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Synthesis and antiplasmodial evaluation of novel chromeno[2,3-*b*]chromene derivatives

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ABSTRACT

5-Methoxyflavenes and 6-methoxyflavenes were found to undergo stereoselective acid-catalyzed rearrangement to generate a range of novel chromeno[2,3-*b*]chromene derivatives. When subjected to an in vitro antiplasmodial growth inhibition assay using *Plasmodium falciparum* (3D7 line) the chromene analogues were shown to display IC_{50} values ranging from 6.8 to 39.8 μM.

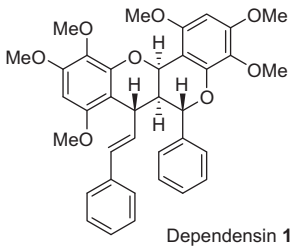
1. Introduction

Flavonoids are a broad class of polyphenolic secondary metabolites abundant in plants and in a variety of common foods such as apples, onions, tea and red wine.[1] Flavonoids possess a broad range of pharmacological properties including antioxidant, anticancer, antiviral and antiinflammatory properties and form the largest class of phytoestrogens known so far.[2]

Biflavonoids are a series of naturally occurring compounds that include flavone–flavone, flavanones–flavone and flavanones–flavanone subunit linkages. More than 100 biflavonoids have been identified from plants since the isolation of gingetin in 1929.[3,4] A variety of biological activities for biflavonoids have been published, including antiinflammatory, antimicrobial, antioxidant, and others.[4,5] Antiplasmodial activity has also been reported, for example the two biflavonoids, sikokianins B and C were isolated from the roots of *Wikstroemia indica* and displayed IC_{50} values of 0.54 and 0.56 μg/ml respectively, against a chloroquine-resistant strain of *Plasmodium falciparum*.[6] Similarly, a biflavanone was isolated as a potent antiplasmodial natural product (IC_{50} value of 80 ng/ml) from the extract of the outer bark of *Ochna integerrima*.[7]

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. Therefore, it is highly desirable to develop efficient synthetic methodologies, which could generate not only the natural products themselves but also synthetic analogues for pharmacological applications.

The typical methods reported for the synthesis of biflavonoids include the Ulmann condensation of halogenoflavonoids,[8] Suzuki coupling reaction of halogenated flavanones with appropriate boronic acid derivatives[9] and Stille coupling of tributyltinflavones with bromoflavones.[10]



Dependensin **1**

The natural product, dependensin **1** is a dimeric flavonoid isolated from the root bark of the Tanzanian medicinal plant, *Uvaria dependens*. The crude extract of this plant shows potent antimalarial activity.[11] 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

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unable to verify it experimentally, obtaining a ring-opened compound instead.[11] However, our research group has reported the successful synthesis of dependensin via the acid-catalyzed reaction of 5,7,8-trimethoxyflavene.[12] 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.[12] But, similar acid-catalyzed reactions of the corresponding 4,5-diacetoxy- and 4,6-diacetoxyflavenes produced a complex mixture of products.[12]

As part of our ongoing study on the synthesis of these dimeric systems, we have investigated the acid catalyzed reactions of 5-methoxy-, 6-methoxy- and 7-methoxyflavenes. Interestingly, we found that the dimerization products obtained were dependent on the position of the methoxy group in the flavene nucleus. 7-Methoxyflavenes and 5,7,8-trimethoxyflavenes underwent acid-catalyzed dimerization as expected to yield analogues of dependensin. Recently, we presented data on the development of efficient synthetic methodologies to these dimeric structures possessing the benzopyrano[4,3-*b*]benzopyran ring system, along with their preliminary antimalarial inhibition assay results.[13]

However, it has been observed that the 5-methoxy- and 6-methoxyflavenes underwent a completely different dimerization reaction to yield a new range of dimeric structures namely the chromeno[2,3-*b*]chromenes.[14] It is postulated that these unique scaffolds will serve as an ideal source for generating chemically diverse libraries for use in future drug discovery programs.

The literature cites examples of molecules from nature bearing close resemblance to the chromeno[2,3-*b*]chromene ring system. For example, albanols A **2** and albanol B **3**, isolated from the root bark of *Morus alba*, Moraceae (mulberry) were noteworthy, as pharmacological tests on this species showed marked hypotensive effect in rabbits.[15] Similarly, a systematic study performed on plants belonging to the Moraceae family showed the presence of antimicrobial activity in the extract of *Sorocea ilicifolia*, from which a number of phenolic components were isolated. One among them was the ketalized Diels–Alder type adduct, named sorocein L **4**.[16]

However, there are no reports in the literature for the synthesis of such fused benzopyran ring systems via the generation of carbocation intermediates mediated by acid-catalyzed reactions. We report herein the facile one step methodology to the synthesis of a series of highly functionalized chromeno[2,3-*b*]chromenes from the acid-catalyzed reactions of 5-methoxyflavenes and 6-methoxyflavenes. Both ring systems, containing the chromeno [2,3-*b*]chromenes and the benzopyrano[4,3-*b*]benzopyrans present in dependensin possess two benzopyran rings, but differ in the position and manner in which they are fused together. Therefore, it was anticipated that the new ring system might show interesting antiplasmodial properties. This paper discusses the full characterization details for the novel chromeno[2,3-*b*]chromenes as well as their biological activity in an in vitro antiplasmodial growth inhibition assay.

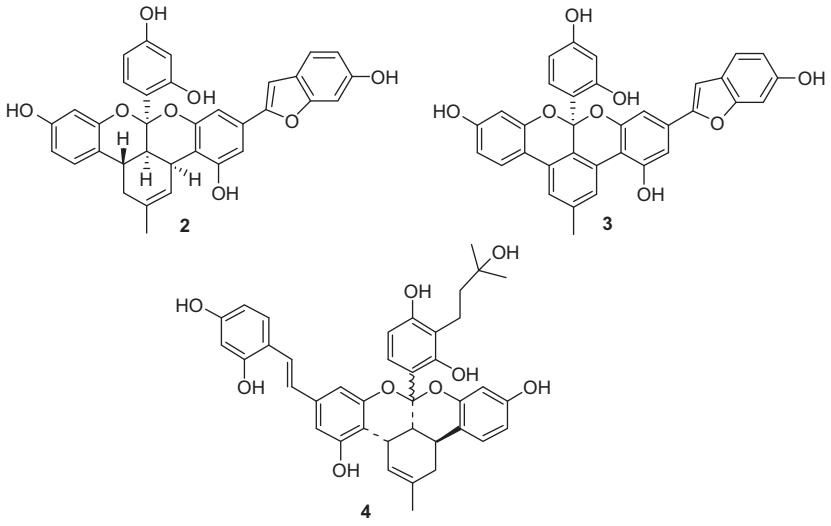
2. Results and discussion

2.1. Synthesis of flavene precursors

The strategy of reductive cyclization reported by Clark-Lewis and Jemison[20] was utilized for the direct conversion of 2′-hydroxychalcones **5a–i** into the corresponding flav-3-enes **6a–i** with the use of $NaBH_4$ in IPA (Scheme 1). This methodology was beneficial as it could furnish the desired flavenes, the essential precursors in fewer steps as well as generate improved overall yields. The flavenes **6a–i** were obtained in yields ranging from 65% to 80%.

