a, 12a-Dihydro-1,3,4,8,10,11-hexamethoxy-6-(4′-bromophenyl)-7-[(1**E**)-2-(4″-bromophenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (15a):*

White solid, Yield: 68%; M.p. 172-174 °C; UV (MeOH): λ_{max} 208 (ε 15726 $cm^{-1}M^{-1}$), 263 (3702) nm; IR (KBr): ν_{max} 3479, 2933, 2839, 1736, 1613, 1503, 1463, 1347, 1241, 1205, 1105, 1064, 961, 928, 809 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (ddd, *J* = 1.5, 2.3, 10.9 Hz, 1H, H6a), 3.31 (dd, *J* = 1.5, 5.3 Hz, 1H, H7), 3.69, 3.73, 3.82, 3.86, 3.89 and 3.90 (6s, 18H, 6 x CH_3O), 4.93 (d, *J* = 10.9 Hz, 1H, H6), 5.36 (d, *J* = 2.3 Hz, 1H, H12a), 5.95 (d, *J* = 15.8 Hz, 1H, H_β), 6.13 (dd, *J* = 5.3, 15.8 Hz, 1H, H_α), 6.16 (s, 1H, H2), 6.18 (s, 1H, H9), 7.15 (d, *J* = 8.6 Hz, 2H, H2′, H6′), 7.18 (d, *J* = 8.3 Hz, 2H, H3″, H5″), 7.35 (d, *J* = 8.3 Hz, 2H, H2″, H6″), 7.52 (d, *J* = 8.6 Hz, 2H, H3′, H5′); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 32.9 (C7), 40.9 (C6a), 55.7, 55.8, 56.0, 56.2, 60.8 and 60.9 (6 x CH_3O), 61.4 (C12a), 75.9 (C6), 89.4 (C2), 89.5 (C9), 102.3 (C12b), 104.2 (C7a), 120.7 (C4′), 122.4 (C4″), 127.7 (C_β), 128.9 (C2′, C6′), 129.0 (C2″, C6″), 129.5 (C3′, C5′), 130.2 (C_α), 131.2 (C11), 131.6 (C4), 132.9 (C3″, C5″), 136.1 (C1″), 137.8 (C1′), 149.1 (C3), 149.9 (C10), 153.2 (C1), 153.9 (C8), 153.9 (C11a), 154.5 (C4a); MS (TOF-ESI) *m/z* Calcd. for $C_{36}H_{34}Br_2O_8Na$ $(M + Na)^+$ 775.05 (Br^{79}). Found 775.02 (Br^{79}); Anal. Calcd. for $C_{36}H_{34}Br_2O_8$: C, 57.31; H, 4.54. Found: C, 57.58; H, 4.82.

*4.1.4.6. 6a, 12a-Dihydro-1,3,4,8,10,11-hexamethoxy-6-(4′-chlorophenyl)-7-[(1**E**)-2-(4″-chlorophenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (15b):*

White solid, Yield: 70%; M.p. 166-168 °C; UV (MeOH): λ_{max} 210 (ε 98869 $cm^{-1}M^{-1}$), 261 (38495) nm; IR (KBr): ν_{max} 3476, 2934, 2839, 1726, 1610, 1502, 1456, 1348, 1242, 1205, 1115, 1061, 974, 938, 812 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.24 (ddd, *J* = 1.1, 2.3, 11.3 Hz, 1H, H6a), 3.31 (dd, *J* = 1.1, 6.0 Hz, 1H, H7), 3.69, 3.73, 3.82, 3.86, 3.88 and 3.90 (6s, 18H, 6 x CH_3O), 4.94 (d, *J* = 11.3 Hz, 1H, H6), 5.37 (d, *J* = 2.3 Hz, 1H, H12a), 5.96 (d, *J* = 15.8 Hz, 1H, H_β), 6.14 (dd, *J* = 6.0, 15.8 Hz, 1H, H_α), 6.18 (2s, 2H, H2, H9), 7.20 (d, *J* = 8.6 Hz, 2H, H2′, H6′), 7.23 (d, *J* = 8.6 Hz, 2H, H3′, H5′), 7.26 (d, *J* = 8.7 Hz, 2H, H3″, H5″), 7.37 (d, *J* = 8.7 Hz, 2H, H2″, H6″); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 32.9 (C7), 41.0 (C6a), 55.7, 56.0, 56.3, 56.4, 60.8 and 60.9 (6 x CH_3O), 61.4 (C12a), 75.9 (C6), 89.4 (C2), 89.5 (C9), 102.4 (C12b), 104.3 (C7a), 127.4 (C3′, C5′), 127.8 (C_β), 128.5 (C2′, C6′), 128.5 (C3″, C5″), 128.7 (C2″, C6″), 130.6 (C_α), 131.3 (C11), 131.5 (C4), 132.8 (C4′), 134.2 (C4″), 135.6 (C1″), 137.3 (C1′), 148.0 (C3), 149.1 (C10), 152.2 (C1), 153.3 (C8), 154.0 (C11a), 154.8 (C4a); MS (TOF-ESI) *m/z* Calcd. for $C_{36}H_{34}Cl_2O_8Na$ $(M + Na)^+$ 687.15. Found 687.06; Anal. Calcd. for $C_{36}H_{34}Cl_2O_8$: C, 64.97; H, 5.15. Found: C, 65.13; H, 5.26.

