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Short communication

Synthesis of prenylated benzaldehydes and their use in the synthesis of analogues of licochalcone A

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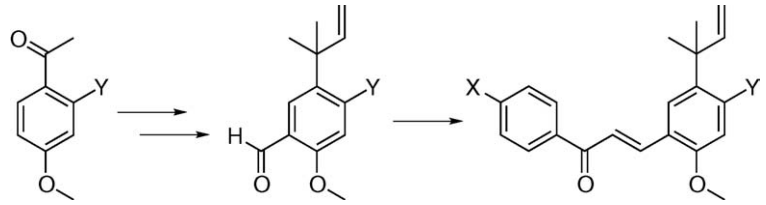
Abstract

In this paper, a general applicable synthesis of prenylated aromatic compounds exemplified by prenylated benzaldehydes starting from readily available acetophenones is described. The synthesized benzaldehydes are used to prepare a number of novel analogues of Licochalcone A, a known antibacterial compound, and for the exploration of the pharmacophoric elements that are essential for the antibacterial activity. It is shown that the hydroxyl group in the A ring is essential for the activity and that the hydroxyl group in the B ring has no influence on the antibacterial effect of Licochalcone A. Furthermore, it is shown that the prenyl group at the position 5 of the B ring also has a dominating influence on the activity. This aliphatic group can be replaced by other lipophilic long chained substituents in order to maintain the activity.



1. Introduction

A large number of naturally occurring compounds contain prenylated aromatic rings as an important part of their structure. These compounds are in many cases biological active and are therefore interesting as potential drug candidates. Examples of this type of compounds are the antibacterial [1,2] oxygenated chalcone Licochalcone A (**1**) isolated from the root of Chinese liquorice [3], the cytotoxic [4] Kazinol K (**2**) isolated from the root bark of *Broussonetia kazinoki* [5] and the antioxidant [6] Garciniaxanthone A (**3**) isolated from heartwood of *Garcinia subelliptica* [7] (Chart 1).

The structure–activity relationship analysis of this type of compounds has however been complicated by the difficult accessible prenylated starting materials. Total synthesis of e.g. Licochalcone A, has been performed by various Claisen-type rearrangements of corresponding isoprenyloxy compounds (Scheme 1) [8]. The conditions under which these reactions have been performed are often very harsh, using high pressure and elevated temperature. In addition, the synthesis is limited to compounds having hydroxyl groups in a favorable position in order for the rearrangement to occur with the desired regioselectivity [9].

In this paper, we describe a general applicable synthesis of prenylated aromatic compounds exemplified by prenylated benzaldehydes. This synthetic strategy is of general use, starting from readily available acetophenones. The synthesized benzaldehydes are used to prepare a number of novel analogues of Licochalcone A, which are used for the explo-

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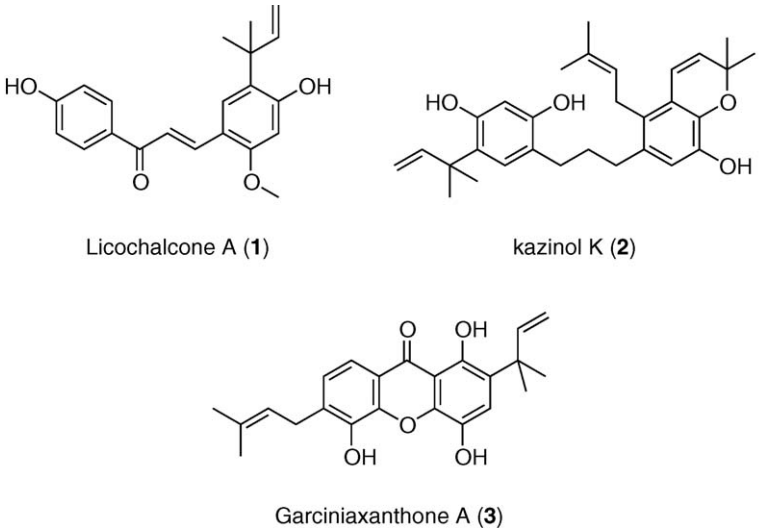
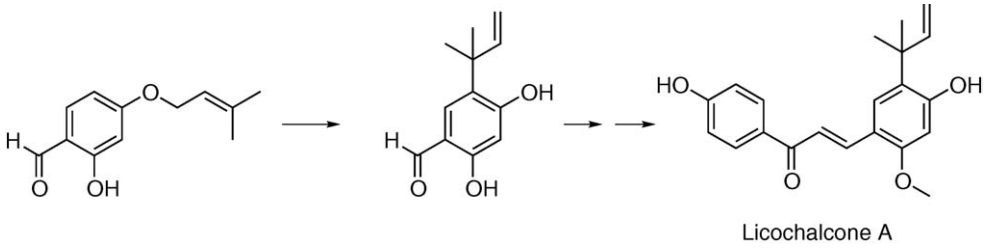


Chart 1. Chemical structures of Licochalcone A, Kazinol K, Garciniaxanthone A.



