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Facile Formation of Methylenebis(chalcone)s through Unprecedented Methylenation Reaction. Application to Antiparasitic and Natural Product Synthesis

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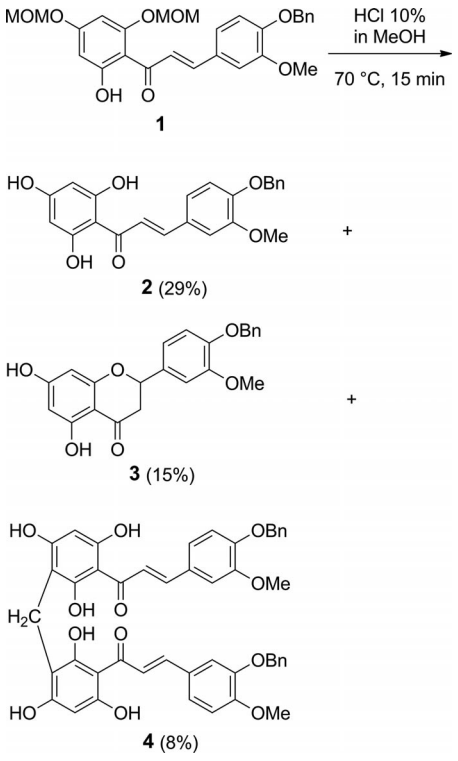
The formation of methylenebis(chalcone)s has been discovered during deprotection with methoxymethyl groups from trihydroxychalcones. Studies on this methylenation reaction led to a mechanism hypothesis that was extended to other chalcones and to dihydrochalcone, acetophenone, benzophenone and flavone derivatives. This new method was applied to the rapid synthesis of natural product piperanduncin C. These original methylenebis compounds were also evaluated for their antiparasitic activity.

Introduction

Protection of hydroxyl groups is widely carried out in multistep organic syntheses. Among common protective groups, methoxymethyl (MOM) ether was described as an easily prepared and removed group.[1] During the total synthesis of a natural flavonoid possessing an antiparasitic activity, we needed to protect and deprotect 2′,4′,6′-trihydroxychalcone **1** (Scheme 1) with a MOM group. The MOM group removal was classically performed with 10% HCl in MeOH leading to desired deprotected chalcone **2** together with two side products. The first side product was designated as flavanone **3**, the formation of which from the 2′-hydroxychalcone derivative in acidic medium is well known.[2] The second compound was isolated and characterized as methylenebis(chalcone) **4**.

To the best of our knowledge, methylenation reaction between two trihydroxychalcone units has never been described under conditions for MOM group removal. Moreover few syntheses of methylenebis(chalcone)s have been published so far.[3] Notably, Reddy et al. obtained bis(chalcone)s from benzaldehydes in the presence of trioxane and sulfuric acid.[4]

Nevertheless, some natural products that contain a methylene bridge were extracted from plants. For instance, bis(flavonoid)s were isolated from a western North America fern, *Pentagramma triangularis*,[5] or a bis(dihydrochalcone) from the slender tree *Piper adundum* has been described by Orjala (piperaduncin C, Figure 1).[6] Recently, Anis and Ali discovered methylenebis(santin) from the shrub *Dodonaea viscosa* (Figure 1).[7]



Scheme 1. Deprotection of MOM group from chalcone **1**.

Therefore, we decided that this original reaction deserved further investigation to facilitate total synthesis of such natural compounds. We present here our study on the formation of methylenebis compounds under MOM group removal conditions, and on its scope and limitations.

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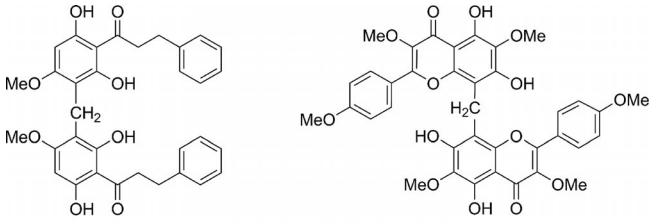
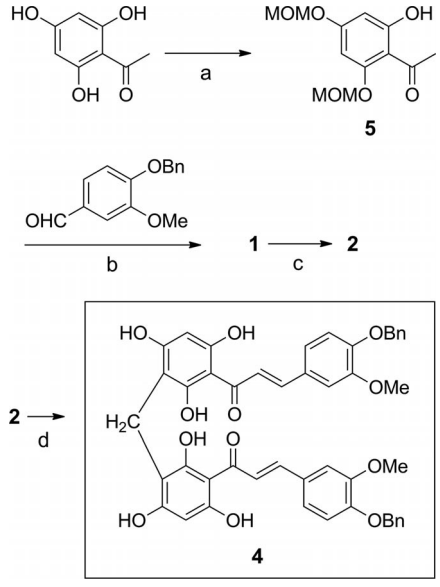


Figure 1. Methylene-bridged bis(flavonoid)s isolated from plants.

Results and Discussion

Synthesis

We first looked at the acidic conditions required to obtain compound **4** from chalcone **1** that was prepared according to Scheme 2. After protection of phloroacetophenone by using MOMCl, acetophenone **5** reacted with aldehyde **6** to afford chalcone **1** by base-catalyzed Claisen condensation reaction in good yield. By optimizing the methylenation reaction conditions, we noticed that an HCl concentration lower than 3 N did not afford compound **4**, and deprotected chalcone **2** and flavanone **3** were mainly obtained. On the contrary, if the HCl solution was more concentrated than 3 N, degradation was observed. Finally, we chose to continue our investigation by using a 3 N concentration. Then, to verify if hydrogen chloride was the only acid that could be used for this reaction from protected chalcone **1**, various acids were tested. Surprisingly, trifluoroacetic acid, acetic acid and triflic acid did not enable CH_2-bridge formation. Indeed, no methylenebis product **4** was observed but mostly starting material **1** and/or a mixture of deprotected chalcone **2** and flavanone **3**. Therefore, hydrogen chloride was required in the methylene reaction mechanism. When *p*-toluenesulfonic acid (PTSA) in EtOH/CH_2Cl_2 (7:1, v/v) was used deprotected chalcone **2** was quantitatively produced in under 2 hours without any side products (Scheme 2).



