

Synthesis of Aromatic Retinoids and Curcuminoids and Evaluation of their Antiproliferative, Antiradical, and Anti-inflammatory Activities

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Natural retinoids and curcuminoids are known for their broad spectrum of biological properties, such as antioxidant, anti-inflammatory, antitumor, and so forth. In this work, a convenient synthesis of aromatic retinoids and curcuminoids from vinyl or allyl ketones, and the corresponding alcohols, using olefin metathesis as a key reaction, was elaborated. The best yields and diastereoselectivities were obtained from allylic or homoallylic alcohols by employing the two-step cross-metathesis/oxidation procedure. The synthesized analogues were tested for

their antiproliferative activity on human cancer cell lines of various origin (leukemia CEM, adenocarcinoma MCF7, cervical carcinoma HeLa) as well as for their antioxidant and anti-inflammatory activity in vitro. All examined derivatives exhibited strong anti-inflammatory activity in vitro without affecting cell viability. They also showed strong cytotoxicity against leukemia cell line CEM, except for **18** and **35**. The antioxidant activity of the tested compounds was rather weak.

1. Introduction

Over the past few decades, olefin metathesis has emerged as a powerful tool in organic synthesis. During this period, efforts have been focused on the development of new metathesis catalysts that show not only high activity, selectivity, and functional-group tolerance, but also operational simplicity and availability.^[1–4] These investigations resulted in the discovery of ruthenium complexes such as **I**, **II**, **III**, **IV**, **V** (Figure 1), which have found successful application in the preparation of various olefins, including natural and biologically active compounds.^[5–8] In this paper, we report the use of cross metathesis for the synthesis of retinoids and curcuminoids. Both of these compound families (Figure 2) exhibit beneficial biological activities. Retinoids, which are natural and synthetic analogues of retinoic acid, play an essential role in a variety of biological processes, such as vision, reproduction, cell differentiation, and immune response.^[9–11] Curcuminoids, which are derivatives of curcumin, a natural pigment isolated from the rhizome of *Curcuma longa*, also show a broad range of biological activities, including antioxidant, anti-inflammatory, antitumor, anti-HIV, antibacterial, antiviral, and antifungal properties.^[12] Additional-

ly, recent clinical trials have demonstrated that curcumin is safe even in high doses.^[13–17] Curcumin underwent clinical trials for cancer^[18] and Alzheimer's disease.^[15] However, its potential use as a therapeutic agent is severely affected by its low water solubility, rapid metabolism, and poor bioavailability.^[19–21]

2. Results and Discussion

2.1. Chemistry

Continuing our studies on the synthesis of aromatic retinoids^[22] and other biologically active compounds,^[23,24] we conceived a strategy for the synthesis of retinoic acid analogues with a carbonyl group instead of an ethylene (–CH=CH–) fragment in the polyene chains, as well as curcumin analogues with an atypical arrangement of carbonyl groups. Both types of compounds may be prepared from the same substrates, that is, vinyl or allyl ketones, by employing cross metathesis (CM) for the synthesis of retinoids and unsymmetrical curcuminoids, or self-metathesis (SM) in the case of symmetrical curcuminoids (Scheme 1).

To validate our strategy, we investigated the metathesis reactions of model vinyl and allyl ketones. The unsaturated ketones **3**, **4**, **7**, and **8** needed for synthesis were prepared from commercially available benzaldehyde and cinnamaldehyde by using the Grignard reaction with vinyl- or allylmagnesium bromide followed by oxidation, as shown in Scheme 2.