Scheme 1. Synthesis of Licochalcone A by Claisen rearrangement.

ration of the pharmacophoric elements that are essential for the antibacterial activity.

2. Results and discussion

2.1. Chemistry

The 4-chloro-5-(1,1-dimethyl-allyl)-2-methoxy-benzaldehyde **14** was prepared in six steps starting from 2′-chloro-4′-methoxyacetophenone **4** as shown in Scheme 2. Reaction of **4** with tosylmethylisocyanide under basic conditions gave the nitrile **5**, which was methylated in α-position with methyliodide resulting in **6**. Bromination of **6** with *N*-bromosuccinimide in trifluoroacetic acid gave **8**, which was reduced by DIBAL-H to yield the aldehyde **10**. Subsequent Wittig-reaction using methyltriphenylphosphonium bromide gave the allylic compound **12** that after treatment with *n*-BuLi and DMF gave the desired benzaldehyde **14**.

The 5-(1,1-dimethyl-allyl)-2-methoxy benzaldehyde **15** was prepared from the nitrile **7** [10] (Scheme 2). Compound **7** was methylated using methyliodide and NaH. The following steps were equivalent to the steps used in the preparation of compound **14**.

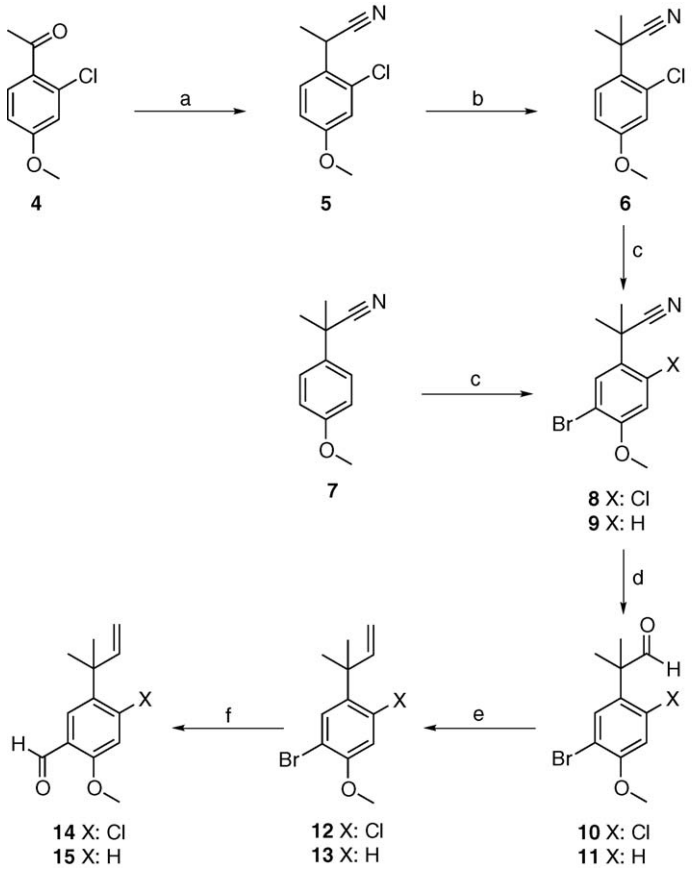
Claisen-Schmidt condensations using hydroxy acetophenones have been described in the literature, using either basic [11,12] or acidic [13–15] catalysis. We obtained the best results when the hydroxy acetophenones were protected as the tetrahydropyranyl ether (THP) **16** [16] yielding chalcones **18–19** (Scheme 3, Route A). 4′-Dehydroxy chalcone (**21**) was synthesized in 1.5 M solution of HCl in absolute ethanol (Scheme 3, Route B).

Compounds **22–25** were prepared by methylation directly on Licochalcone A using methyliodide under basic conditions (Scheme 4). It has previously been described that the 4′-hydroxy group in the A ring of Licochalcone A is more readily alkylated than the 4-hydroxy group in the B ring, probably due to less sterical hindrance of the former [17]. The synthesis of compound **22** takes advantage of this fact, using 1.2 equivalents methyl iodide. If a large excess of methyl iodide is used alkylation of the hydroxyl groups in both the A- and B rings is achieved yielding compound **23**. Compound **25** was prepared by THP-protection of the 4′-hydroxy group in the A ring (Compound **24**) followed by methylation of the 4-hydroxy group in the B ring and subsequent cleavage of the THP-ether under acidic conditions.

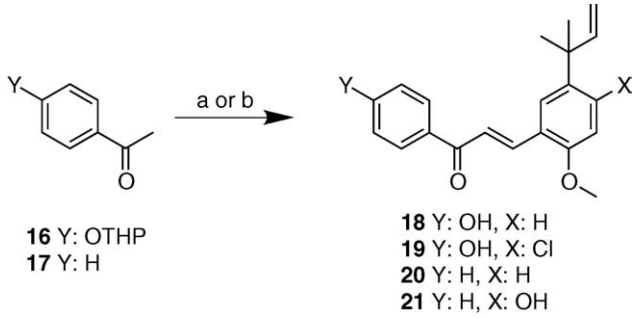
The compounds shown in Table 2 were prepared as previously described [16,18,19].