Scheme 2. Synthetic pathway to methylenebis(chalcone) **4**. Reagents and reaction conditions: (a) MOMCl, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 0 °C to room temp., 2.5 h, 72%; (b) KOH, EtOH, 70 °C, 4 d, 60%; (c) PTSA.H_2O, EtOH/CH_2Cl_2 (7:1, v/v), 1.5 h, 100%; (d) See Table 1.

It was expected that a MOM degradation product would be responsible for methylenation, therefore the reactivity of chalcone **2** in the presence of MOMCl in acidic medium was studied. Thus a mixture of chalcone **2** and MOMCl in 3 N HCl in MeOH was heated to reflux. After 10 min, all starting material disappeared and methylenebis(chalcone) **4** was obtained in almost quantitative yield (Table 1, Entry 1). Hence, chloromethyl methyl ether proved to be the methylene source of this reaction when HCl and methanol were present in the reaction medium. As previously described for compound **1**, the reaction was carried out with various acids under these conditions. In all tests methylenebis compound **4** was detected in the reaction mixture. In the presence of acetic acid or triflic acid a mixture of compounds **2**, **3** and **4** was obtained. Nevertheless, trifluoroacetic acid was the only other acid able to afford compound **4** in good yield (74%) as the major product (Table 1, Entry 3). To avoid the use of the very toxic commercial solution of MOMCl, we generated it from dimethoxymethane in the presence of acetyl chloride (Table 1, Entry 2).[8] As expected, the methylenation reaction turned out well.

Table 1. Reaction conditions to afford methylenebis(chalcone) **4** from trihydroxychalcone **2**.

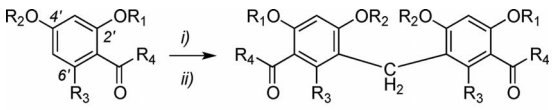
Entry	Conditions	Yield
1	MOMCl, 3 N HCl in MeOH, 70 °C, 15 min	98%
2	(i) $CH_2(OMe)_2$, AcCl, $ZnBr_2$ cat., CH_2Cl_2, room temp., 5 h; (ii) **2**, HCl in MeOH, 70 °C, 10 min	97%
3	MOMCl, TFA, MeOH, 70 °C, 20 min	74%[a]
4	HCHO, HCl, MeOH, room temp., 4 h	79%

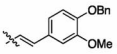
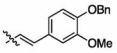
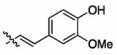
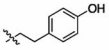
[a] Yields calculated from NMR spectroscopic data.

Therefore all experiments for the formation of methylene-bridged compounds presented in Table 2 were performed with in situ formed MOMCl.

Based on previous results[9] and on our work, we suggested a possible mechanism for methylenation reaction in three steps (Scheme 3). The first step, consisting of the removal of the MOM group by HCl, leads to the formation of a methoxymethylene cation.[9a] Then, this carbocation reacts with water in acidic medium to give methoxymethanol by releasing HX (Scheme 3, Equation 1), In the presence of any acid (H^+X^-), this molecule decomposes into methanol and formaldehyde.[9b] In the early eighties, Bigi proposed a mechanism in which phenol in the presence of formaldehyde formed a methylene-bridged dimer.[9c] Based on this hypothesis, we could easily explain the formation of the methylenebis compound [(Scheme 3, Equation 3). To confirm that formaldehyde was the CH_2 source in our case, the methylenation reaction was performed in the presence of HCHO and HCl in MeOH. However, when the reaction

Table 2. Methylenation reaction on phloroacetophenone, chalcones, dihydrochalcones and benzophenones.[a]



Entry		Monomer 7 R_1	R_2	R_3	R_4	Yield of **8** (%)
1	**a**	H	H	OH		65[b]
2	**b**	Me	H	OH		91
3	**c**	Me	Me	OH		27[b]
4	**d**	Me	Me	OMe		0
5	**e**	H	H	OH		80[b]
6	**f**	H	Me	OH		61
7	**g**	H	H	OH		29[b]
8	**h**	H	H	OH		73
9	**i**	H	Me	OH		50
10	**j**	H	H	OH		29
11	**k**	H	H	OH	H	24
12	**l**	H	H	H		traces

[a] Reactions were carried out with (i) $CH_2(OMe)_2$ (4 equiv.), AcCl, $ZnBr_2$ cat., CH_2Cl_2, room temp., 5 h; (ii) **7**, 3 N HCl in MeOH, 70 °C, 5 min to 2 h. [b] Yields calculated from NMR spectroscopic data.

medium was heated at 70 °C under these conditions, degradation was observed. Therefore the reaction was carried out at room temperature and consequently required longer reaction times to obtain compound **4** in good yield (Table 1, Entry 4). Therefore we demonstrated that formaldehyde could be the methylene source in this reaction, supporting our proposed mechanism. An alternative mechanism could be proposed in which the methoxymethylene cation reacts directly with compound **7** before transformation into formaldehyde. However there is no evidence for either mechanism. We think that the involvement of formaldehyde is more probable because our reaction was not carried out under anhydrous conditions and water is present in the reaction medium because concentrated aqueous HCl was used to make HCl 3 N in methanol.

To extend the method and to see its limits, some chalcones, dihydrochalcones, benzophenones, phloroacetophenone and a flavone were submitted to methylenation reaction conditions. The structure of the methylene bridged-derivatives was confirmed by X-ray analysis of methylenebis(chalcone) **8a** (Figure 2). Then the role of hydroxyl groups in 2′,4′ and/or 6′ positions was investigated by replacing them by one to three methoxy groups.