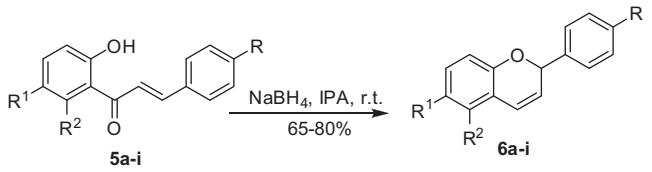
2.2. Acid-catalyzed reactions of 6-methoxyflavenes

The 6-methoxyflavenes **6a–d** were subjected to the dimerization reaction in the presence of few drops of TFA or glacial AcOH as catalysts in MeOH, which unexpectedly generated a series of chromeno[2,3-*b*]chromenes **7a–d** (Scheme 2), instead of analogues of the natural product dependensin.



The strategies employed for the synthesis of this ring system include the condensation of dimethylol ketones with 2-naphthol using Amberlyst-15 as catalyst,[17] reaction of phenolic Mannich bases with α-chloroacrylonitrile involving [4+2] cycloaddition[18] and the Diels–Alder cylcoaddition reactions of *o*-quinone methides, generated from 4*H*-1,2-benzoxazines with various dienophiles.[19]

This was evident from the ^{1}H NMR spectrum of compound **7b** which showed the presence of four aliphatic protons corresponding to H11, H11a and H12 between δ 2.84 and 3.46 ppm. The three protons corresponding to H11a and H12 appeared as a singlet at δ 2.84 ppm and the proton corresponding to H11 was seen as a doublet of doublets at δ 3.46 (J = 3.4, 9.8 Hz). The protons on the *trans*



chalcone	R^1	R^2	R	flavene	yields[%]
5a	OMe	H	H	**6a**	72
5b	OMe	H	Br	**6b**	65
5c	OMe	H	Cl	**6c**	68
5d	OMe	H	Me	**6d**	74
5e	H	OMe	H	**6e**	77
5f	H	OMe	Br	**6f**	70
5g	H	OMe	Cl	**6g**	70
5h	H	OMe	OMe	**6h**	80
5i	H	OMe	Me	**6i**	79

Scheme 1. Synthesis of flavene precursors.

double bond, H_α and H_β appeared as a doublet of doublets and a doublet at δ 6.24 (J = 9.8, 15.8 Hz) and δ 6.47 (J = 15.8 Hz) respectively. The HMBC experiment showed two bond and three bond proton to carbon couplings from the four aliphatic protons to C5a at 100.4 ppm. DEPT-135 along with a broadband decoupled ^{13}C NMR spectrum indicated the presence of one CH_2 group at C12 in the structure. Further, a single crystal of compound **7b** was obtained for X-ray crystallographic analysis[14] confirming the structure indicated.

We have previously proposed a rationale[14] for the observed acid-catalyzed dimerization reaction. The reaction mechanism for the formation of this ring system is postulated to involve the protonation of the flavene, followed by ring opening leading to the formation of a highly stabilized benzylic carbocation intermediate. A second molecule of the flav-3-ene can possibly undergo prototropic rearrangement in situ to the corresponding flav-2-ene isomer under acidic conditions. This can attack the carbocation intermediate giving rise to a second stable benzylic intermediate. The latter on ring closure produces the required dimeric ring system.

By correlation of the ^1H and ^{13}C NMR spectra, the other dimeric products of 6-methoxyflavenes **7a**, **7c** and **7d** were assigned to contain the same ring system. These dimeric structures **7a–d** were obtained in 70–74% yields.

2.3. Acid-catalyzed reactions of 5-methoxyflavenes

Similar acid-catalyzed reactions were attempted on the 5-methoxyflavenes **6e–i** and they produced the chromeno [2,3-*b*]chromenes **7e–i** in 68–76% yields (Scheme 3), in parallel to the 6-methoxyflavenes.

The ^1H NMR spectra of these dimeric compounds **7e–i** were found to vary from those obtained previously via the dimerization of 6-methoxyflavenes **7a–d**, with respect to the coupling patterns of the aliphatic protons. The aliphatic protons in **7g** corresponding to H11, H11a and H12, were identified as doublet of doublet at δ 2.59 (J = 11.7, 17.3 Hz) and a doublet at δ 2.77 (J = 17.3 Hz) correlating to H12, and doublet of doublets at δ 2.91 (J = 6.4, 11.7 Hz) and δ 3.74 (J = 6.4, 9.0 Hz) correlating to H11a and H11, respectively.

The presence of a diastereotopic methylene unit in **7g** was evident in its COSY spectrum (correlation between the protons at H12). In addition, analysis of the COSY NMR data led to the identification of the two 1,4-disubstituted and two trisubstituted benzene rings. The presence of a *cis* fused ring system is evident from a NOESY correlation between H11a and H2″. The other important NOESY correlations of interest were those from H12 to H11a and H_α to H2′, giving evidence of the structure formed.

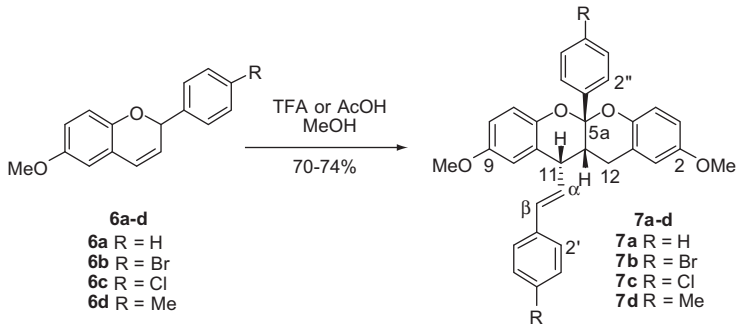
Further assignments for **7g** were determined on the basis of HMQC and HMBC data. The presence of a methylene group at H12 was also evident from its DEPT spectrum. HMBC correlations of the methylene proton at δ 2.59 to C5a, C11 and C12a and the other methylene proton at δ 2.77 to C5a, C11, C11a and C1 were noteworthy, indicating the presence of two fused benzopyran units. The aliphatic proton, H11a showed HMBC connections to C11, C12a, C1′ and C1″ and the other aliphatic proton, H11 showed HMBC connections to C5a, C6a, C10a, C_β and C1′, thereby indicating the attachment of the styryl group to position 11. This was further confirmed by the HMBC correlations from the vinylic protons, H_α and H_β to both the benzopyran ring as well as to the 1,4-disubstituted benzene ring. Lastly, the attachment of the second 1,4-disubstituted benzene ring to C5a was evident from the HMBC connections from H2″ to C4″ and C5a, in accordance with the assigned structure.

Similar coupling patterns were observed in the other dimeric structures **7e**, **7f**, **7h** and **7i**. The dimerization reaction mechanism is expected to follow the same pathway taken by the 6-methoxyflavenes **6a–d**.