In the first experiments, the metathesis reactions of phenyl vinyl ketone (**3**) were studied. Having in mind the synthesis of oxoretinoids and curcumin analogues, two series of reactions of **3**, that is, its CM and SM reactions were investigated. Based on our previous studies related to the synthesis of etretinate

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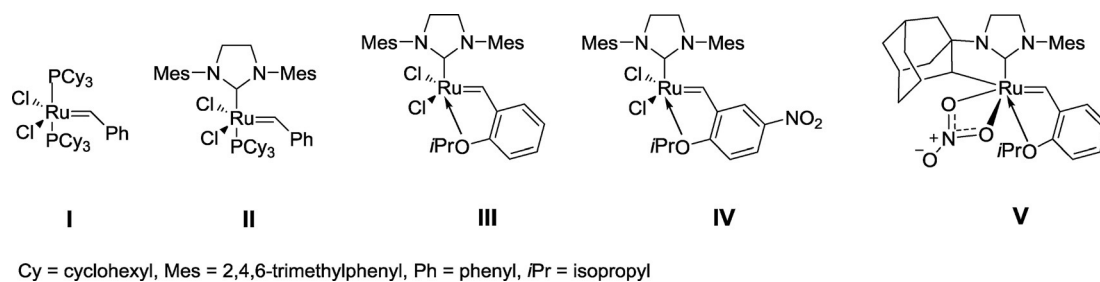


Figure 1. Examples of commercially available metathesis catalysts.

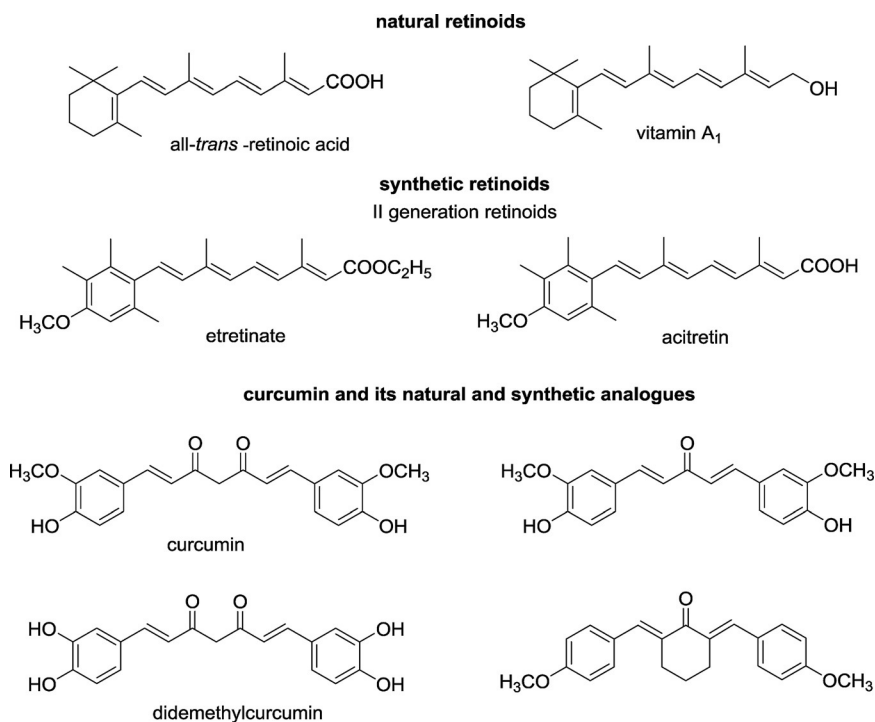
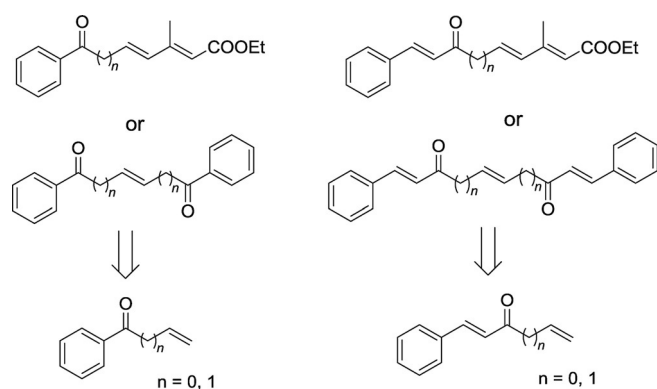


Figure 2. Natural and synthetic retinoids and curcuminoids.