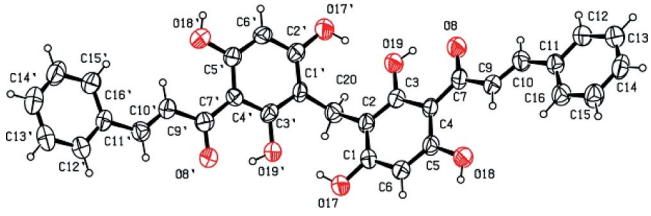
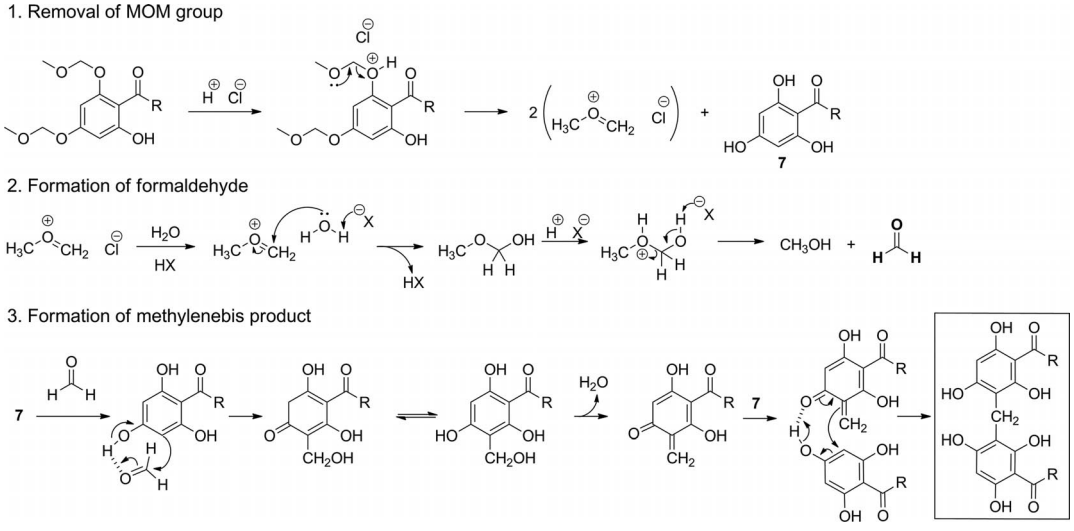


Figure 2. ORTEP representation of methylenebis(chalcone) **8a**.

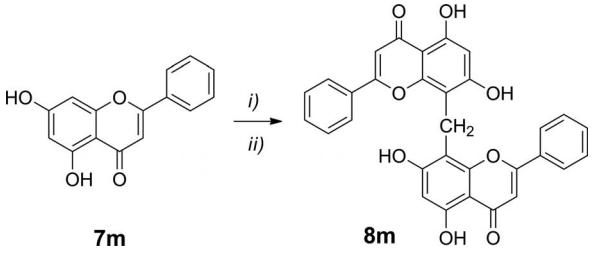
The presence of at least one free hydroxyl group is required to undergo methylenation as shown by the absence of conversion observed with 2′,3′,4′-trimethoxychalcone **7d** (Table 2, Entry 4). In cases in which there was at least one hydroxyl group available, a methylene bridge was created between the two monomer units under our conditions. Gen-



Scheme 3. Plausible mechanism for the methylenation reaction from MOM-protected acetophenones, chalcones, dihydrochalcones or benzophenones.

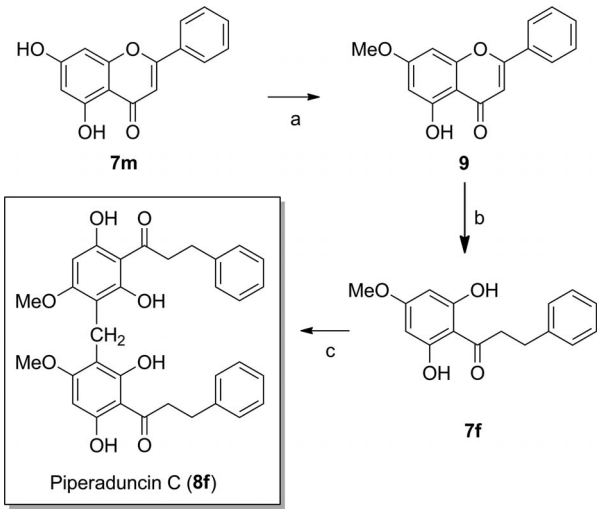
erally, good to excellent yields were obtained with 2′,4′,6′-trisubstituted substrates bearing two OH groups in relative *meta* position (65–91%, Table 2, Entries 1, 2, 5 and 8) except for dihydrochalcones **7g** and **7j** which bear free hydroxyl groups on the B cycle and for phloroacetophenone 7**k** (Table 2, Entries 7, 10, 11). However, when two out of three hydroxyl groups were methylated, much lower conversion was noted (Table 2, Entry 3). Likewise, when 4′-OH involved in our proposed mechanism was absent, methylenation yield decreased (Table 2, Entry 8 relative to Entry 9).