2.2. In vitro activity

Tables 1 and 2 summarize the antibacterial activity of the synthesized chalcones, expressed in terms of antibacterial minimum inhibitory concentrations (MIC) against a Gram-positive bacteria strain—*Staphylococus aureus*.



Scheme 2. Synthesis of prenylated benzaldehydes. Reagents and conditions: (a) Tosylmethylisocyanide, *t*-BuOK, DME, *t*-BuOH; (b) CH_3I, NaH, DMF; (c) NBS, TFA, rt.; (d) DIBAL-H, THF; (e) Methyl triphenylphosphonium bromide, *n*-BuLi, THF; (f) (i): *n*-BuLi, THF, –78 °C, (ii): DMF.



Scheme 3. Claisen–Schmidt condensation. Reagents and conditions: (a) Route A: (i) 0.1 eq. NaOH, prenylated benzaldehyde, EtOH, rt. (ii) 2 N HCl; (b) Route B: 1.5 M HCl in EtOH, prenylated benzaldehyde, rt.

Methylation of the hydroxyl group in the A ring of Licochalcone A (compound **22**) and synthesis of the 4′-dehydroxy analogue (compound **21**) show that the free hydroxyl group in position 4′ of the A ring is necessary for the antibacterial activity of Licochalcone A. On the other hand, no change in activity is observed when the hydroxyl group in the 4-position of the B ring is removed (compound **18**), blocked by a methyl (compound **25**), or replaced by a chlorine (compound **19**). Removal of both hydroxyl groups (compound **20**) or blockage of both hydroxyl groups by methylation (compound **23**) eliminates the activity completely in accordance with the observations above.

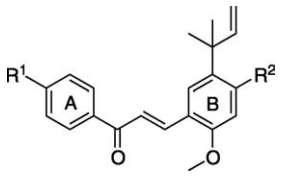
When the lipophilic prenyl group is removed (compound **27** [18]), a total loss of activity is observed (Table 2). Compound **26** [19] in which only the two hydroxyl groups are remaining is also inactive, in accordance with the results above. If the prenyl group is exchanged by a propyl group (compound **28** [16]) a moderate antibacterial effect is observed. The introduction of the longer hexyl group (compound **29** [16]) results in a compound that is more potent than Licochalcone A. When comparing ClogP with the antibacterial activity of the compounds (Table 2), a clear correlation between lipophilicity and antibacterial activity, is seen. These findings are in accordance with the findings by Haraguchi et al. [23] which reported that the presence of the hydrophobic prenyl group is important for the antibacterial activity of naturally occurring retrochalcones, isolated from *G. Inflate*.

In this paper, the results show that the lipophilic character of Licochalcone A is essential for the antibacterial activity. Furthermore, it is shown that the prenyl group in position 5 can be replaced by other aliphatic groups, if they mimic the lipophilic character of it.

3. Conclusion

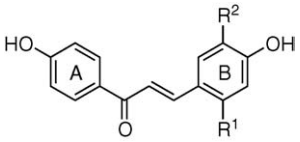
In this article, we described the synthesis of novel prenylated benzaldehydes from readily available acetophenones.

Table 1
In vitro antibacterial activity of prepared analogues of Licochalcone A (MIC, μM). Each value represents the mean of three experiments



Compound	R^1	R^2	MIC (μm) *S. aureus ATCC29213*
1	OH	OH	20
18	OH	H	20
25	OH	OCH_3	20
22	OCH_3	OH	>300
19	OH	Cl	10
20	H	H	>300
21	H	OH	>300
Ciprofloxacin [20]			2
Linezolid [21,22]			2

Table 2
In vitro antibacterial activity of selected analogues of Licochalcone A (MIC, μM). Each value represents the mean of three experiments