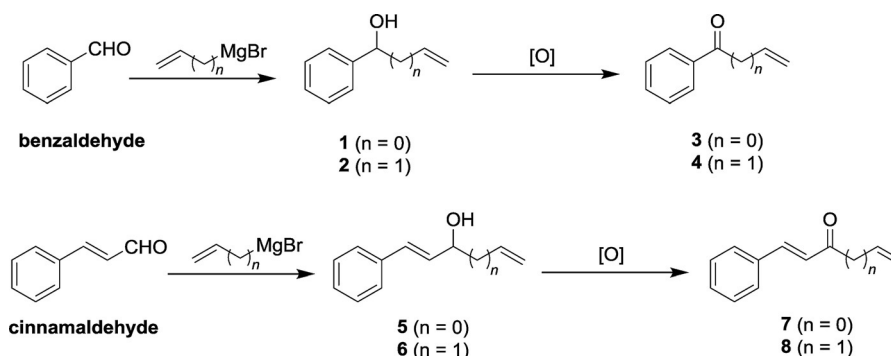


Scheme 1. Retrosynthesis of etretinate and curcumin analogues.

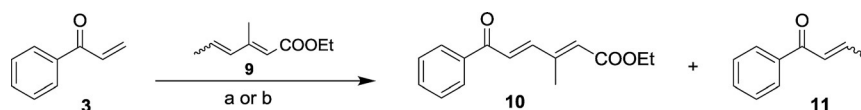
analogues,^[22] we chose ethyl (2*E*,4*E/Z*)-3-methylhexa-2,4-dienoate (**9**) as a convenient partner for the cross-coupling reactions. When the CM of ketone **3** was carried out under optimal

reaction conditions, as previously established for etretinate analogues (3 equiv diene **9**, 10 mol% catalyst **III**, toluene, 50 °C),^[22] the desired product **10** was obtained in less than 5% yield. The main product appeared to be ketone **11** formed through an undesirable metathesis pathway (Scheme 3). This reaction outcome could not be changed either by using different amounts of the catalyst (5 or 20 mol%) or its type (complex **II**). However, when the cross-coupling reaction was carried out in dichloromethane instead of toluene, the desired oxoester **10** was obtained in 18% yield. Although the product yield was low, the reaction proved completely *E*-stereoselective. Attempts to optimize the reaction conditions did not result in any further improvement.

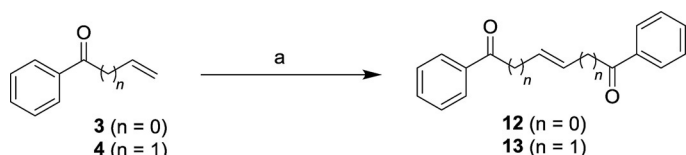
Then, to test the usefulness of phenyl vinyl ketone for the synthesis of symmetrical curcuminoids, the SM of **3** in the presence of **III** in CH₂Cl₂ or toluene (Scheme 4) was studied. Under optimal conditions (15 mol% **III**, CH₂Cl₂, reflux), alkene **12** was obtained in a moderate yield (45%). In the case of symmetrical 1,2-disubstituted olefins, analysis of the *E/Z* product configura-



Scheme 2. Preparation of substrates for metathesis reactions. Reagents and conditions: a) **1**: 85%, **2**: 90%, b) **3**: 53%, [O]=PDC, **4**: 77%, [O]=DMP, c) **5**: 91%, **6**: 98%, d) **7**: 60%, [O]=MnO₂, **8**: 67%, [O]=DMP.



Scheme 3. CM reaction between phenyl vinyl ketone (**3**) and ethyl (2*E*,4*E/Z*)-3-methylhexa-2,4-dienoate (**9**). Reagents and conditions: a) 10 mol% of catalyst **III**, toluene, 50 °C, **10**: < 5%, **11**: 30%, b) 10 mol% of catalyst **III**, CH₂Cl₂, reflux, **10**: 18%, **11**: < 2%.