In the case of chalcone **6c**, the only available OH group was located at the *ortho* position relative to the carbonyl moiety and was thus involved in a hydrogen bound, making it less reactive. That is why, we suggested that formaldehyde preferentially reacts with the OH group at the 4′-position and in its absence, the reaction would occur at the other positions but more sluggishly. Consequently, methylenebis products were obtained in lower yields as observed for methylenebis(chalcone)s **8c** and **8i** (Table 2, Entries 3 and 9) relative to their trihydroxy analogues **4** and **8h** (Table 1, Entry 1, and Table 2, Entry 8), respectively. Although methylenation readily took place with dihydrochalcones **7e** and **7f** (Table 2, Entries 5 and 6), modest yields were obtained for the synthesis of bis(dihydrochalcone)s **8g** and **8j** (Table 2, Entries 7 and 10) or bis(acetophenone) **8k** (Table 2, Entry 11). Indeed, in those cases, the reaction was not complete but longer reaction times only led to degradation product formation. Likewise, the conversion was low with commercially available 2′,4′-dihydroxychalcone **7l** (Table 2, Entry 12) or chrysin **7m** (Scheme 4), partly owing to starting material poor solubility. Conditions would have to be optimized in those cases. Hence the presence of three free hydroxyl groups seemed to greatly facilitate the methylene bridge formation. Besides, it should be noted that solubility issues of final products led to challenging purification and consequently were responsible for significant differences between the calculated NMR spectroscopic and isolated yields.



Scheme 4. Methylenation reaction from chrysin **7m**. Reagents and reaction conditions: (i) $CH_2(OMe)_2$ (4 equiv.), AcCl, $ZnBr_2$ cat., CH_2Cl_2, room temp., 5 h; (ii) Chrysin **7m**, 3 N HCl in MeOH, MW (70 °C, 10 min), 1% and 10% isolated and calculated NMR spectroscopic yields, respectively.

The optimization of this methylenation reaction was applied to the rapid synthesis of a natural compound, piperaduncin C (Figure 1). After methylation of chrysin, flavone **9** was submitted to hydrogenation to obtain dihydrochalcone **7f** that was submitted to methylenation affording piperaduncin C (**8f**; Scheme 5). Hence the total synthesis of this antibacterial methylenebis(dihydrochalcone)[6] was performed in only three steps with 37% overall yield.



Scheme 5. Total synthesis of piperaduncin C (**8f**). Reagents and reaction conditions: (a) MeI (1.1 equiv.), K_2CO_3 (1.1 equiv.), dimethylformamide, room temp., 24 h, 66%; (b) NH_4HCO_2 (9 equiv.), 10% Pd/C, reflux, 2 h, 92%; (c) (i) $CH_2(OMe)_2$ (4 equiv.), AcCl, $ZnBr_2$ cat., CH_2Cl_2, room temp., 5 h; (ii) **7f**, 3 N HCl in MeOH, 70 °C, 1.5 h, 61%.

Biological Evaluation

As part of our work on antiparasitic agents, these new derivatives deserved to be evaluated for their inhibitory activity against parasitic growth. Therefore, some of them were assayed on the intraerythrocytic stages of *Plasmodium falciparum*,[9] responsible for malaria and in the bloodstream forms of *Trypanosoma brucei gambiense*, the pathogenic agent of African sleeping sickness.[10] To see whether methylenation would be beneficial to activity, the corresponding monomers were also evaluated. Results are summarized in Table 3.

In general, bis(benzophenone) derivatives **8h**–**8j** and bis(acetophenone) **8k** along with their respective monomers displayed no antiparasitic activity. On the contrary, chalcone, dihydrochalcone and flavone derivatives were generally active against both parasites, except chalcone **7a** and chrysin **7m**. Therefore, a longer link between A and B cycles seemed to be required to display inhibition. Moreover, we observed that for all chalcones, the most potent compounds of this series, the formation of the methylene bridge between two monomer units was beneficial to activity on both parasites. Thus methylenebis(chalcone)s **4**, **8a** and **8b** displayed better results than their corresponding precursors **2**, **7a** and **7b** (Table 3). Notably, dimer **8a** became a potential growth inhibitor of *P. falciparum* and *T. brucei* after methylenation of inactive monomer **7a**. Likewise, bis(flavone) **8m** showed antiplasmodial activity whereas chrysin **7m** demonstrated no significant inhibition of *Plasmodium falciparum* proliferation. Finally, methylenebis(chalcone) **8b** possessing

Table 3. Inhibitory activity of monomer units and their methylenebis derivatives.

	IC_{50} on *T. brucei* [μM]	IC_{50} on *P. falciparum* [μM]
2	8.2 ± 0.3	27.4 ± 3.2
4	5.8 ± 0.5	7.7[a]
7a	> 50	> 50
8a	12.7 ± 1.1	12.3[a]
7b	5.6 ± 0.2	34.8 ± 3.1
8b	1.0 ±0.1	8.4 ± 1.7
7e	17.4 ± 1.0	9.8 ± 0.3
8e	32.5 ± 2.1	9.6 ± 0.2
7f	7.8 ± 0.2	27 ± 2.3
8f	1.3 ± 0.1	7.8 ± 0.2
7g	30.1 ± 0.2	9.6 ± 0.5
8g	52.4 ± 1.1	29.8 ± 0.9
7h	> 50	> 50
8h	> 50	> 50
7j	> 50	> 50
8j	> 50	> 50
7k	> 50	> 50
8k	> 50	> 50
7m	14.4 ± 2.0	> 50
8m	30.9 ± 1.2	31.3[a]
Chloroquine		0.072 ± 0.0074
Pentamidine	0.011 ± 0.0017	

[a] Determined from only 2 experiments.

a methoxy group on 2′-position displayed the highest activities with IC_{50} = 1.0 ± 0.1 μM against *T. brucei gambiense*. The most potent antiplasmodial agent was bis(chalcone) **4** with IC_{50} = 7.7 μM.