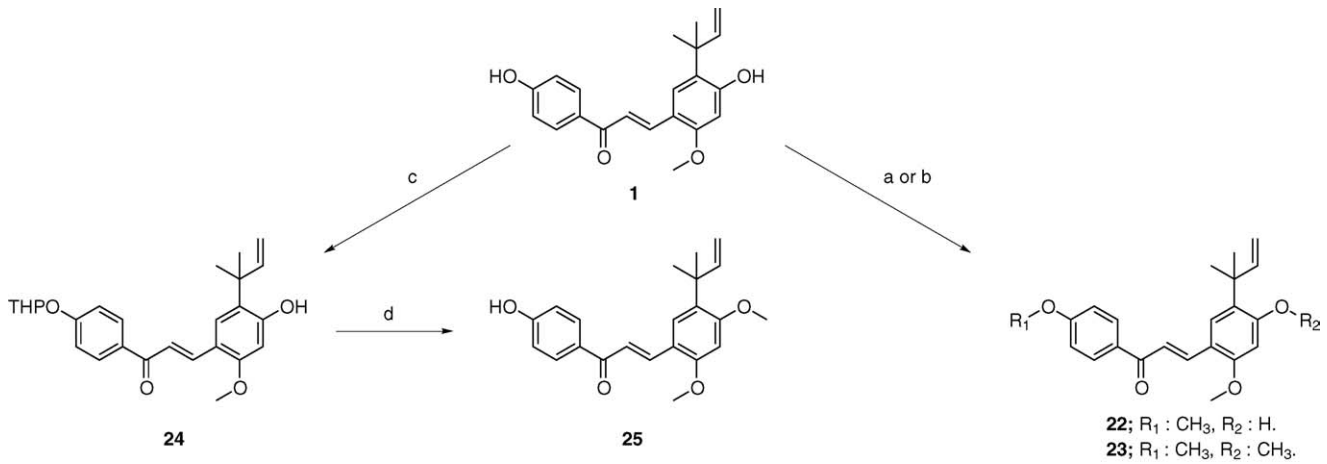
Compound	R^1	R^2	ClogP	MIC (μm) *S. aureus ATCC29213*
1	OCH_3	Prenyl	4.9	20
26 [19]	H	H	3.1	>300
27 [18]	OCH_3	H	3.2	>300
28 [16]	OCH_3	Propyl	4.7	75
29 [16]	OCH_3	Hexyl	6.3	10

The prenylated benzaldehydes were used to identify the pharmacophoric elements that are responsible for the antibacterial activity of Licochalcone A. It was shown that the hydroxyl group at position 4′ of the A ring was essential for the activity. On the other hand, the hydroxyl group at position 4 of the B ring has no effect on the antibacterial activity of Licochalcone A. Furthermore it was shown that the prenyl group at position 5 of the B ring has also a dominating influence on the activity. The aliphatic group can be replaced by other lipophilic long chained aliphatic substituents. It is believed that the strong lipophilic character of the molecule plays an essential role in the antibacterial effect.

4. Experimental section

4.1. Chemistry

Thin-layer chromatography (TLC) was performed on silica gel F_{254} plates (Merck). All compounds were detected using UV light. ^{1}H-NMR and ^{13}C spectra were recorded on a 400 MHz Varian Gemini spectrometer or a Bruker AC-300 F spectrometer, using $CDCl_3$ or DMSO-d_6 as the solvent. Chemical shifts are given in ppm (δ) using TMS as internal standard, and coupling constants (J) are given in Hertz. Mass spectra (GC-MS) were recorded using a Agilent Technologies 6890N and were all >95% pure. Mass spectra (LC-MS) were recorded using a Waters Alliance HPLC-system coupled to a Quatro Micro triple quadropol mass spectrometer (Micromass) operating in positive (ESI) mode. Separation was performed on a XTerra MS C_{18} column (150 × 2.1 mm I.D., 3,5 μm particle size). Purity of the final products (>95%) was determined using a Waters Alliance 2690 separation module and Waters 996 PDA-detector. Separation was performed on a XTerra MS C_{18} column (150 × 2.1 mm I.D., 3.5 μm particle size) using 40% mobile phase A (acetonitrile) and 60% mobile phase B (10 mM ammonium acetate pH 9.5). During the first 20 min, the mobile phase was changed



Scheme 4. Synthesis of methylated analogues of Licochalcone A. Reagents and conditions: (a) 1.2 eq. CH_3I, 2.0 eq. NaOH, DMSO, rt; (b) 2.4 eq. CH_3I, 2.0 eq. NaOH, DMSO, rt.; (c) 3,4-dihydro-*2H*-pyran, pyridinium *p*-toluenesulfonate; (d) i: CH_3I, NaOH, DMSO, rt, ii: HCl (ag).

via a linear gradient to 90% A and 10% B. The accurate mass measurements were obtained on a VG AutoSpec mass spectrometer (Micromass, Manchester, UK) equipped with an EI source. The instrument was operated at a resolution of 10000 FWHM.

Column chromatography (CC) was performed on Merck silica gel 60 (0.063–0.200 mm). All solvents and reagents were obtained from Fluka or Aldrich and used without further purification except DMF and THF, which was stored over 3 Å molecular sieves. Compound **26–29** (>95% pure) were prepared as described previously [24,25].

*4.2. 2-(2-Chloro-4-methoxyphenyl)propionitrile (**5**)*

A solution of 2-chloro-4-methoxyacetophenone (**4**) (18.5 g, 0.10 mol) and tosylmethylisocyanide (TOSMIC, 21.5 g, 0.11 mol) in dry DME (100 ml) was cooled to –10 °C. A solution of *t*-BuOK (22.4 g, 0.20 mol) in dry *t*-BuOH (250 ml) was added slowly keeping the temperature below 5 °C. The homogeneous orange solution was stirred for 2 h at 0 °C and 1 h at 25 °C. The resulting suspension was evaporated to a slurry. Water (200 ml) was added and extracted with Et_2O (3 × 150 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure leaving an orange oil. Yield: 19 g (97%). GC-MS: 196 (M). ^{1}H-NMR (DMSO-d_6): δ 7.49 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 2.7 Hz, 1H), 7.02 (dd, J = 8.7 Hz, J = 2.7 Hz, 1H), 4.42 (q, J = 7.2 Hz, 1H), 3.80 (s, 3H), 1.55 (d, J = 7.1 Hz, 3H).