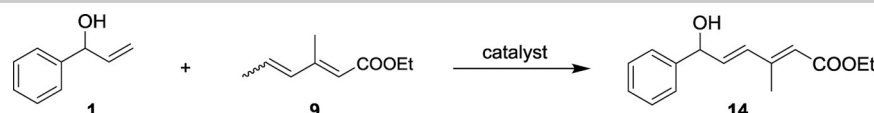
Scheme 4. SM reactions of unsaturated ketones **3** and **4**. Reagents and conditions: a) 15 mol% of catalyst **III**, CH₂Cl₂, reflux, 16 h, **12**: 45%, *E/Z* = 17:1, **13**: 49%, *E/Z* = 23:1.

tion by using the NMR method is a challenging task. The coupling constant ³*J*(H,H) is not directly accessible from the ¹H NMR spectrum, owing to the equivalence of the alkene protons. Determining a double-bond configuration requires a new method to be employed, which uses the low natural abundance of the NMR-active ¹³C isotope to break the symmetry of the carbon-bound vicinal protons.^[25] The 2D NMR technique uses a combination of INEPT and selective TOCSY pulse sequences to retrieve the coupling constant value of chemically equivalent alkene protons. Analysis of the correlation spectra for ketone **12** allowed us to identify the alkene proton signals and then to determine, from the peak areas, that the alkene was formed as a mixture of *E* and *Z* isomers in a ratio of 17:1. The SM of allyl ketone **4** was also investigated (Scheme 4). Despite expecting that this substrate should be more reactive than the electron-deficient vinyl ketone **3**, product **13**, in the presence of catalyst **III**, was only produced in a 49% yield as a mixture of *E* and *Z* isomers in a ratio of 23:1, as determined by using the same method.

As these initial results of the vinyl and allyl ketone metathesis reactions appeared unsatisfactory, in view of the planned retinoid and curcuminoid synthesis, we decided to modify our strategy. In the next reactions, we used the corresponding alcohols **1** and **2** as substrates, which were expected to be more

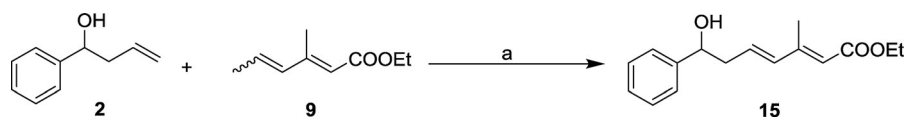
reactive, instead of the unsaturated ketones. Allyl alcohol **1** was subjected to CM with diene **9** under various reaction conditions. The results of these experiments are summarized in Table 1.

When the reaction was carried out with three equivalents of diene **9** in the presence of a second-generation catalyst (**II** or **III**) in toluene at room temperature, product **14** was formed in low yields (ca. 10%), but with complete *E* selectivity (Table 1, entries 1 and 2). Additionally, homocoupled alcohol and unreacted substrates were observed in the reaction mixture. By using dichloromethane as a solvent and increasing the temperature to 40 °C, better yields of the desired product **14** were obtained (Table 1, entries 4–8). Interestingly, in this solvent, slow SM of ethyl 3-methylhexa-2,4-dienoate (**9**) was observed, which never occurred in toluene. The best product yield was obtained when alcohol **1** was reacted with five equivalents of diene **9** in the presence of catalyst **III** in refluxing dichloromethane (Table 1, entry 6). When alcohol **1** was used in excess, a significant decrease in the yield of product **14** was observed (Table 1, entry 7). This result may suggest that SM of alcohol **1** is faster than its reaction with **9**, and the corresponding dimer is resistant to secondary metathesis reactions. By changing the solvent from dichloromethane to toluene or 1,2-dichloroethane and increasing the reaction temperature to 65 °C, the yield decreased from 71 to 22 or 36%, respectively (Table 1, entries 3 and 9). It seems that lower yields of **14** at elevated temperature can be attributed to faster catalyst and product decomposition. In all cases, catalyst **III** proved to be more efficient in promoting this transformation than complex **II**. The best reaction conditions were also optimal for the CM of homoallylic alcohol **2** with ethyl 3-methylhexa-2,4-dienoate (Scheme 5), affording product **15** in 80% yield. In both cases, the desired products (**14** or **15**) were formed with complete *E* stereoselectivity.