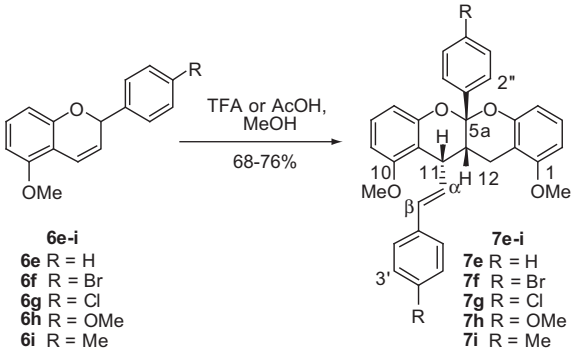
The identity and purity of all compounds were confirmed by ^1H and ^{13}C NMR spectroscopy, IR spectroscopy, mass spectrometry, thin layer chromatography and elemental analyses.

2.4. Biological activity of the chromeno[2,3-*b*]chromene derivatives

The new chromeno[2,3-*b*]chromene derivatives were all screened for antiplasmodial activity using a chloroquine sensitive *P. falciparum* line (3D7) and a standard in vitro growth inhibition assay. Preliminary cytotoxicity data for **7a–i** were all obtained using NFF (neonatal foreskin fibroblasts) cells, which are routinely used in toxicity studies to determine compound selectivity for the malaria parasite. Table 1 shows the biological data of all tested compounds.



Scheme 2. Synthesis of chromeno[2,3-*b*]chromenes **7a–d**.



Scheme 3. Synthesis of chromeno[2,3-*b*]chromenes **7e–i**.

Table 1
Antiplasmodial activities and cytotoxicity data for compounds **7a–i**

Compound	*P. falciparum* 3D7 IC_{50} ± SD (μM)	NFF[a] cytotoxicity % inhibition ± SD at 100 μM
7a	10.2 ± 1.2	51.4 ± 19.5
7b	17.2 ± 3.0	51.0 ± 11.9
7c	35.6 ± 3.7	40.4 ± 22.3
7d	8.9 ± 0.2	40.0 ± 15.8
7e	17.3 ± 0.3	48.6 ± 12.9
7f	39.8 ± 3.6	38.0 ± 16.4
7g	8.3 ± 2.7	83.6 ± 15.3
7h	8.2 ± 0.4	54.6 ± 15.6
7i	6.8 ± 2.7	60.1 ± 21.4
Dependensin	3.9 (±1.5)	46.9 (±0.3) [b]
Chloroquine	0.03 (±0.01)	[c]

[a] NFF, neonatal foreskin fibroblast cells.
[b] % Inhibition (±SD) at 50 μM.
[c] Chloroquine IC_{50} = 34.0 ± 0.3 μM.

Five compounds displayed antiplasmodial IC_{50} values <10 μM, with **7i** showing the most potent antiparasitic activity with an IC_{50} of 6.8 μM. All molecules displayed some toxicity towards the NFF cells at 100 μM. A comparison of the IC_{50} values of the chromeno[2,3-*b*]chromene derivatives with the natural product, dependensin and previously reported analogues[13] shows that the scaffold present in **7a–i** exhibits less antiplasmodial activity than the dependensin scaffold. Therefore, the manner in which the benzopyran rings are fused together is important for antiplasmodial activity. Although compounds **7a–i** show some selectivity towards the malaria parasite, the low selectivity and moderate to low antiplasmodial activity does not warrant further medicinal chemistry efforts. However, these dimeric structures will be screened in other biological assays in the future in order to identify their potential use in other areas of drug discovery.

3. Conclusion

A facile simple one-step methodology to the synthesis of a series of novel chromeno[2,3-*b*]chromenes has been developed via the acid-catalyzed dimerization reactions of 5-methoxyflavenes and 6-methoxyflavenes. All synthetic chromene analogues **7a–i** displayed moderate to low antiplasmodial activity with IC_{50} values ranging from 6.8 to 39.8 μM.

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. ^1H and ^{13}C NMR spectra were recorded in the designated solvents on a Bruker Avance DPX300 (300 MHz) spectrometer at the designated frequency and were internally referenced to the solvent peaks.

Low-resolution mass spectrometric analysis was carried out at the Biomedical Mass Spectrometry Facility, UNSW, and the spectra were recorded on Q-TOF Ultima API (Micro mass). High-resolution mass spectra were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. Infrared spectra were recorded with a Thermo Nicolet 370 FTIR spectrometer. Ultraviolet–visible spectra were recorded using a Varian Cary 100 Scan spectrometer, and the absorption maxima together with the molar absorptivity (ε) are reported. Gravity column chromatography was carried out using Grace Davison LC60A 40–63 micron silica gel while vacuum column chromatography was carried out using Grace Davison LC60A 6–35 micron silica gel. Reactions were monitored using thin layer chromatography, performed on Merck DC aluminium plates coated with silica gel GF_{254}.

4.1.1. General procedure for the synthesis of flavenes (6a–i)

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 min. The reaction mixture was cooled to rt 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 × 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.

4.1.1.1. 6-Methoxyflav-3-ene (6a)[21]. Colorless sticky residue, Yield: 72%; UV (MeOH): λ_{max} 204 (ε 11257 cm^{-1} M^{-1}), 231 (46379), 293 (14841) nm; IR (KBr): ν_{max} 3425, 3018, 2959, 2926, 2852, 1615, 1493, 1465, 1431, 1269, 1203, 1159, 1039, 968, 807 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.77 (s, 3H, CH_3O), 5.84 (dd, *J* = 3.0, 10.9 Hz, 1H, H3), 5.88 (dd, *J* = 1.5, 3.0 Hz, 1H, H2), 6.52 (dd, *J* = 1.5, 10.9 Hz, 1H, H4), 6.60 (d, *J* = 3.0 Hz, 1H, H5), 6.68 (dd, *J* = 3.0, 8.7 Hz, 1H, H7), 6.75 (d, *J* = 8.7 Hz, 1H, H8), 7.32–7.47 (m, 5H, H2, H3, H4, H5, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.7 (CH_3O), 76.9 (C2), 111.7 (C5), 114.4 (C7), 116.5 (C8), 122.0 (C4a), 124.1 (C4), 125.8 (C3), 127.0 (C2, C6), 128.2 (C4), 128.6 (C3, C5), 140.6 (C1), 147.0 (C8a), 154.0 (C6).

4.1.1.2. 4-Bromo-6-methoxyflav-3-ene (6b). White solid, Yield: 65%; mp 98–100 °C; UV (MeOH): λ_{max} 202 (ε 34171 cm^{-1} M^{-1}), 232 (39101), 330 (4329) nm; IR (KBr): ν_{max} 3441, 3006, 2958, 2931, 2831, 1608, 1580, 1490, 1461, 1427, 1271, 1219, 1152, 1028, 971, 821 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 3H, CH_3O), 5.80 (dd, *J* = 3.0, 10.9 Hz, 1H, H3), 5.83 (dd, *J* = 1.5, 3.0 Hz, 1H, H2), 6.52 (dd, *J* = 1.5, 10.9 Hz, 1H, H4), 6.61 (d, *J* = 3.0 Hz, 1H, H5), 6.68 (dd, *J* = 3.0, 8.7 Hz, 1H, H7), 6.74 (d, *J* = 8.7 Hz, 1H, H8), 7.32 (d, *J* = 8.3 Hz, 2H, H2, H6), 7.49 (d, *J* = 8.3 Hz, 2H, H3, H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6 (CH_3O), 76.0 (C2), 111.8 (C5), 114.6 (C7), 116.5 (C8), 121.8 (C4a), 122.2 (C4), 124.5 (C4), 125.1 (C3), 128.7 (C2, C6), 131.7 (C3, C5), 139.6 (C1), 146.7 (C8a), 154.1 (C6); MS (TOF-ESI) *m/z* Calcd for $C_{16}H_{13}{}^{79}BrO_2$ $(M+1)^+$ 317.02. Found

316.95; Anal. Calcd for $C_{16}H_{13}BrO_2$: C, 60.59; H, 4.13. Found: C, 60.82; H, 4.26.