*4.3. 2-(2-Chloro-4-methoxyphenyl)-2-methylpropionitrile (**6**)*

A solution of 2-(2-chloro-4-methoxyphenyl)propionitrile (**5**) (19 g, 0.097 mol) and methyl iodide (7 ml, 0.11 mol) in dry DMF (100 ml) was flushed with argon for 2 min and cooled to 0 °C. Sodium hydride (60% oil susp., 4.4 g, 0.11 mol) was added in small portions. The thick suspension was stirred for another 18 h at 25 °C and then poured into water (300 ml) and extracted with Et_2O (3 × 100 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure leaving yellow oil, which was distilled. b.p. 103–106 °C/0.06 mbar, clear oil that solidifies on standing. Yield: 17.5 g (83%). GC-MS: 210 (M). ^{1}H-NMR (DMSO-d_6): δ 7.43 (d, J = 8.9 Hz, 1H), 7.13 (d, J = 2.9 Hz, 1H), 6.98 (dd, J = 8.9 Hz, J = 2.8 Hz, 1H), 3.80 (s, 3H), 1.77 (s, 6H).

*4.4. 2-(5-Bromo-2-chloro-4-methoxyphenyl)-2-methylpropionitrile (**8**)*

A solution of 2-(2-chloro-4-methoxyphenyl)-2-methylpropionitrile (**6**) (17.5 g, 0.084 mol) in TFA (100 ml) was cooled to 0 °C. *N*-bromosuccinimide (14.9 g, 0.084 mol) was added in small portions keeping the temperature below 5 °C. The orange solution was stirred for 2 h at 25 °C and evaporated to dryness. Water (200 ml) was added and the mixture was stirred vigorously for 1 h. The crude product was filtered off and recrystallized from boiling MeOH. The pure product was isolated as white needles. Yield: 13 g (54%). GC-MS: 289 (M). ^{1}H-NMR (DMSO-d_6): δ 7.56 (s, 1H), 7.23 (s, 1H), 3.84 (s, 3H), 1.70 (s, 6H).

*4.5. 2-(3-Bromo-4-methoxyphenyl)-2-methylpropionitrile (**9**)*

Same procedure as described for compound **8**. Recrystallisation from *n*-heptane gave **9** as white needles. Yield: 19.3 g (76%). GC-MS: 254 (M). ^{1}H NMR (DMSO-d_6): δ 7.68 (d, J = 2.5 Hz, 1H), 7.50 (dd, J = 8.7 Hz, J = 2.4 Hz, 1H), 7.16 (d, J = 8.8 Hz, 1H), 3.86 (s, 3H), 1.70 (s, 6H).

*4.6. 2-(5-Bromo-2-chloro-4-methoxyphenyl)-2-methylpropionaldehyde (**10**)*

A solution of 2-(5-bromo-2-chloro-4-methoxyphenyl)-2-methylpropionitrile (**8**) (13.0 g, 0.045 mol) in dry THF (80 ml) was cooled to –10 °C under argon. DIBAL-H (1 M in THF, 100 ml, 0.10 mol) was added keeping the temperature below 0 °C. The mixture was stirred for 30 min at 0 °C and then 2 h at 25 °C. The clear solution was carefully poured into icecold HCl (2 M, 100 ml). THF was removed under reduced pressure. The aqueous phase was cooled and the crude product was filtered off and recrystallized from MeOH. GC-MS: 292 (M). Yield: 7.8 g (59%). ^{1}H-NMR (DMSO-d_6): δ 9.61 (s, 1H), 7.68 (s, 1H), 7.27 (s, 1H), 3.89 (s, 3H), 1.40 (s, 6H).

*4.7. 2-(3-Bromo-4-methoxyphenyl)-2-methylpropionaldehyde (**11**)*

Same procedure as described for compound **10**. THF was removed under reduced pressure to give clear oil. The oil was distilled (b.p. 114–130 °C at 4.3 × 10^{-3} mbar) Yield: 7.40 g (58%). GC-MS: 257 (M). ^{1}H-NMR ($CDCl_3$): δ 9.44 (s, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.15 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 3.89 (s, 3H), 1.43 (s, 6H).