Conclusions

In conclusion, we discovered a previously unreported, new reaction that occurs during MOM group removal. We synthesized various chalcone, dihydrochalcone, benzophenone, acetophenone and flavone derivatives to study the reactivity of these monomers and understand the mechanism that we herein propose. During deprotection with the MOM group, formaldehyde is formed. The next step seemed to require at least one free OH group in the monomer to occur. Though reaction conditions should be optimized for each derivative, this reaction provides a new method to synthesize bis(methylene) natural products, as was demonstrated by the rapid total synthesis of piperaduncin C. Furthermore, application of this new reaction on various flavonoids afforded some new methylene-bridged compounds that were evaluated for their inhibitory activity on parasite growth. All methylenebis(chalcone)s displayed higher activities than their monomer homologues. Finally, the most potent trypanocidal compound of this series, methylenebis(chalcone) **8b**, showed a promising activity with an IC_{50} value of 1.0 μM. Accordingly these encouraging results are paving the way for further investigations on antiparasitic activity of methylenebis(chalcone)s.

Experimental Section

General: All commercial reagents were used as received without further purification. Analytical thin-layer chromatography was carried out on pre-coated silica gel aluminum plates (SDS TLC plates, silica gel $60F_{254}$). Column chromatography was performed with pre-packed Redisep columns. Preparative TLC (PLC) was performed with Merck TLC with silica gel $60F_{254}$. NMR spectra, including ^1H, ^{13}C (HMQC and HMBC) experiments, were recorded with a Bruker Avance 300 (300 MHz) and Avance 500 (500 MHz) spectrometers. Chemical shifts are given in ppm relative to $CDCl_3$ (δ =7.26 ppm; 77.2 ppm), [D_6]acetone (δ =2.05 ppm; 30.5 ppm) or [D_6] Dimethyl sulfoxide (DMSO; δ = 2.50 ppm; 39.5 ppm). Splitting patterns are designed as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and combinations thereof. IR spectra were recorded with a Perkin–Elmer Spectrum BX. Mass spectra were recorded with Thermoquest AQA Navigator with a TOF detection (ESI-HRMS). UHPLC analyses were realized with Waters Acquity UPLC. Melting points were measured with a Büchi b-450 instrument.

The purity of all target compounds was measured by using reversed-phase UHPLC (HSS C-18, 2.1 × 50 mm column): compounds were eluted with 95:5 A/B for 0.5 min then with a gradient of 5–100% B/A for 3.5 min followed by 0:100 isocratic for 1 min at a flow rate of 0.6 mL/min, in which solvent A was 0.1% formic acid in H_2O, and solvent B was 0.1% formic acid in CH_3CN. Purity was determined with TAC (total absorbance current from 200 to 400 nM).

General Procedure for the Methylenation Reaction: Acetyl chloride (4 mmol) and $ZnBr_2$ (0.01 mol-%) were added under argon to a solution of dimethoxymethane (4 mmol) in CH_2Cl_2 (0.25 mL). The solution was stirred at room temperature under argon for 5 h. The resulting solution of in situ generated chloromethyl methyl ether (4 mmol) was then added to an HCl solution (3 N in MeOH, 12 mL) of the phenolic compound (1 mmol). The reaction mixture was stirred at 70 °C (unless otherwise indicated) until the reaction was complete. The resulting solution was cooled to room temperature. After addition of water to the reaction mixture, the precipitate (if formed, unless indicated) was filtered, washed with water and dried to yield the desired methylenebis product.

(2*E*,2′*E*)-1,1′-[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]bis{3-[4-(benzyloxy)-3-methoxyphenyl]prop-2-en-1-one} (4): General procedure (15 min at 70 °C) applied to chalcone **2** (50.0 mg) to afford methylenebis(chalcone) **4** (50.0 mg, 98%) as an orange amorphous solid. ^1H NMR ([D_6]DMSO): δ = 14.4 (s, 2 H), 10.8 (s, 2 H), 10.1 (s, 2 H), 8.04 (d, J = 15.5 Hz, 2 H), 7.66 (d, J = 15.5 Hz, 2 H), 7.47–7.34 (m, 10 H), 7.28 (d, J = 1.8 Hz, 2 H), 7.23 (dd, J = 8.4, 1.8 Hz, 2 H), 7.11 (d, J = 8.4 Hz, 2 H), 5.96 (s, 2 H), 5.15 (s, 4 H), 3.83 (s, 6 H), 3.64 (s, 2 H) ppm. ^{13}C NMR ([D_6]DMSO): δ = 191.7, 164.2, 163.0, 159.7, 149.8, 149.2, 141.6, 136.7, 128.4, 128.3, 127.9, 127.9, 125.6, 122.2, 113.4, 111.1, 105.7, 104.1, 94.6, 69.9, 55.5, 15.5 ppm. MS (ESI$^-$, MeOH + CH_2Cl_2): m/z = 795.2 [M – H]$^-$. HRMS: calcd. for $C_{47}H_{41}O_{12}^+$ [M + H]$^+$ 797.2598; found 797.2609. IR (neat): $\tilde{\nu}$ = 3240, 1719, 1604, 1250, 1220, 1086, 1026 cm^{-1}, m.p. 200 °C. UPLC method (H_2O/MeCN): room temp., 5.99 min, 94%.

(2*E*,2′*E*)-1,1′-[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]bis-(3-phenylprop-2-en-1-one) (8a): General procedure (10 min at 70 °C) applied to chalcone **7a** (100 mg). Purification by flash chromatography on silica gel [gradient heptane to heptane/EtOAc (6:4, v/v) in 25 min] to afford methylenebis(chalcone) **8a** (6.0 mg, isolated yield: 8%; NMR yield before purification: 65%) as an orange amorphous solid. ^1H NMR ([D_6]acetone): δ = 10.8 (s, 2 H), 8.32 (d, J = 15.5 Hz, 2 H), 7.87 (d, J = 15.5 Hz, 2 H), 7.73–7.71 (m, 4 H), 7.47–7.45 (m, 6 H), 6.12 (s, 2 H), 3.81 (s, 2 H) ppm. ^{13}C NMR ([D_6]acetone): δ = 193.7, 163.7, 143.9, 136.4, 131.3, 129.9, 129.4, 127.9, 106.7, 105.5, 97.1, 16.1 ppm. MS (ESI$^+$, MeOH + CH_2Cl_2):

m/*z* = 523.1 [M + H]$^+$. HRMS: calcd. for $C_{31}H_{25}O_8^+$ [M + H]$^+$ 523.1453; found 523.1393. IR (neat): ṽ = 3249, 1625, 1592, 1285, 1225, 1180, 1087 cm^{-1}, m.p. 145 °C. UPLC method (H_2O/MeCN): room temp., 5.52 min, 82%.