4.1.1.3. 4-Chloro-6-methoxyflav-3-ene (6c). White solid, Yield: 68%; mp 65–67 °C; UV (MeOH): λ_{max} 204 (ε 45814 cm^{-1} M^{-1}), 229 (33355), 331 (2951) nm; IR (KBr): ν_{max} 3456, 3006, 2950, 2937, 2833, 1610, 1576, 1491, 1468, 1433, 1271, 1215, 1148, 1042, 966, 834 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 3H, CH_3O), 5.80 (dd, J = 3.8, 10.9 Hz, 1H, H3), 5.84 (dd, J = 1.9, 3.8 Hz, 1H, H2), 6.52 (dd, J = 1.9, 10.9 Hz, 1H, H4), 6.59 (d, J = 2.6 Hz, 1H, H5), 6.67 (dd, J = 2.6, 8.7 Hz, 1H, H7), 6.72 (d, J = 8.7 Hz, 1H, H8), 7.32 (d, J = 8.3 Hz, 2H, H2, H6), 7.35 (d, J = 8.3 Hz, 2H, H3, H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6 (CH_3O), 76.0 (C2), 111.8 (C5), 114.6 (C7), 116.5 (C8), 121.8 (C4a), 124.5 (C4), 125.2 (C3), 128.3 (C2, C6), 128.7 (C3, C5), 134.1 (C4), 139.0 (C1), 146.7 (C8a), 154.1 (C6); MS (TOF-ESI) m/z Calcd for $C_{16}H_{13}{}^{35}ClO_2$ $(M+1)^+$ 273.07. Found 273.00; Anal. Calcd for $C_{16}H_{13}ClO_2.1/10H_2O$: C, 70.00; H, 4.85. Found: C, 69.97; H, 4.96.

4.1.1.4. 6-Methoxy-4-methylflav-3-ene (6d). Colorless sticky residue, Yield: 74%; UV (MeOH): λ_{max} 204 (ε 81758 cm^{-1} M^{-1}), 255 (27838), 290 (16061) nm; IR (KBr): ν_{max} 3435, 3011, 2999, 2921, 2833, 1610, 1583, 1495, 1464, 1430, 1263, 1201, 1153, 1036, 971, 804 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 3.76 (s, 3H, CH_3O), 5.82 (dd, J = 3.4. 9.4 Hz, 1H, H3), 5.86 (dd, J = 1.1, 3.4 Hz, 1H, H2), 6.51 (dd, J = 1.1, 9.4 Hz, 1H, H4), 6.59 (d, J = 2.6 Hz, 1H, H5), 6.67 (dd, J = 2.6, 8.7 Hz, 1H, H7), 6.72 (d, J = 8.7 Hz, 1H, H8), 7.17 (d, J = 8.3 Hz, 2H, H3, H5), 7.34 (d, J = 8.3 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1 (CH_3) 55.6 (CH_3O), 76.5 (C2), 111.6 (C5), 114.4 (C7), 116.5 (C8), 122.0 (C4a), 124.1 (C4), 125.9 (C3), 127.0 (C2, C6), 129.2 (C3, C5), 137.6 (C4), 138.1 (C1), 147.0 (C8a), 153.9 (C6); (TOF-ESI) m/z Calcd for $C_{17}H_{16}O_2K$ $(M+K)^+$ 291.12. Found 291.08; Anal. Calcd for $C_{17}H_{16}O_2.1.6H_2O$: C, 72.63; H, 6.88. Found: C, 72.55; H, 6.69.

4.1.1.5. 5-Methoxyflav-3-ene (6e)[22]. White solid, Yield: 77%; mp 89–91 °C; UV (MeOH): λ_{max} 204 (ε 13733 cm^{-1} M^{-1}), 228 (12076), 283 (4156) nm; IR (KBr): ν_{max} 3418, 3013, 2961, 2938, 2840, 1601, 1582, 1466, 1438, 1272, 1246, 1202, 1107, 1033, 837, 752 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H, CH_3O), 5.77 (dd, J = 3.4, 10.4 Hz, 1H, H3), 5.86 (dd, J = 1.9, 3.4 Hz, 1H, H2), 6.45 (d, J = 8.3 Hz, 2H, H6, H8), 6.90 (dd, J = 1.9, 10.4 Hz, 1H, H4), 7.06 (t, J = 8.3 Hz, 1H, H7), 7.28–7.42 (m, 5H, H2, H3, H4, H5, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6 (CH_3O), 76.6 (C2), 103.4 (C6), 109.1 (C8), 110.8 (C4a), 118.7 (C4), 122.8 (C3), 127.0 (C2, C6), 128.2 (C4), 128.5 (C3, C5), 129.2 (C7), 140.8 (C1), 153.9 (C5), 155.3 (C8a).

4.1.1.6. 4-Bromo-5-methoxyflav-3-ene (6f). White solid, Yield: 70%; mp 56–58 °C; UV (MeOH): λ_{max} 203 (ε 66797 cm^{-1} M^{-1}), 226 (63329), 283 (18772) nm; IR (KBr): ν_{max} 3423, 3007, 2963, 2934, 2838, 1601, 1580, 1467, 1439, 1272, 1244, 1196, 1098, 1012, 899, 742 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 3H, CH_3O), 5.84 (dd, J = 3.4, 9.8 Hz, 1H, H3), 5.88 (dd, J = 1.9, 3.4 Hz, 1H, H2), 6.39 (d, J = 8.3 Hz, 1H, H6), 6.53 (d, J = 8.3 Hz, 1H, H8), 6.78 (dd, J = 1.9, 9.8 Hz, 1H, H4), 7.06 (t, J = 8.3 Hz, 1H, H7), 7.34 (d, J = 8.6 Hz, 2H, H2, H6), 7.54 (d, J = 8.6 Hz, 2H, H3, H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6 (CH_3O), 75.7 (C2), 103.5 (C6), 109.0 (C8), 110.7 (C4a), 119.2 (C4), 122.1 (C3), 122.3 (C4), 128.7 (C2, C6), 129.4 (C7), 131.6 (C3, C5), 139.7 (C1), 153.6 (C5), 155.3 (C8a); MS (TOF-ESI) m/z Calcd for $C_{16}H_{13}{}^{79}BrO_2$ $(M+1)^+$ 317.02. Found 316.99; Anal. Calcd for $C_{16}H_{13}BrO_2$: C, 60.59; H, 4.13. Found: C, 60.78; H, 4.24.