*4.8. 1-Bromo-4-chloro-5-(1,1-dimethylallyl)-2-methoxybenzene (**12**)*

A suspension of methyltriphenylphosphonium bromide (11.4 g, 0.032 mol) in dry THF (100 ml) was cooled to 0 °C under argon. *n*-BuLi (2.5 M, 12 ml, 0.030 mol) was added slowly. The resulting clear orange solution of the ylide was stirred for another 15 min at 0 °C. 2-(3-Bromo-4-methoxyphenyl)-2-methylpropionaldehyde (**10**) (7.8 g, 0.027 mol) was dissolved in dry THF (50 ml) and added to ylide solution. The mixture was stirred for 3 h at 25 °C and the resulting suspension was quenched with MeOH (10 ml). The solvent was removed under reduced pressure and the crude product was purified by CC using *n*-heptane as eluent. Yield: 3.92 g (50%) as a clear oil. GC-MS: 290 (M). ^{1}H-NMR (DMSO-d_6): δ 7.55 (s, 1H), 7.14 (s, 1H), 6.05 (dd,

J = 17.5 Hz, J = 10.5 Hz, 1H), 5.04 (dd, J = 10.5 Hz, J = 1.2 Hz, 1H), 4.92 (dd, J = 9.3 Hz, J = 0.9 Hz, 1H), 3.87 (s, 3H), 1.45 (s, 6H).

*4.9. 2-Bromo-4-(1,1-dimethylallyl)-1-methoxybenzene (**13**)*

Same procedure as described for compound **12**. Yield: 3.1 g (84%) as a clear oil. GC-MS: 255 (M). ^{1}H-NMR ($CDCl_3$): δ 7.50 (d, J = 2.4 Hz, 1H), 7.23 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 6.83 (d, J = 8.7 Hz, 1H), 5.97 (dd, J = 17.7 Hz, J = 10.3 Hz, 1H), 5.06 (dd, J = 17.7 Hz, J = 1.4 Hz, 1H), 5.02 (dd, J = 10.4 Hz, J = 1.2 Hz, 1H), 3.87 (s, 3H), 1.44 (s, 6H).

*4.10. 4-Chloro-5-(1,1-dimethylallyl)-2-methoxybenzaldehyde (**14**)*

A solution of 1-bromo-4-chloro-5-(1,1-dimethylallyl)-2-methoxybenzene (**12**) (3.9 g, 0.014 mol) in dry THF (30 ml) was cooled to –78 °C under argon. *n*-BuLi (2.5 M, 6 ml, 0.0145 mol) was added keeping the temperature below –70 °C. The yellow mixture was stirred for another 15 min and quenched with dry DMF (1.2 ml, 0.015 mol). The cooling bath was removed and the mixture was allowed to warm to 25 °C. Saturated $NaHCO_3$ (30 ml) was added and the solution was extracted with EtOAc (3 × 50 ml). The organic phase was dried (Na_2SO_4) and evaporated to dryness. The crude product was recrystallized from MeOH. Yield: 3.00 g (93%). GC-MS: 239 (M). ^{1}H-NMR (DMSO-d_6): δ 10.30 (s, 1H), 7.80 (s, 1H), 7.30 (s, 1H), 6.08 (dd, J = 17.3 Hz, J = 10.5 Hz, 1H), 5.06 (dd, J = 10.5 Hz, J = 1.2 Hz, 1H), 4.91 (dd, J = 17.3 Hz, J = 0.9 Hz, 1H), 3.93 (s, 3H), 1.49 (s, 6H).

*4.11. 5-(1,1-Dimethylallyl)-2-methoxybenzaldehyde (**15**)*

Same procedure as described for compound **14**. Evaporation of the organic phase gave yellow oil. Yield: 2.31 g (94%). GC-MS: 204 (M). ^{1}H-NMR ($CDCl_3$): δ 10.48 (s, 1H), 7.84 (d, J = 2.5 Hz, 1H), 7.55 (dd, J = 8.8 Hz, J = 2.6 Hz, 1H), 6.94 (d, J = 8.7 Hz, 1H), 6.00 (dd, J = 17.7 Hz, J = 10.2 Hz, 1H), 5.05–5.01 (m, 2H), 3.93 (s, 3H), 1.41 (s, 6H).

4.12. General procedures for the Claisen–Schmidt condensation

4.12.1. Route A

To a solution of acetophenone (2 mmol) and prenylated benzaldehyde (2 mmol) in EtOH (10 ml) was added a small NaOH pellet (50 mg). The reaction was stirred overnight at rt. Addition of aqueous HCl (2 M, 10 ml) gave a slurry of crystals that was filtered off.

4.12.2. Route B

A solution of acetophenone (2 mmol) and prenylated benzaldehyde (2 mmol) in a 1.5 M solution of HCl in EtOH (10 ml) was stirred at rt over night. Water (30 ml) was added and the solution was extracted with EtOAc (3 × 20 ml). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by CC using EtOAc/*n*-heptane as eluent.