Table 1. CM reaction between 1-phenylprop-2-en-1-ol (**1**) and ethyl 3-methylhexa-2,4-dienoate (**9**) under various reaction conditions.



Entry	Equiv of 9	Catalyst ^[a]	Solvent	T [°C]	Product yield ^[b,c] [%]
1	3	III	toluene	rt	12
2	3	II	toluene	rt	10
3	3	III	toluene	65	22
4	3	III	CH ₂ Cl ₂	40	29
5	3	II	CH ₂ Cl ₂	40	25
6	5	III	CH ₂ Cl ₂	40	71
7	0.2	III	CH ₂ Cl ₂	40	26
8	5	II	CH ₂ Cl ₂	40	43
9	5	III	C ₂ H ₄ Cl ₂ ^[d]	65	36
10	5	II	C ₂ H ₄ Cl ₂	65	22

[a] In all cases, 10 mol% of the catalyst was used and reaction was carried out for 16 h. [b] Product was obtained as all-*E* isomer. [c] The remaining material comprised mainly unreacted substrates and homocoupled alcohol. [d] C₂H₄Cl₂-1,2-dichloroethane.

**Scheme 5.** CM reaction between homoallyl alcohol **2** and ethyl (2*E*,4*E/Z*)-3-methylhexa-2,4-dienoate (**9**). Reagents and conditions: a) 10 mol% of catalyst III, 5 equiv of **9**, CH₂Cl₂, reflux, 16 h, **15**: 80%, 100% *E*.

As in the case of unsaturated ketones, allylic (**1**) and homoallylic (**2**) alcohols were also subjected to SM. Metathetic homocoupling of secondary allylic and homoallylic alcohols seemed to be an easy goal, as these substrates could be considered as type II and type I olefins, respectively, according to the Grubbs model of olefin reactivity.^[26] Indeed, the optimized yields of SM products **16** and **17**, as reported in Table 2, were satisfactory.

However, both products were formed as inseparable mixtures of diastereoisomers (*RS*, *RR/SS*, both *E* or *Z*), although the stereochemical issue will not be discussed here. In the case of reaction of homoallylic alcohol **2**, the best yields (over 90%) of product **17** were achieved with 5 mol% of complexes III or II (Table 2, entries 2 and 4). Lowering the catalyst loading from 5 to 3 mol% decreased the product yield from 94 to 31% for

Table 2. SM reactions of 1-phenylprop-2-en-1-ol (**1**) and 1-phenylbut-3-en-1-ol (**2**).


Entry	Substrate	Catalyst (amount)	Reaction conditions ^[a]	SM product (yield)
1	2	III (3 mol%)	CH ₂ Cl ₂ , 40 °C	17 (31%)
2	2	III (5 mol%)	CH ₂ Cl ₂ , 40 °C	17 (94%)
3	2	II (3 mol%)	CH ₂ Cl ₂ , 40 °C	17 (52%)
4	2	II (5 mol%)	CH ₂ Cl ₂ , 40 °C	17 (90%)
5	2	I (5 mol%)	CH ₂ Cl ₂ , 40 °C	17 (77%)
6	2	VI ^[27] (5 mol%)	CH ₂ Cl ₂ , 40 °C	17 (66%)
7	1	III (10 or 5 mol%)	CH ₂ Cl ₂ , 40 °C	16 (0%) + 18 (40%)
8	1	III (10 mol%)	CH ₂ Cl ₂ , rt	16 (42%) + 18 (40%)
9	1	III (10 mol%)	toluene, 50 °C	16 (0%) + 18 (31%)
10	1	III (10 mol%)	toluene, rt	16 (66%) + 18 (23%)
11	1	III (5 mol%)	toluene, rt	16 (66%) + 18 (<10%)
12	1	II (5 mol%)	CH ₂ Cl ₂ , 40 °C	16 (78%)
13	1	II (5 mol%)	toluene, rt	16 (55%) + 18 (<5%)
14	1	VI ^[27] (5 mol%)	CH ₂ Cl ₂ , 40 °C	16 (69%)