4.1.1.7. 4-Chloro-5-methoxyflav-3-ene (6g). Yellow sticky residue, Yield: 70%; UV (MeOH): λ_{max} 204 (ε 79277 cm^{-1} M^{-1}), 222 (58394), 275 (18204) nm; IR (KBr): ν_{max} 3439, 3043, 2983, 2945, 2825, 1736, 1611, 1593, 1470, 1438, 1377, 1268, 1154, 1091, 1013, 829, 780, 750 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 3.76 (s, 3H, CH_3O), 5.83 (dd, J = 3.8, 9.8 Hz, 1H, H3), 5.88 (dd, J = 1.9, 3.8 Hz, 1H, H2), 6.40 (d, J = 8.3 Hz, 1H, H6), 6.52 (d, J = 8.3 Hz, 1H, H8), 6.79 (dd, J = 1.9, 9.8 Hz, 1H, H4), 7.04 (t, J = 8.3 Hz, 1H, H7), 7.39–7.43 (m, 4H, H2, H3, H5, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6 (CH_3O), 75.7 (C2), 103.5 (C6), 109.0 (C8), 110.7 (C4a), 119.1 (C4), 122.2 (C3), 128.4 (C2, C6), 128.7 (C3, C5), 129.4 (C7), 134.0 (C4), 139.2 (C1), 153.6 (C5), 155.3 (C8a); MS (TOF-ESI) m/z Calcd for $C_{16}H_{13}{}^{35}ClO_2Na$ $(M+Na)^+$ 295.05. Found 295.03; Anal. Calcd for $C_{16}H_{13}ClO_2.H_2O$: C, 66.10; H, 5.20. Found: C, 66.20; H, 5.45.

4.1.1.8. 4,5-Dimethoxyflav-3-ene (6h). White solid, Yield: 80%; mp 61–63 °C; UV (MeOH): λ_{max} 203 (ε 21185 cm^{-1} M^{-1}), 229 (20204), 282 (7936) nm; IR (KBr): ν_{max} 3428, 3013, 2965, 2940, 2843, 1601, 1584, 1466, 1426, 1271, 1240, 1204, 1103, 1029, 833, 747 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 3.70 (s, 3H, CH_3O), 3.76 (s, 3H, CH_3O), 5.83 (dd, J = 3.8, 9.4 Hz, 1H, H3), 5.85 (dd, J = 1.9, 3.8 Hz, 1H, H2), 6.33 (d, J = 8.3 Hz, 1H, H6), 6.42 (d, J = 8.3 Hz, 1H, H8), 6.78 (dd, J = 1.9, 9.4 Hz, 1H, H4), 6.89 (d, J = 8.7 Hz, 2H, H3, H5), 7.03 (t, J = 8.3 Hz, 1H, H7), 7.29 (d, J = 8.7 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.2 (CH_3O), 55.6 (CH_3O), 76.3 (C2), 103.3 (C6), 109.1 (C8), 110.8 (C4a), 113.9 (C3, C5), 118.7 (C4), 122.9 (C3), 128.7 (C2, C6), 129.2 (C7), 132.8 (C1), 153.8 (C5), 155.3 (C8a), 159.6 (C4); HRMS (ESI) m/z Calcd for $C_{17}H_{16}O_3Na$ $(M+Na)^+$ 291.0999. Found 291.1011; Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 76.11; H, 6.04.

4.1.1.9. 5-Methoxy-4-methylflav-3-ene (6i). Colorless sticky residue, Yield: 79%; UV (MeOH): λ_{max} 205 (ε 27890 cm^{-1} M^{-1}), 224 (16588), 275 (6161) nm; IR (KBr): ν_{max} 3454, 3002, 2978, 2924, 2838, 1605, 1583, 1468, 1439, 1268, 1235, 1098, 1018, 818, 745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH_3), 3.86 (s, 3H, CH_3O), 5.79 (dd, J = 3.4, 9.8 Hz, 1H, H3), 5.87 (dd, J = 1.9, 3.4 Hz, 1H, H2), 6.46 (d, J = 8.3 Hz, 1H, H6), 6.50 (d, J = 8.3 Hz, 1H, H8), 6.94 (dd, J = 1.9, 9.8 Hz, 1H, H4), 7.09 (t, J = 8.3 Hz, 1H, H7), 7.21 (d, J = 8.6 Hz, 2H, H3, H5), 7.27 (d, J = 8.6 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.1 (CH_3), 55.6 (CH_3O), 76.5 (C2), 103.3 (C6), 109.1 (C8), 110.8 (C4a), 118.7 (C4), 123.0 (C3), 127.1 (C2, C6), 129.2 (C3, C5), 129.7 (C7), 137.8 (C4), 138.0 (C1), 153.9 (C5), 155.3 (C8a); MS (TOF-ESI) m/z Calcd for $C_{17}H_{16}O_2$ $(M+H)^+$ 253.12. Found 253.12; Anal. Calcd for $C_{17}H_{16}O_2.H_2O$: C, 75.53; H, 6.71. Found: C, 75.77; H, 6.38.

4.1.2. General procedure for acid-catalyzed dimerization reactions (7a–i)

To a solution of the appropriate flavene (1.0 equiv) in MeOH (20 mL) was added 10 drops of acid (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.

4.1.2.1. (*E*)-2,9-Dimethoxy-5a-phenyl-11-styryl-11a,12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7a). White solid, Yield: 70%; mp 204–206 °C; UV (MeOH): λ_{max} 204 (ε 45414 cm^{-1} M^{-1}), 231 (14449), 256 (16881), 293 (8914) nm; IR (KBr): ν_{max} 3440, 3003, 2933, 2837, 1612, 1498, 1464, 1430, 1273, 1231, 1203, 1153, 1035, 972, 952, 823, 802 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.88 (s, 3H, H11a, H12), 3.49 (dd, J = 3.0, 9.0 Hz, 1H, H11), 3.69 (s, 3H, CH_3O), 3.73 (s, 3H, CH_3O), 6.28 (dd, J = 9.0, 15.8 Hz, 1H, H_α), 6.51 (d, J = 15.8 Hz, 1H, H_β), 6.63 (d, J = 3.0 Hz, 2H, H1, H10),

6.72 (dd, J = 3.0, 8.7 Hz, 1H, H3), 6.80 (dd, J = 3.0, 8.7 Hz, 1H, H8), 6.88 (d, J = 8.7 Hz, 1H, H4), 6.98 (d, J = 8.7 Hz, 1H, H7), 7.28–7.52 (m, 10H, H2, H3, H4, H5, H6, H2, H3, H4, H5, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.2 (C12), 37.9 (C11a), 42.4 (C11), 55.5 (CH_3O), 55.6 (CH_3O), 100.9 (C5a), 113.6 (C1), 113.8 (C10), 113.9 (C3), 114.3 (C8), 117.1 (C7), 117.5 (C4), 121.6 (C12a), 122.6 (C10a), 125.9 (ArCH), 126.3 (ArCH), 126.8 (ArCH), 127.7 (ArCH), 127.9 (ArCH), 128.6 (ArCH), 128.8 (C_α), 133.7 (C_β), 136.7 (C1), 140.5 (C1), 145.7 (C4a), 146.3 (C6a), 154.1 (C9), 154.2 (C2); HRMS (ESI) m/z Calcd for $C_{32}H_{28}O_4Na$ $(M+Na)^+$ 499.1888. Found 499.1860; Anal. Calcd for $C_{32}H_{28}O_4.1/2EtOH$: C, 79.33; H, 6.25. Found: C, 79.23; H, 6.29.