*4.1.4.7. 6a, 12a-Dihydro-1,3,4,8,10,11-hexamethoxy-6-(4′-methoxyphenyl)-7-[(1**E**)-2-(4″-methoxyphenylethenyl)]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (15c):*

White solid, Yield: 71%; M.p. 188-190 °C; UV (MeOH): λ_{max} 210 (ε 68254 $cm^{-1}M^{-1}$), 265 (18123) nm; IR (KBr): ν_{max} 3442, 2934, 2838, 1735, 1609, 1510, 1464, 1349, 1246, 1204, 1112, 1060, 974, 928, 820 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (ddd, *J* = 1.1, 2.3, 11.3 Hz, 1H, H6a), 3.34 (dd, *J* = 1.1, 5.3 Hz, 1H, H7), 3.67, 3.73, 3.76, 3.83, 3.85, 3.86, 3.89 and 3.90 (8s, 24H, 8 x CH_3O), 4.91 (d, *J* = 11.3 Hz, 1H, H6), 5.39 (d, *J* = 2.3 Hz, 1H, H12a), 5.95 (d, *J* = 15.8 Hz, 1H, H_β), 6.05 (dd, *J* = 5.3, 15.8 Hz, 1H, H_α), 6.14 (s, 1H, H2), 6.17 (s, 1H, H9), 6.78 (d, *J* = 8.7 Hz, 2H, H3′, H5′), 6.92 (d, *J* = 8.6 Hz, 2H, H3″, H5″), 7.20 (d, *J* = 8.7 Hz, 2H, H2′, H6′), 7.23 (d, *J* = 8.6 Hz, 2H, H2″, H6″); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 32.9 (C7), 41.1 (C6a), 55.1, 55.8, 56.0, 56.2, 56.3, 56.4, 60.8 and 60.9 (8 x CH_3O), 61.6 (C12a), 76.2 (C6), 89.2 (C2), 89.6 (C9), 103.3 (C12b), 104.6 (C7a), 113.7 (C3′, C5′), 113.8 (C3″, C5″), 127.2 (C2′, C6′), 127.6 (C_β), 128.5 (C2″, C6″), 130.1 (C1″), 130.4 (C1′), 130.4 (C_α), 131.3 (C4), 131.5 (C11), 147.1 (C3), 149.5 (C10), 151.9 (C1), 152.2 (C8), 152.9 (C11a), 153.5 (C4a), 158.9 (C4′), 159.7 (C4″); MS (TOF-ESI) *m/z* Calcd. for $C_{38}H_{40}O_{10}Na$ $(M + Na)^+$ 679.25. Found 679.10; Anal. Calcd. for $C_{38}H_{40}O_{10}$: C, 69.50; H, 6.14. Found: C, 69.69; H, 6.41.

*4.1.4.8. 6a, 12a-Dihydro-1,3,4,8,10,11-hexamethoxy-6-tolyl-7-[(1**E**)-2-tolylethenyl]-6**H**,7**H**-[1]benzopyrano[4,3-**b**][1]benzopyran (15d):*