[a] In all cases, reaction was carried out for 16 h.

complex III and from 90 to 52% for complex II (Table 2, entries 1 and 3). Other catalysts, such as I and VI (Figure 3), also proved effective (Table 2, entries 5 and 6).

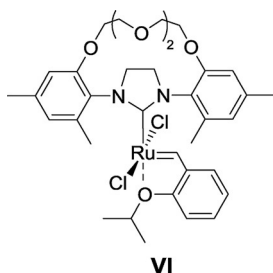


Figure 3. New modified ruthenium metathesis catalyst with polyether clamp embracing *N,N'*-2,4-dimethylphenyl substituents in the NHC ligand.^[27]

Homometathesis of allylic alcohol 1 turned out to be more challenging. When the coupling reaction was performed with catalyst III (10 mol%) in DCM at reflux, instead of the desired diol 16, compound 18 (40%, Figure 4) was isolated (Table 2,

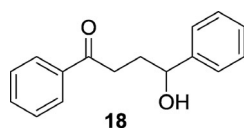


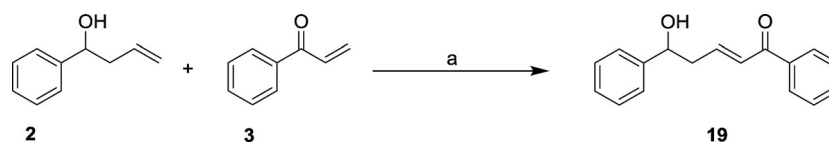
Figure 4. Isomerization product formed in the SM reaction of 1-phenylprop-2-en-1-ol (1).

entry 7), which was formed through SM followed by isomerization, in addition to a small amount of the isomerized substrate, propiophenone. Different reaction conditions were attempted to avoid the formation of product 18 and to improve the yield of the SM product (Table 2, entries 8–14). At first, the influences of temperature, catalyst amount, and solvent were examined for reactions promoted by catalyst III (Table 2, entries 7–11). It was found that the yield of by-product 18 could be decreased by lowering both the reaction temperature (Table 2, entries 8 and 10) and catalyst loading (Table 2, entry 11). From entries 8 and 10/11 in Table 2 it can be seen that the choice of solvent is also important. When the reaction was carried out in the presence of catalyst III in toluene, the conversion was higher and less of the isomerization product was formed. However, this solvent effect was not obvious for reactions catalyzed by complex II (Table 2, entries 12 and 13). Fortunately, changing the promoter for compound II or VI (Figure 3) and lowering the catalyst loading from 10 to 5 mol% allowed us to

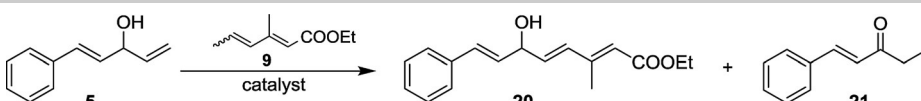
hamper the isomerization process completely. The desired product 16 was produced in good yields (78% in the presence of II, 69% in the presence of VI) without a trace of the undesired isomerized product 18 (Table 2, entries 12 and 14).