4.1.2.2. (*E*)-5a-(4-Bromophenyl)-11-(4-bromostyryl)-2,9-dimethoxy-11a,12-dihydro-5a*H*, 11*H*-chromeno[2,3-*b*]chromene (7b). White solid, Yield: 73%; mp 212–214 °C; UV (MeOH): λ_{max} 203 (ε 61384 cm^{-1} M^{-1}), 220 (35949), 230 (28486), 263 (34167), 291 (17870), 299 (12616) nm; IR (KBr): ν_{max} 3444, 3006, 2929, 2833, 1615, 1489, 1464, 1427, 1275, 1230, 1200, 1154, 1041, 971, 951, 803 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 3H, H11a, H12), 3.46 (dd, J = 3.4, 9.8 Hz, 1H, H11), 3.70 (s, 3H, CH_3O), 3.73 (s, 3H, CH_3O), 6.24 (dd, J = 9.8, 15.8 Hz, 1H, H_α), 6.47 (d, J = 15.8 Hz, 1H, H_β), 6.55 (d, J = 2.3 Hz, 1H, H10), 6.60 (d, J = 3.0 Hz, 1H, H1), 6.72 (dd, J = 3.0, 8.6 Hz, 1H, H3), 6.80 (dd, J = 2.3, 8.3 Hz, 1H, H8), 6.87 (d, J = 8.6 Hz, 1H, H4), 6.96 (d, J = 8.3 Hz, 1H, H7), 7.24 (d, J = 8.6 Hz, 2H, H2, H6), 7.37 (d, J = 8.3 Hz, 2H, H3, H5), 7.41 (d, J = 8.3 Hz, 2H, H2, H6), 7.44 (d, J = 8.6 Hz, 2H, H3, H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.6 (C12), 37.8 (C11a), 42.3 (C11), 55.5 (CH_3O), 55.6 (CH_3O), 100.4 (C5a), 113.6 (C1), 113.7 (C10), 114.0 (C3), 114.4 (C8), 117.2 (C7), 117.5 (C4), 121.3 (C4), 121.6 (C12a), 122.1 (C10a), 123.1(C4), 125.8 (C2, C6), 127.8 (C2, C6), 128.5 (C_α), 131.6 (C3, C5), 131.7 (C3, C5), 133.2 (C_β), 135.4 (C1), 139.5 (C1), 145.5 (C4a), 145.9 (C6a), 154.2 (C9), 154.3 (C2); HRMS (ESI) m/z Calcd for $C_{32}H_{26}{}^{79}Br_2O_4Na$ $(M+Na)^+$ 655.0098. Found 655.0086); Anal. Calcd for $C_{32}H_{26}Br_2O_4$: C, 60.59; H, 4.13. Found: C, 60.32; H, 4.13.

4.1.2.3. (*E*)-5a-(4-Chlorophenyl)-11-(4-chlorostyryl)-2,9-dimethoxy-11a,12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7c). White solid, Yield: 74%; mp 180–182 °C; UV (MeOH): λ_{max} 203 (ε 11232 cm^{-1} M^{-1}), 221 (6487), 268 (18721) nm; IR (KBr): ν_{max} 3434, 3003, 2942, 2833, 1614, 1492, 1464, 1426, 1278, 1231, 1197, 1152, 1038, 976, 953, 820, 809 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (s, 3H, H11a, H12), 3.46 (dd, J = 3.4, 9.0 Hz, 1H, H11), 3.70 (s, 3H, CH_3O), 3.73 (s, 3H, CH_3O), 6.23 (dd, J = 9.0, 15.8 Hz, 1H, H_α), 6.48 (d, J = 15.8 Hz, 1H, H_β), 6.56 (d, J = 3.0 Hz, 1H, H10), 6.60 (d, J = 3.0 Hz, 1H, H1), 6.72 (dd, J = 3.0, 8.7 Hz, 1H, H3), 6.80 (dd, J = 3.0, 9.0 Hz, 1H, H8), 6.87 (d, J = 8.7 Hz, 1H, H4), 6.97 (d, J = 9.0 Hz, 1H, H7), 7.25 (d, J = 8.3 Hz, 2H, H2, H6), 7.28 (d, J = 8.3 Hz, 2H, H3, H5), 7.34 (d, J = 8.6 Hz, 2H, H3, H5), 7.45 (d, J = 8.6 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.6 (C12), 37.8 (C11a), 42.3 (C11), 55.5 (CH_3O), 55.6 (CH_3O), 100.4 (C5a), 113.6 (C1), 113.7 (C10), 114.0 (C3), 114.3 (C8), 117.2 (C4), 117.5 (C7), 121.3 (C12a), 122.2 (C10a), 127.5 (C3, C5), 128.0 (C_α), 128.3 (C2, C6), 128.7 (C3, C5), 128.8 (C2, C6), 133.1 (C_β), 133.4 (C4), 134.8 (C4), 135.0 (C1), 138.9 (C1), 145.5 (C4a), 145.9 (C6a), 154.2 (C9), 154.3 (C2); HRMS (ESI) m/z Calcd for $C_{32}H_{26}{}^{35}Cl_2O_4Na$ $(M+Na)^+$ 567.1108. Found 567.1095; Anal. Calcd for $C_{32}H_{26}Cl_2O_4.EtOH$: C, 69.04; H, 5.45. Found: C, 69.27; H, 5.14.

4.1.2.4. (*E*)-2,9-Dimethoxy-11-(4-methylstyryl)-5a-*p*-tolyl-11a,12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7d). White solid, Yield: 72%; mp 217–219 °C; UV (MeOH): λ_{max} 204 (ε 31109 cm^{-1} M^{-1}), 229 (15067), 261 (11049), 290 (5107) nm; IR (KBr): ν_{max} 3435, 2998, 2922, 2832, 1614, 1492, 1464, 1427, 1272, 1232, 1200, 1153, 1039, 971, 951, 820, 803 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.85 (s, 3H, H11a, H12), 3.50 (dd, J = 3.0, 9.4 Hz, 1H, H11), 3.69 (s, 3H, CH_3O), 3.73 (s, 3H, CH_3O), 6.22 (dd, J = 9.4, 15.8 Hz, 1H, H_α), 6.43 (d, J = 15.8 Hz, 1H, H_β), 6.53 (d, J = 3.0 Hz, 1H, H10), 6.60 (d, J = 3.0 Hz, 1H, H1), 6.72 (dd, J = 3.0, 9.0 Hz, 1H, H3), 6.78 (dd, J = 3.0, 8.7 Hz, 1H, H8), 6.87 (d, J = 9.0 Hz, 1H, H4), 6.97 (d, J = 8.7 Hz, 1H, H7), 7.14 (d, J = 8.3 Hz, 2H, H3, H5), 7.18 (d, J = 8.3 Hz, 2H, H3, H5), 7.29 (d, J = 8.3 Hz, 2H, H2, H6), 7.42 (d, J = 8.3 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9 (CH_3), 21.2 (CH_3), 23.9 (C12), 37.8 (C11a), 42.4 (C11), 55.5 (CH_3O), 55.6 (CH_3O), 100.9 (C5a), 113.5 (C1), 113.7 (C10), 113.9 (C3), 114.2 (C8), 117.1 (C7), 117.5 (C4), 121.6 (C12a), 122.7 (C10a), 125.8 (C3, C5), 126.5 (C2, C6), 128.8 (C_α), 129.0 (C2, C6), 129.2 (C3, C5), 133.9 (C_β), 134.0 (C1), 137.4 (C4), 137.5 (C4), 138.6 (C1), 145.7 (C4a), 146.3 (C6a), 153.9 (C9), 154.1 (C2); HRMS (ESI) m/z Calcd for $C_{34}H_{32}O_4Na$ $(M+Na)^+$ 527.2201. Found 527.2187; Anal. Calcd for $C_{34}H_{32}O_4.EtOH$: C, 78.52; H, 6.96. Found: C, 78.71; H, 6.66.