(2*E*,2′*E*)-1,1′-[Methylenebis(2,4-dihydroxy-6-methoxy-3,1-phenylene)]bis(3-phenylprop-2-en-1-one) (8b): General procedure (2 h at 70 °C) applied to chalcone **7b** (31.0 mg) to afford methylenebis(chalcone) **8b** (28.0 mg, 91%) as an orange amorphous solid. ^{1}H NMR ([D$_6$]DMSO): δ = 13.9 (s, 2 H), 9.91 (s, 2 H), 7.46 (d, *J* = 15.5 Hz, 2 H), 7.26 (d, *J* = 7.5 Hz, 4 H), 7.22 (d, *J* = 15.5 Hz, 2 H), 7.00–6.99 (m, 6 H), 5.57 (s, 2 H), 3.41 (s, 6 H), 3.27 (s, 2 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 191.6, 165.1, 163.4, 160.4, 141.2, 135.0, 130.2, 129.0, 128.3, 127.6, 106.8, 104.4, 91.0, 55.7, 15.6 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 553.2 [M + H]$^+$. HRMS: calcd. for $C_{33}H_{29}O_8^+$ [M + H]$^+$ 553.1862; found 553.1843. IR (neat): ṽ = 1741, 1624, 1591, 1285, 1232, 1118 cm^{-1}, m.p. 259 °C. UPLC method (H_2O/MeCN): room temp., 6.72 min, 92%.

1,1′-[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]bis[3-(4-hydroxy-3-methoxyphenyl)propan-1-one] (8e): General procedure (40 min at 70 °C) applied to dihydrochalcone **7e** (20.0 mg). Purification by flash chromatography on silica gel [gradient heptane to heptane/EtOAc (4:6, v/v) in 15 min] to afford methylenebis(dihydrochalcone) **8e** (2.0 mg, isolated yield: 10%; NMR yield before purification: 80%) as a pale yellow amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 14.5 (br. s, 2 H), 10.7 (s, 2 H), 9.53 (br. s, 2 H), 7.26 (s, 2 H), 6.88 (s, 2 H), 6.74–6.70 (m, 4 H), 6.07 (s, 2 H), 3.82 (s, 6 H), 3.76 (s, 2 H), 3.40 (t, *J* = 8.0 Hz, 4 H), 2.91 (t, *J* = 8.0 Hz, 4 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 206.7, 163.1, 162.7, 162.5, 148.2, 145.7, 133.9, 121.6, 115.7, 113.0, 106.4, 105.3, 96.8, 56.2, 46.8, 31.1, 15.9 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 621.2 [M + H]$^+$. HRMS: calcd. for $C_{33}H_{33}O_{12}^+$ [M + H]$^+$ 621.1972; found 621.2018. IR (neat): ṽ = 3272, 2923, 2853, 1603, 1515, 1452, 1365, 1259, 1207, 1123, 1081, 1032 cm^{-1}. UPLC method (H_2O/MeCN): room temp., 4.36 min, 100%.

Piperaduncin C (8f): General procedure (1.5 h at 70 °C) applied to dihydrochalcone **7f** (50.0 mg) to afford piperaduncin C (**8f**; 31.0 mg, 61%) as a beige amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 12.1 (br. s, 2 H), 11.5 (s, 2 H), 7.27–7.15 (m, 10 H), 6.11 (s, 2 H), 3.86 (s, 6 H), 3.80 (s, 2 H), 3.41 (t, *J* = 8.0 Hz, 4 H), 2.97 (t, *J* = 8.0 Hz, 4 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 206.1, 165.0, 163.6, 161.6, 142.9, 129.3, 129.2, 126.7, 107.5, 105.5, 92.3, 56.2, 46.6, 31.4, 15.8 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 557.2 [M + H]$^+$. HRMS: calcd. for $C_{33}H_{33}O_8^+$ [M + H]$^+$ 557.2175; found 557.2171. IR (neat): ṽ = 1622, 1422, 1218, 1140, 699 cm^{-1}, m.p. 169 °C; UPLC method (H_2O/MeCN): room temp., 6.07 min, 91%.

1,1′-[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]bis[3-(4-hydroxyphenyl)propan-1-one] (8g): General procedure (overnight at room temperature) applied to commercially available phloretin 7**g** (10.0 mg). Purification by flash chromatography on silica gel [gradient heptane to heptane/EtOAc (65:35, v/v) in 15 min] to afford methylenebis(phloretin) **8g** (1.0 mg, isolated yield: 10%; NMR yield before purification: 29%) as a beige amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 14.5 (br. s, 2 H), 10.7 (s, 2 H), 8.07 (s, 2 H), 7.10 (d, *J* = 8.0 Hz, 4 H), 6.75 (d, *J* = 8.0 Hz, 4 H), 6.06 (s, 2 H), 3.75 (s, 2 H), 3.39 (t, 4 H), 2.90 (t, 4 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 206.7, 163.2, 162.8, 162.6, 156.6, 133.3, 130.3, 116.1, 106.4, 105.3, 96.9, 46.9, 30.6, 16.0 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 561.2 [M + H]$^+$. HRMS: calcd. for $C_{31}H_{29}O_{10}^+$ [M + H]$^+$ 561.1760; found 561.1756. IR (neat): ṽ = 3197, 1603, 1515, 1454, 1259, 1091, 1015, 796 cm^{-1}, m.p. 225 °C. UPLC method (H_2O/MeCN): room temp., 4.24 min, 100%.