When planning the synthesis of unsymmetrical curcuminoids, homoallylic alcohol 2 was coupled with allylic alcohol 1 in the presence of a second-generation catalyst (II or III). Although there are examples of selective CM reactions between type I and type II olefins,^[26] in the case of the reaction of two model substrates, a mixture of products was formed as a result of CM and SM of both substrates, even if the allylic alcohol was used in excess. When we used the less reactive vinyl ketone 3 instead of allylic alcohol 1 as a cross partner for 2, the reaction proved to be much more selective, and the desired product 19, under optimized reaction conditions, was obtained in 77% yield with high diastereoselectivity ($E > 98\%$, Scheme 6). The result of this reaction suggested that phenyl vinyl ketone 3 may be efficiently coupled with more reactive olefins of type 1 or 2, according to the Grubbs classification. However, in the case of a less reactive partner (as diene 9), the cross-coupled product is formed only in low yields (Table 1). In subsequent experiments, we investigated the reactions of ketones 7 and 8 as well as of alcohols 5 and 6 derived from cinnamaldehyde (Scheme 2). The presence of an additional internal double bond in these substrates makes their CM reactions more challenging. However, it could be expected that the terminal monosubstituted double bond is more reactive. A series of reactions was carried out for olefin 5 with ethyl sorbate 9 (Table 3). The main product of reactions in toluene (entries 1–4) was 1-phenylpent-1-en-3-one (21), produced by ruthenium complex-catalyzed isomerization of substrate 5. Even the addition of chloroborocatechol or 1,4-benzoquinone^[28–30] to prevent isomerization was unsuccessful. Only traces of the expected product 20 were formed. The same happened when 1,2-dichloroethane was used as a solvent at 65 °C (entries 9 and 10). However, reactions carried out in CH_2Cl_2 (entries 5–7) or in ethyl ether (entry 8) at reflux produced mostly product 20, albeit in low yields. Interestingly, the influence of the catalyst type, its concentration, and the reaction temperature on the reaction course proved to be less important. The above CM appeared to be regioselective—the product of the internal double-bond reaction in substrate 5 was formed in negligible amounts.

The problem of the competitive isomerization process was also encountered during the study of the SM of 5. Even the reactions of compound 5 in the presence of catalyst II carried out in refluxing CH_2Cl_2 led to the formation of ketone 21 as the main product, in addition to the unreacted substrate. In

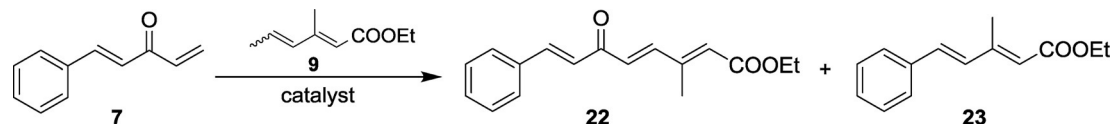


Scheme 6. CM reaction between phenyl vinyl ketone (3) and 1-phenylbut-3-en-1-ol (2). Reagents and conditions: a) 10 mol% of catalyst III, 4 equiv of 3, CH_2Cl_2 , reflux, 16 h, 19: 77%, $E > 98\%$.

Table 3. CM of 1-phenylpenta-1,4-dien-3-ol (**5**) with ethyl 3-methylhexa-2,4-dienoate (**9**) under various conditions.


Entry	Catalyst (amount)	Reaction conditions ^[a]	Product yield [%]	
			20 ^[b]	21
1	II (10 mol%)	3 equiv of 9 , toluene, rt	< 2	70
2	II (5 mol%)	3 equiv of 9 , toluene, rt	< 2	64
3	II (10 mol%)	chloroborocatechol, 3 equiv of 9 , toluene, rt	< 2	70
4	III (5 mol%)	3 equiv of 9 , toluene, rt	0	65
5	II (10 mol%)	3 equiv of 9 , CH ₂ Cl ₂ , 40 °C	12	7
6	II (10 mol%)	5 equiv of 9 , CH ₂ Cl ₂ , 40 °C	15	6
7	III (10 mol%)	5 equiv of 9 , CH ₂ Cl ₂ , 40 °C	12	7
8	II (10 mol%)	Cu ₂ I ₂ (15 mol%), 3 equiv of 9 , Et ₂ O, 35 °C, 5 h	13	4
9	II (10 mol%)	5 equiv of 9 , C ₂ H ₄ Cl ₂ , 65 °C	< 2	43
10	III (10 mol%)	5 equiv of 9 , C ₂ H ₄ Cl ₂ , 65 °C	3	40