4.1.2.5. (*E*)-1,10-Dimethoxy-5a-phenyl-11-styryl-11a,12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7e). White solid, Yield: 75%; mp 127–129 °C; UV (MeOH): λ_{max} 206 (ε 48225 cm^{-1} M^{-1}), 258 (10664) nm; IR (KBr): ν_{max} 3418, 3026, 2933, 2836, 1594, 1489, 1469, 1438, 1349, 1266, 1208, 1096, 1029, 961, 830, 780 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.71 (dd, J = 12.4, 17.3 Hz, 1H, H12), 2.79 (d, J = 17.3 Hz, 1H, H12), 2.99 (dd, J = 6.4, 12.4 Hz, 1H, H11a), 3.64 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 3.78 (dd, J = 6.4, 9.0 Hz, 1H, H11), 6.19 (dd, J = 9.0, 15.4 Hz, 1H, H_α), 6.33 (d, J = 15.4 Hz, 1H, H_β), 6.39 (d, J = 8.3 Hz, 1H, H2), 6.51 (d, J = 8.3 Hz, 1H, H9), 6.69 (d, J = 8.3 Hz, 1H, H4), 6.72 (d, J = 8.3 Hz, 1H, H7), 7.06 (t, J = 8.3 Hz, 2H, H3, H8), 7.10–7.56 (m, 10H, H2, H3, H4, H5, H6, H2, H3, H4, H5, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.3 (C12), 37.0 (C11a), 38.8 (C11), 55.3 (CH_3O), 55.6 (CH_3O), 100.5 (C5a), 103.0 (C2), 104.0 (C9), 109.1 (C4), 109.7 (C7), 110.7 (C12a), 111.3 (C10a), 125.7 (C4), 125.9 (C2, C6), 126.0 (C3), 126.6 (C8), 127.9 (C_α), 128.1 (C4), 128.3 (C3, C5), 128.5 (C2, C6), 129.7 (C3, C5), 131.2 (C_β), 136.9 (C1), 140.5 (C1), 152.8 (C4a), 153.7 (C6a), 157.4 (C1), 158.7 (C10); HRMS (ESI) m/z Calcd for $C_{32}H_{28}O_4Na$ $(M+Na)^+$ 499.1888. Found 499.1878; Anal. Calcd for $C_{32}H_{28}O_4.1/4EtOH$: C, 79.98; H, 6.09. Found: C, 80.18; H, 6.32.

4.1.2.6. (*E*)-5a-(4-Bromophenyl)-11-(4-bromostyryl)-1,10-dimethoxy-11a,12-dihydro-5a*H*, 11*H*-chromeno[2,3-*b*]chromene (7f). White solid, Yield: 68%; mp 118–120 °C; UV (MeOH): λ_{max} 204 (ε 103747 cm^{-1} M^{-1}), 265 (11329) nm; IR (KBr): ν_{max} 3459, 3005, 2932, 2830, 1593, 1487, 1468, 1350, 1269, 1210, 1088, 1054, 978, 832, 770 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.59 (dd, J = 12.0, 17.7 Hz, 1H, H12), 2.77 (d, J = 17.7 Hz, 1H, H12), 2.94 (dd, J = 6.4, 12.0 Hz, 1H, H11a), 3.61 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 3.72 (dd, J = 6.4, 9.0 Hz, 1H, H11), 6.05 (dd, J = 9.0, 15.8 Hz, 1H, H_α), 6.30 (d, J = 15.8 Hz, 1H, H_β), 6.35 (d, J = 8.3 Hz, 1H, H2), 6.49 (d, J = 8.3 Hz, 1H, H9), 6.64 (d, J = 8.3 Hz, 2H, H4, H7), 6.79 (t, J = 8.3 Hz, 2H, H3, H8), 7.06 (d, J = 8.3 Hz, 2H, H2, H6), 7.16 (d, J = 8.3 Hz, 2H, H3, H5), 7.27 (d, J = 8.3 Hz, 2H, H2, H6), 7.49 (d, J = 8.3 Hz, 2H, H3, H5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.5 (C12), 36.7 (C11a), 38.8 (C11), 55.3 (2×CH_3O), 99.7 (C5a), 103.4 (C2), 104.3 (C9), 107.5 (C4), 108.8 (C7), 109.4 (C12a), 110.8 (C10a), 119.9 (C4), 122.8 (C4), 126.5 (C3), 127.8 (C2, C6), 128.0 (C2, C6), 128.6 (C_α), 128.9 (C8), 130.4 (C3, C5), 131.0 (C3, C5), 131.9 (C_β), 135.9 (C1), 139.8 (C1), 152.3 (C4a), 153.8 (C6a), 157.8 (C1), 158.4 (C10); MS (TOF-ESI) m/z Calcd for $C_{32}H_{26}{}^{79}Br_2O_4$ $(M+1)^+$ 633.03. Found 633.02; Anal. Calcd for $C_{32}H_{26}Br_2O_4$: C, 60.59; H, 4.13. Found: C, 60.32; H, 4.36.

4.1.2.7. (*E*)-5a-(4-Chlorophenyl)-11-(4-chlorostyryl)-1,10-dimethoxy-11a,12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7g). White solid, Yield: 72%; mp 120–122 °C; UV (MeOH): λ_{max} 205 (ε 56444 cm^{-1} M^{-1}), 264 (15800) nm; IR (KBr): ν_{max} 3420, 3008, 2930, 2837, 1594, 1491, 1469, 1439, 1347, 1267, 1210,

1094, 1052, 967, 831, 772 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.59 (dd, J = 11.7, 17.3 Hz, 1H, H12), 2.77 (d, J = 17.3 Hz, 1H, H12), 2.91 (dd, J = 6.4, 11.7 Hz, 1H, H11a), 3.61 (s, 3H, CH_3O), 3.70 (s, 3H, CH_3O), 3.74 (dd, J = 6.4, 9.0 Hz, 1H, H11), 6.04 (dd, J = 9.0, 15.8 Hz, 1H, H_α), 6.29 (d, J = 15.8 Hz, 1H, H_β), 6.32 (d, J = 8.3 Hz, 1H, H2), 6.49 (d, J = 8.3 Hz, 1H, H9), 6.65 (d, J = 8.3 Hz, 2H, H4, H7), 6.85 (t, J = 8.3 Hz, 2H, H3, H8), 7.11 (d, J = 8.3 Hz, 2H, H2, H6), 7.18 (d, J = 8.3 Hz, 2H, H3, H5), 7.29 (d, J = 8.7 Hz, 2H, H3, H5), 7.44 (d, J = 8.7 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8 (C12), 36.4 (C11a), 38.5 (C11), 55.3 (CH_3O), 55.6 (CH_3O), 100.0 (C5a), 103.1 (C2), 104.0 (C9), 108.9 (C7), 109.7 (C4), 110.5 (C12a), 111.0 (C10a), 127.1 (C3), 127.3 (C3, C5), 127.5 (C3, C5), 128.1 (C8), 128.4 (C_α), 128.6 (C2, C6), 128.9 (C2, C6), 131.3 (C_β), 132.1 (C4), 134.6 (C4), 136.2 (C1), 139.2 (C1), 152.3 (C4a), 153.7 (C6a), 157.3 (C1), 158.4 (C10); HRMS (ESI) *m/z* Calcd for $C_{32}H_{26}{}^{35}Cl_2O_4Na$ $(M+Na)^+$ 567.1108. Found 567.1079; Anal. Calcd for $C_{32}H_{26}Cl_2O_4.1/4H_2O$: C, 69.89; H, 4.86. Found: C, 70.08; H, 4.87.