[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]bis(phenylmethanone) (8h): General procedure (4 days at room temperature) applied to benzophenone 7**h** (16.0 mg) to afford methylenebis(benzophenone) **8h** (12.0 mg, 75%) as a pale yellow amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 12.1 (s, 2 H), 9.81 (s, 2 H), 9.65 (s, 2 H), 7.63 (d, *J* = 7.5 Hz, 4 H), 7.51 (t, *J* = 7.5 Hz, 2 H), 7.42 (t, *J* = 7.5 Hz, 4 H), 6.11 (s, 2 H), 3.82 (s, 2 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 200.2, 162.5, 161.1, 160.7, 142.1, 132.0, 129.1, 128.5, 106.6, 96.9, 16.3 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 473.1 [M + H]$^+$. HRMS: calcd. for $C_{27}H_{21}O_8^+$ [M + H]$^+$ 473.1236; found 473.1235. IR (neat): ṽ = 3295, 1739, 1596, 1287, 1175, 1113, 1079 cm^{-1}, m.p. 243 °C.

[Methylenebis(2,6-dihydroxy-4-methoxy-3,1-phenylene)]bis(phenylmethanone) (8i): General procedure (16 h at room temperature) applied to benzophenone **7i** (8.0 mg) to afford methylenebis(benzophenone) **8i** (4.0 mg, 50%) to as a pale yellow amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 10.3 (s, 2 H), 10.1 (s, 2 H), 7.62 (d, *J* = 7.5 Hz, 4 H), 7.51 (t, *J* = 7.5 Hz, 2 H), 7.42 (t, *J* = 7.5 Hz, 4 H), 6.17 (s, 2 H), 3.86 (s, 6 H), 3.81 (s, 2 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 200.0, 164.7, 161.5, 159.6, 142.3, 132.1, 129.2, 128.5, 107.9, 106.2, 92.5, 56.4, 16.2 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 501.2 [M + H]$^+$. HRMS: calcd. for $C_{29}H_{25}O_8^+$ [M + H]$^+$ 501.1549; found 501.1595. IR (neat): ṽ = 3338, 1737, 1624, 1594, 1239, 1201, 1124, 1100, 1077, 1025 cm^{-1}, m.p. 174 °C.

[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]bis[(3,4-dihydroxyphenyl)methanone] (8j): General procedure (overnight at room temperature) applied to commercially available maclurin **7j** (200 mg). No precipitate was formed. Water was added to the reaction mixture that was extracted twice with ethyl acetate. The combined organic layers were washed twice with brine, dried with $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel [gradient heptane to heptane/EtOAc/2-propanol (3:6:1, v/v/v) in 25 min] to afford methylenebis(maclurin) **8j** (59.0 mg, 29%) as a pale yellow amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 9.43 (s, 2 H), 8.54 (br. s, 2 H), 8.22 (br. s, 2 H), 7.27 (d, *J* = 2.5 Hz, 2 H), 7.18 (dd, *J* = 8.0; 2.5 Hz, 2 H), 6.84 (d, *J* = 8.0 Hz, 2 H), 6.12 (s, 2 H), 3.80 (s, 2 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 197.8, 160.7, 159.5, 158.9, 150.3, 145.0, 133.3, 123.7, 117.1, 115.1, 106.8, 106.5, 96.7, 16.6 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 537.1 [M + H]$^+$. HRMS: calcd. for $C_{27}H_{21}O_{12}^+$ [M + H]$^+$ 537.1033; found 537.1033. IR (neat): ṽ = 3258, 1686, 1591, 1514, 1436, 1282, 1236, 1161, 1113, 1082 cm^{-1}, m.p. 219 °C; UPLC method (H_2O/MeCN): room temp., 2.60 min, 100%.

1,1′-[Methylenebis(2,4,6-trihydroxy-3,1-phenylene)]diethanone (8k): General procedure (overnight at room temperature) applied to phloroacetophenone **7k** (100 mg) to afford methylenebis(acetophenone) **8k** (25.0 mg, 24%) as a beige amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 14.5 (br. s, 2 H), 10.6 (s, 2 H), 9.50 (s, 2 H), 6.06 (s, 2 H), 3.73 (s, 2 H), 2.66 (s, 6 H) ppm. ^{13}C NMR ([D$_6$]acetone): δ = 204.8, 163.2, 162.7, 106.2, 105.5, 96.7, 32.6, 15.8 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): *m*/*z* = 349.1 [M + H]$^+$. HRMS: calcd. for $C_{17}H_{17}O_8^+$ [M + H]$^+$ 349.0923; found 349.0935. IR (neat): ṽ = 3198, 1608, 1588, 1448, 1365, 1262, 1099, 1073 cm^{-1}, m.p. 210 °C; UPLC method (H_2O/MeCN): room temp., 3.77 min, 97%.

6,6′-Methylenebis(5,7-dihydroxy-2-phenyl-4*H*-chromen-4-one) (8m): General procedure (10 min at 70 °C under microwave irradiation) applied to commercially available chrysin **7m** (50.0 mg). Purification by preparative TLC (eluent: heptane/EtOAc/2-propanol, 5:4:1, v/v/v) afforded methylenebis(flavone) **8m** (0.6 mg, isolated yield: 1%; NMR yield before purification: 10%) as a pale yellow amorphous solid. ^{1}H NMR ([D$_6$]acetone): δ = 13.0 (d, 2 H), 8.00 (d, *J* =

7.00 Hz, 4 H), 7.54–7.48 (m, 6 H), 6.73 (s, 2 H), 6.33 (s, 2 H), 4.47 (s, 2 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 182.3, 163.2, 162.8, 159.3, 154.9, 131.9, 131.2, 129.1, 126.4, 104.8, 105.2, 103.7, 98.5, 16.7 ppm. MS (ESI$^+$, MeCN + CH_2Cl_2): m/z = 521.1 [M + H]$^+$. HRMS: calcd. for $C_{31}H_{21}O_8^+$ [M + H]$^+$ 521.1236; found 521.1265. IR (neat): $\tilde{\nu}$ = 2961, 1737, 1643, 1604, 13701, 1259, 1096, 1032 cm^{-1}. UPLC method (H_2O/MeCN): room temp., 4.41 min, 91%.