*4.13. 3-[5-(1,1-Dimethylallyl)-2-methoxyphenyl]-1-(4-hydroxyphenyl)-propenone (**18**)*

General procedure A gave the desired product **18** as yellow crystals. Yield: 12%. LC-MS: 323.4 (M + 1). ^{1}H-NMR (DMSO-d_6): δ 10.40 (bs, 1H), 8.03 (d, J = 7.0 Hz, 2H), 7.98 (d, J = 15.8 Hz, 1H), 7.82 (d, J = 15.4 Hz, 1H), 7.79 (s, 1H), 7.36 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H), 7.04 (d, J = 7.9 Hz, 1H), 6.91 (d, J = 8.7 Hz, 2H), 6.08 (dd, J = 17.7 Hz, J = 10.2 Hz, 1H), 5.07–5.01 (m, 2H), 3.87 (s, 3H), 1.41 (s, 6H). ^{13}C-NMR ($CDCl_3$): 190.4; 160.5; 157.2; 147.9; 140.9; 131.3; 131.1; 129.6; 127.6; 123.5; 122.8; 115.6; 111.1; 111.0; 55.7; 40.6; 28.4. HRMS ($C_{21}H_{22}O_3$) calculated. 322.1569; found: 322.1580.

*4.14. 3-[4-Chloro-5-(1,1-dimethylallyl)-2-methoxyphenyl]-1-(4-hydroxyphenyl)-propenone (**19**)*

General procedure A gave the desired product **19** as yellow crystals. Yield: 42%. LC-MS: 357.8 (M + 1). ^{1}H-NMR (DMSO-d_6): δ 10.41 (bs, 1H), 8.03 (d, J = 8.7 Hz, 2H), 7.88–7.83 (m, 3H), 7.14 (s, 1H), 6.91 (d, J = 8.9 Hz, 2H), 6.09 (dd, J = 17.5 Hz, J = 8.7 Hz, 1H), 5.03 (dd, J = 10.7 Hz, J = 1.3 Hz, 1H), 4.93 (dd, J = 17.6 Hz, J = 1.1 Hz, 2H), 3.92 (s, 3H), 1.53 (s, 6H). ^{13}C (DMSO-d_6): 196.6; 162.1; 156.8; 146.7; 136.8; 136.6; 135.7; 131.0; 130.2; 129.1; 122.7; 121.7; 115.3; 114.9; 111.1; 56.1; 55.5; 40.9; 28.0. HRMS ($C_{21}H_{21}ClO_3$) calculated: 356.1179; found: 356.1183.

*4.15. 3-[5-(1,1-Dimethylallyl)-2-methoxyphenyl]-1-phenylpropenone (**20**)*

General procedure A gave the desired product **20** as yellow oil. Yield: 51%. LC-MS: 307.4 (M + 1). ^{1}H-NMR ($CDCl_3$): δ 8.08 (d, J = 16.1 Hz, 1H), 8.05–8.02 (m, 2H), 7.64 (d, J = 16.1 Hz, 1H), 7.60–7.50 (m, 4H), 7.38 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 6.91 (d, J = 8.6 Hz, 1H), 6.04 (dd, J = 17.5 Hz, J = 10.3 Hz, 1H), 5.10 (dd, J = 17.6 Hz, J = 2.4 Hz, 2H), 5.08 (dd, J = 10.4 Hz, J = 1.4 Hz, 1H), 3.92 (s, 3H), 1.44 (s, 6H). ^{13}C ($CDCl_3$): 191.5; 157.2; 147.9; 141.1; 140.9; 132.4; 129.6; 129.1 (2 × C); 128.6; 128.5; 128.2; 127.8; 127.7; 123.2; 111.1; 111.0; 55.7; 40.6; 28.4; 28.1. HRMS ($C_{21}H_{22}O_2$) calculated 306.1620; found: 306.1637.

*4.16. 3-[5-(1,1-Dimethylallyl)-4-hydroxy-2-methoxyphenyl]-1-phenylpropenone (**21**)*

General procedure B gave the desired product **21** as yellow crystals. Yield: 16%. LC-MS: 323.4 (M + 1). ^{1}H-NMR ($CDCl_3$): δ 8.03–7.98 (m, 3H), 7.59–7.47 (m, 5H), 6.45 (s,

1H), 6.25 (s, 1H), 6.20 (dd, J = 17.7 Hz, J = 8.8 Hz, 1H), 5.43–5.34 (m, 2H), 3.88 (s, 3H), 1.55 (s, 6H).

^{13}C NMR (DMSO-d_6): 196.5; 160.2; 148.3; 141.1; 138.2; 130.4; 129.3; 129.1; 127.3; 127.1; 127.0; 126.3; 125.1; 122.2; 111.4; 111.1; 55.4; 40.6; 28. HRMS ($C_{21}H_{22}O_3$) calculated 322.1569; found: 322.1578.