4.1.2.8. (*E*)-1,10-Dimethoxy-5a-(4-methoxyphenyl)-11-(4-methoxystyryl)- 11a,12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7h). White solid, Yield: 76%; mp 134–136 °C; UV (MeOH): λ_{max} 204 (ε 36939 cm^{-1} M^{-1}), 267 (9405) nm; IR (KBr): ν_{max} 3429, 3001, 2933, 2835, 1594, 1489, 1468, 1439, 1348, 1251, 1215, 1089, 1034, 981, 834, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.70 (dd, *J* = 12.1, 17.1 Hz, 1H, H12), 2.81 (d, *J* = 17.1 Hz, 1H, H12), 2.91 (dd, *J* = 6.4, 12.1 Hz, 1H, H11a), 3.61 (s, 3H, CH_3O), 3.68 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3O), 3.79 (s, 3H, CH_3O), 3.80 (dd, *J* = 6.4, 9.8 Hz, 1H, H11), 6.04 (dd, *J* = 9.8, 16.2 Hz, 1H, H_α), 6.31 (d, *J* = 16.2 Hz, 1H, H_β), 6.36 (d, *J* = 8.3 Hz, 1H, H2), 6.47 (d, *J* = 8.3 Hz, 1H, H9), 6.60–6.86 (m, 4H, H3, H4, H7, H8), 6.94 (d, *J* = 8.7 Hz, 2H, H3, H5), 7.15 (d, *J* = 9.0 Hz, 2H, H3, H5), 7.37 (d, *J* = 8.7 Hz, 2H, H2, H6), 7.44 (d, *J* = 9.0 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.9 (C12), 38.8 (C11a), 39.8 (C11), 55.1 (CH_3O), 55.2 (CH_3O), 55.4 (2×CH_3O), 99.6 (C5a), 102.8 (C2), 103.6 (C9), 109.5 (C4), 109.7 (C7), 113.5 (C12a), 113.6 (C10a), 126.9 (C3, C5), 127.0 (C3), 127.2 (C3, C5), 127.8 (C8), 128.3 (C_α), 128.8 (C2, C6), 129.9 (C2, C6), 131.8 (C_β), 136.3 (C1), 139.6 (C1), 152.8 (C4a), 152.9 (C6a), 157.6 (C1), 158.5 (C10), 158.9 (C4), 159.7 (C4); HRMS (ESI) *m/z* Calcd for $C_{34}H_{32}O_6Na$ $(M+Na)^+$ 559.2099. Found 559.2071; Anal. Calcd for $C_{34}H_{32}O_6$: C, 76.10; H, 6.01. Found: C, 76.22; H, 6.29.

4.1.2.9. (*E*)-1,10-Dimethoxy-11-(4-methylstyryl)-5a-*p*-tolyl-11a, 12-dihydro-5a*H*,11*H*-chromeno[2,3-*b*]chromene (7i). White solid, Yield: 74%; mp 186–188 °C; UV (MeOH): λ_{max} 204 (ε 50304 cm^{-1} M^{-1}), 261 (11142) nm; IR (KBr): ν_{max} 3432, 3023, 2933, 2837, 1593, 1485, 1468, 1437, 1350, 1268, 1208, 1087, 1055, 976, 825, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.63 (dd, *J* = 12.4, 17.3 Hz, 1H, H12), 2.72 (d, *J* = 17.3 Hz, 1H, H12), 2.81 (dd, *J* = 6.3, 12.4 Hz, 1H, H11a), 3.61 (dd, *J* = 6.3, 8.3 Hz, 1H, H11), 3.66 (s, 3H, CH_3O), 3.78 (s, 3H, CH_3O), 5.33 (dd, *J* = 8.3, 15.5 Hz, 1H, H_α), 6.09 (d, *J* = 15.5 Hz, 1H, H_β), 6.47 (d, *J* = 8.3 Hz, 2H, H2, H9), 6.61 (d, *J* = 8.3 Hz, 1H, H4), 6.70 (d, *J* = 8.3 Hz, 1H, H7), 6.87 (t, *J* = 8.3 Hz, 2H, H3, H8), 7.00 (d, *J* = 8.3 Hz, 2H, H3, H5), 7.08 (d, *J* = 8.7 Hz, 2H, H3, H5), 7.16 (d, *J* = 8.3 Hz, 2H, H2, H6), 7.33 (d, *J* = 8.7 Hz, 2H, H2, H6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.0 (2×CH_3), 22.9 (C12), 38.7 (C11a), 39.8 (C11), 55.3 (CH_3O), 55.4 (CH_3O), 99.7 (C5a), 102.7 (C2), 103.5 (C9), 109.5 (C4), 109.6 (C7), 109.7 (C12a), 111.5 (C10a), 125.8 (C3, C5), 126.9 (C_α), 127.2 (C3), 128.3 (C8), 128.8 (C2, C6), 129.0 (C2, C6), 129.3 (C3, C5), 130.9 (C_β), 133.9 (C1), 136.2 (C4), 137.5 (C4), 138.5 (C1), 152.7 (C4a), 152.9 (C6a), 157.5 (C1), 158.9 (C10); HRMS (ESI) *m/z* Calcd for $C_{34}H_{32}O_4Na$ $(M+Na)^+$ 527.2201. Found 527.2193; Anal. Calcd for $C_{34}H_{32}O_4$.EtOAc: C, 77.00; H, 6.80. Found: C, 77.30; H, 6.54.

4.2. Biological experiments

4.2.1. Method for in vitro antiplasmodial growth inhibition assay

The *P. falciparum* growth inhibition assays were carried using an isotopic microtest, as previously described.[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. Following 48 h incubation under standard *P. falciparum* culture conditions, 0.5 μCi [^3H]-hypoxanthine was added to each well after which the plates were cultured for a further 24 h. Cells were harvested onto 1450 MicroBeta filter mats (Wallac) and ^3H 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.[24] The mean IC_{50} is shown for two independent experiments, each carried out in triplicate.

4.2.2. Method for in vitro cytotoxicity assay[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 (3000/well) into 96-well tissue culture plates (Corning, USA) and were grown for 24 h before treatment. Compounds were dissolved in 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.[25] Compounds were tested in triplicate in three independent experiments.

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