Biological Evaluation

Assay for in Vitro Inhibition of *P. Falciparum* Growth: Chloroquine-resistant strain FcB1/Colombia of *Plasmodium falciparum* was maintained in vitro on human erythrocytes in RPMI 1640 reaction mixture supplemented by 8% (v/v) heat-inactivated human serum, at 37 °C, under an atmosphere of 3% CO_2, 6% O_2, and 91% N_2. In vitro drug susceptibility assays were measured by [^{3}H]-hypoxanthine incorporation as described.[9,10] Drugs were prepared in DMSO at 10 mM. Compounds were serially diluted twofold with 100 μL culture medium in 96-well plates. Asynchronous parasite cultures (100 μL, 1% parasitaemia and 1% final hematocrite) were then added to each well and incubated for 24 h at 37 °C prior to the addition of 0.5 μCi of [^{3}H]-hypoxanthine (GE Healthcare, France, 1 to5 Ci·mmol/mL) per well. After a further incubation period of 24 h, plates were frozen and thawed. Cell lysates were then collected onto glass-fiber filters and counted in a liquid scintillation spectrometer. The growth inhibition for each drug concentration was determined through comparison of the radioactivity incorporated in the treated culture with that in the control culture maintained on the same plate. The concentration which resulted in 50% growth inhibition (IC_{50}) was obtained from the drug concentration-response curve and the results were expressed as the mean values ± standard deviations determined from several independent experiments. Chloroquine was used as antimalarial drug control.

Assay for in Vitro Inhibition of *T. Brucei Gambiense* Growth: Bloodstream forms of *Trypanosoma brucei gambiense* strain Feo were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% CO_2.[10,11] In all experiments, log-phage cell cultures were harvested by centrifugation at 3,000 × *g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells.[12] Drug stock solutions were prepared in pure DMSO. *T. b. gambiense* bloodstream forms (3×10^4 cells/ml) were cultured as described above in 96-well plates (200 μL per well) either in the absence or in the presence of different concentrations of inhibitors and with a final DMSO concentration that did not exceed 1%. After a 72-h incubation, period resazurin solution was added in each well at the final concentration of 45 μM. Fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths after a further 4 h incubation period. Each inhibitor concentration was tested in triplicate and the experiment repeated twice. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that in the absence of drug. DMSO was used as a control. IC_{50} values were determined from the dose-response curves with drug concentrations ranging from 100 μM to 50 nM. IC_{50} value is the mean ± standard deviation of three independent experiments. Pentamidine was used as anti-trypanosomal drug control.

Supporting Information (see footnote on the first page of this article): Experimental procedures, characterization data and NMR spectra of all compounds.

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[1] T. W. Greene, P. G. M. Wuts, in: *Protective Groups in Organic Synthesis* 4th ed. John Wiley & Sons, New York, **1999**.
[2] H. Kagawa, A. Shigematsu, S. Ohta, Y. Harigaya, *Chem. Pharm. Bull.* **2005**, *53*, 547–554.
[3] a) S. K. Grover, A. C. Jain, T. R. Seshadri, *Tetrahedron* **1964**, *20*, 555–564; b) A. E. A. Sammour, *Tetrahedron* **1964**, *20*, 1067–1071; c) J. A. Donnelly, D. E. Maloney, *Tetrahedron* **1979**, *35*, 2883–2888; d) F. Hashimoto, G.-i. Nonaka, I. Nishioka, *Chem. Pharm. Bull.* **1989**, *37*, 3255–3263.
[4] A. Nagaraj, C. S. Reddy, *J. Heterocycl. Chem.* **2007**, *44*, 1181–1185.
[5] a) J. N. Roitman, R. Y. Wong, E. Wollenweber, *Phytochemistry* **1993**, *34*, 297–301; b) M. Iinuma, T. Tanaka, K. Suzuki, F. A. Lang, *Phytochemistry* **1994**, *35*, 1043–1047.
[6] J. Orjala, A. D. Wright, H. Behrends, G. Folkers, O. Sticher, H. Rüegger, T. Rali, *J. Nat. Prod.* **1994**, *57*, 18–26.
[7] A. Muhammad, I. Anis, Z. Ali, S. Awadelkarim, A. Khan, A. Khalid, M. R. Shah, M. Galal, I. A. Khan, M. Iqbal Choudhary, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 610–612.
[8] M. Berliner, K. Belecki, *Org. Synth.* **2007**, *84*, 102–110.
[9] a) Y. Quindon, H. E. Morton, C. Yoakim, *Tetrahedron Lett.* **1983**, *24*, 3969–3972; b) E. Wedekind, *Ber. Dtsch. Chem. Ges.* **1903**, *36*, 1383–1386; c) G. Casiraghi, G. Casnati, M. Cornia, G. Sartori, F. Bigi, *Makromol. Chem.* **1981**, *182*, 2973–2979.
[10] D. Bosc, S. Lethu, E. Mouray, P. Grellier, J. Dubois, *MedChemComm* **2012**, *3*, 1512–1517.
[11] M. Thévenin, S. Thoret, P. Grellier, J. Dubois, *Bioorg. Med. Chem.* **2013**, *21*, 4885–4892.
[12] D. Bosc, E. Mouray, P. Grellier, S. Cojean, P. M. Loiseau, J. Dubois, *MedChemComm* **2013**, *4*, 1034–1041.

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