*4.17. 3-[5-(1,1-Dimethylallyl)-4-hydroxy-2-methoxyphenyl]-1-(4-methoxyphenyl)-propenone (**22**)*

To a solution of Licochalcone A (500 mg, 1.5 mmol) in DMSO (5 ml) were added methyl iodide (110 μl, 1.8 mmol) and NaOH (120 mg, 3.0 mmol). The reaction was stirred at rt for 3 h and water was added (40 ml). The aqueous solution was extracted with CH_2Cl_2 (3 × 20 ml) and the combined organic phases were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by CC using EtOAc/*n*-heptane as eluent and recrystallized from EtOAc/*n*-heptane. Yield: 250 mg (50%) as yellow crystals. LC-MS: 353.4 (M + 1). ^{1}H-NMR ($CDCl_3$): δ 8.03 (d, J = 9.0 Hz, 2H), 7.98 (d, J = 15.8 Hz, 1H), 7.58 (d, J = 15.8 Hz, 1H), 7.48 (s, 1H), 7.97 (d, J = 9.0 Hz, 2H), 6.45 (s, 1H), 6.26 (s, 1H), 6.21 (dd, J = 17.8 Hz, J = 8.6 Hz, 1H), 5.37 (dd, J = 17.6 Hz, J = 1.0 Hz, 2H), 5.34 (dd, J = 10.6 Hz, J = 1.0 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 1.46 (s, 6H). ^{13}C ($CDCl_3$): 189.8; 163.1; 159.6; 158.2; 147.9; 140.6; 131.9; 131.0; 130.8; 128.7; 124.7; 120.6; 116.8; 113.9; 113.8; 101.3; 55.7; 55.5; 39.8; 27.4; 27.2. HRMS ($C_{22}H_{24}O_4$) calculated: 352.1675; found: 352.1677.

*4.18. 3-[5-(1,1-Dimethylallyl)-2,4-dimethoxyphenyl]-1-(4-methoxyphenyl)-propenone (**23**)*

Same procedure as described for compound **22** using 2.3 eq. methyl iodide gave the desired compound as yellow crystals. Yield: 15%. LC-MS: 367.5 (M + 1) ^{1}H-NMR ($CDCl_3$): δ 8.03 (d, J = 15.9 Hz, 1H), 8.02 (d, J = 9.0 Hz, 2H), 7.53 (d, J = 15.8 Hz, 1H), 7.52 (s, 1H), 6.98 (d, J = 9.0 Hz, 2H), 6.46 (s, 1H), 6.16 (dd, J = 17.3 Hz, J = 10.8 Hz, 1H), 4.97 (dd, J = 10.7 Hz, J = 1.3 Hz, 2H), 4.94 (dd, J = 17.3 Hz, J = 1.3 Hz, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 1.46 (s, 6H). ^{13}C NMR ($CDCl_3$): 189.7; 163.1; 159.1; 147.9; 140.3; 132.0; 130.7; 129.3; 128.7; 120.2; 115.9; 113.7; 110.1; 96.3; 55.7; 55.5; 55.3; 43.5; 40.1; 27.4. HRMS ($C_{23}H_{26}O_4$) calculated: 366.1831; found: 366.1810.

*4.19. 3-[5-(1,1-Dimethylallyl)-2,4-dimethoxyphenyl]-1-(4-hydroxyphenyl)-propenone (**25**)*

Licochalcone A (500 mg, 1.5 mmol), 3,4-dihydro-*2H*-pyran (167 mmol) and a few crystals of pyridinium *p*-toluenesulfonate in CH_2Cl_2 (5 ml) was stirred over night at rt. The mixture was washed with 1 N Na_2CO_3 (3 × 10 ml) and dried (Na_2SO_4). Evaporation under reduced pressure gave a yellow oil. The oil was dissolved in DMSO (5 ml) and methyl iodide (230 μl, 3.5 mmol) and NaOH (120 mg, 3.0 mmol) were added. The reaction was stirred at rt overnight and HCl (2M, 40 ml) was added. The aqueous solution was extracted with CH_2Cl_2 (3 × 20 ml) and the combined organic phases were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was purified by CC using EtOAc/*n*-heptane as eluent and recrystallized from EtOAc/*n*-heptane. Yield: 180 mg (36%) as yellow crystals. LC-MS: 353.4 (M + 1) ^{1}H-NMR ($CDCl_3$): δ 8.04 (d, J = 15.8 Hz, 1H), 7.97 (d, J = 8.7 Hz, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.51 (s, 1H), 6.93 (d, J = 8.6 Hz, 2H), 6.45 (s, 1H), 6.16 (dd, J = 17.4 Hz, J = 8.6 Hz, 1H), 6.15 (bs, 1H), 4.97 (dd, J = 16.8 Hz, J = 1.4 Hz, 1H), 4.93 (dd, J = 13.4 Hz, J = 1.3 Hz, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 1.45 (s, 6H). ^{13}C NMR (DMSO-d_6): 197.2; 161.7; 161.4; 161.5; 158.6; 157.1; 155.9; 147.9; 138.2; 130.7; 130.1; 128.2; 125.8; 118.9; 114.6; 109.4; 96.7; 55.8; 55.1; 37.3; 27.3. HRMS ($C_{22}H_{24}O_4$) calculated 352.1675; found: 352.1690.

5. Determination of MIC

MIC was determined in triplicate in a microdilution assay using MH-broth as described by *NCLLS* (National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*—Fifth Edition. M7-A5 NCCLS 2000). This method was modified to include uninoculated dilution series of test compounds to facilitate MIC determination if the test compound should precipitate. MIC was determined as the lowest concentration of test compound able to inhibit visible growth of bacteria.

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