White solid, Yield: 73%; M.p. 167-169 °C; UV (MeOH): λ_{max} 210 (ε 38692 $cm^{-1}M^{-1}$), 263 (8242) nm; IR (KBr): ν_{max} 3477, 2932, 2839, 1726, 1610, 1504, 1456, 1348, 1243, 1205, 1119, 1061, 961, 939, 807 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (ddd, *J* = 1.1, 2.3, 10.9 Hz, 1H, H6a), 2.31 and 2.39 (2s, 6H, 2 x CH_3), 3.37 (dd, *J* = 1.1, 5.6 Hz, 1H, H7), 3.68, 3.75, 3.82, 3.83, 3.85 and 3.86 (6s, 18H, 6 x CH_3O), 4.93 (d, *J* = 10.9 Hz, 1H, H6), 5.39 (d, *J* = 2.3 Hz, 1H, H12a), 5.96 (d, *J* = 15.8 Hz, 1H, H_β), 6.14 (dd, *J* = 5.6, 15.8 Hz, 1H, H_α), 6.15 and 6.17 (2s, 2H, H2, H9), 7.05 (d, *J* = 8.3 Hz, 2H, H3′, H5′), 7.13 (d, *J* = 8.6 Hz, 2H, H3″, H5″,), 7.15 (d, *J* = 8.3 Hz, 2H, H2′, H6′), 7.19 (d, *J* = 8.6 Hz, 2H, H2″, H6″); ^{13}C NMR (75.6 MHz, $CDCl_3$): δ 21.0 and 21.2 (2 x CH_3), 32.9 (C7), 40.9 (C6a), 55.7, 55.9, 56.2, 56.4, 60.8 and 60.9 (6 x CH_3O), 61.5 (C12a), 76.5 (C6), 89.2 (C2), 89.6 (C9), 103.2 (C12b), 104.5 (C7a), 126.0 (C2′, C6′), 127.1 (C_β), 127.2 (C3′, C5′), 129.0 (C2″, C6″), 131.3 (C3″, C5″), 131.5 (C_α), 134.5 (C4), 135.8 (C11), 136.0 (C1″), 136.7 (C1′), 137.9 (C4′), 138.0 (C4″), 149.5 (C3), 149.9 (C10), 152.2 (C1), 153.4 (C8), 154.0 (C11a), 154.6 (C4a); HRMS (ESI) *m/z* Calcd. for $C_{38}H_{40}O_8Na$ $(M + Na)^+$ 647.2623. Found 647.2615; Anal. Calcd. for $C_{38}H_{40}O_8$: C, 73.06; H, 6.45. Found: C, 72.80; H, 6.70.

4.2. Biological Experiments

4.2.1. Method for antimalarial growth inhibition assays

P. falciparum growth inhibition assays were carried using an isotopic microtest, as previously described (Andrews, Walduck et al. 2000).[23] Briefly, ring-stage *P. falciparum* 3D7 infected erythrocytes (0.5% parasitemia and 2.5% hematocrit) were seeded into triplicate wells of 96 well tissue culture plates containing serial dilutions of control (chloroquine) or test compounds. Follwing 48 hours incubation under standard *P. falciparum* culture conditions, 0.5 µCi [^{3}H]-hypoxanthine was added to each well after which the plates cultured for a further 24 hours. Cells were harvested onto 1450 MicroBeta filter mats (Wallac) and ^{3}H incorporation was determined using a 1450 MicroBeta liquid scintillation counter. Percentage inhibition of growth was compared to matched DMSO controls (0.5%). IC_{50} values were calculated using linear interpolation of inhibition curves (Huber and Koella 1993).[24] The mean IC_{50} (+/- SD) is shown for three independent experiments, each carried out in triplicate.

4.2.2 Method for *in vitro* cytotoxicity assays[25]

Neonatal foreskin fibroblast (NFF) cells were cultured in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS (CSL Biosciences, Parkville, Victoria, Australia), 1% streptomycin (Life Technologies, Inc., Rockville, MD; complete medium) at 37°C and 5% CO_2. Cells were maintained in log phase growth and then seeded (3,000/well) into 96-well tissue culture plates (Corning, USA) and were grown for 24h before treatment. Compounds were dissolved in 100% DMSO and diluted in complete medium; the DMSO concentration in the medium did not exceed 1%. Control cells were treated with the equivalent dose of DMSO. Three days after treatment initiation, the cells were washed with PBS and fixed in methylated spirits and total protein was determined using sulforhodamine B as described previously (Skehan, Storeng et al. 1990). Compounds were tested in triplicate in two independent experiments.

Acknowledgments

We thank the University of New South Wales, and the Australian Research Council (ARC) for Linkage Project (LP0455373) funding to NK and DB, and Future Fellowship funding to KTA, and the Australian Red Cross Blood Service for providing human blood and sera for *P. falciparum* culture.

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