[a] In all cases, reaction was carried out for 16 h. [b] *E*-isomer of product **20** was formed (> 97%).

Table 4. CM of 1-phenylpenta-1,4-dien-3-one (**7**) and ethyl 3-methylhexa-2,4-dienoate (**9**) under various reaction conditions.


Entry	Catalyst (amount)	Reaction conditions ^[a]	Product yield ^[b] [%]	
			22	23
1	III (10 mol%)	2 equiv of 9 , toluene, 50 °C	15	29
2	III (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , 40 °C	35	22
3	III (10 mol%)	1 equiv of 9 , CH ₂ Cl ₂ , 40 °C	25	10
4	III (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , rt	15	16
5	IV (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , 40 °C	40	26
6	IV (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , rt	30	13
7	IV (10 mol%)	2 equiv of 9 , toluene, 50 °C	12	16
8	II (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , rt	15	29
9	II (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , 40 °C	30	66
10	II (10 mol%)	Cu ₂ I ₂ (15 mol%), 2 equiv of 9 , Et ₂ O, 35 °C, 5 h	18	51
11	VI ^[27] (10 mol%)	2 equiv of 9 , CH ₂ Cl ₂ , 40 °C	30	< 1

[a] In all cases, reaction was carried out for 16 h. [b] In all cases, *E*-isomers of products were formed > 96%.

subsequent experiments, to overcome the problem of isomerization, the corresponding unsaturated ketone **7** was subjected to CM and SM reactions. The results of reaction between vinyl ketone **7** and ethyl 3-methylhexa-2,4-dienoate **9** in the presence of various catalysts are summarized in Table 4. In all reactions, the desired product **22** was accompanied by ester **23**, resulting from the CM reaction on the internal double bond and products of SM of the starting ketone on both double bonds, although to a much lesser extent.

The highest conversion was obtained in reactions promoted by catalyst **II** in refluxing CH₂Cl₂ or under Lipshutz conditions (**II**, Cu₂I₂, refluxing ether)^[31] (Table 4, entries 9 and 10). However, this complex promoted the metathesis reaction on both double bonds present in the substrate. To our surprise, the product on the internal double bond **23** dominated in the reaction mixture. With the use of other metathesis catalysts, such as **III** or **IV**, unselective scission also occurred; however,

these complexes favored the formation of the desired ester **22** over the short-chain ester **23** (Table 4, entries 2–6). A completely regioselective reaction was observed when complex **VI** (entry 11) was used as a catalyst (Figure 3). The retinoid analogue **22** was obtained in 30% yield with high diastereoselectivity. The catalyst's bulkiness likely prevented a reaction on the internal, more hindered double bond. The considerable reactivity of the internal double bond in **7** can be explained as follows. As the steric factors favor a reaction on the less hindered terminal double bond, the electronic effects seemed to be responsible for the increased reactivity of this ketone's internal double bond. The conjugation of benzene π electrons with the α,β -unsaturated ketone system caused higher electron density on this double bond, as compared with the electron-deficient terminal double bond. This assumption seems to be confirmed by the high reactivity of cinnamaldehyde in CM reactions^[22] as well as by the resistance of the analogous

double bond in the corresponding alcohol (no conjugation) to the metathesis reactions, as described above (Table 2).

Then, we attempted metathesis reactions of allylic ketone **8**. Although its CM with ethyl sorbate **9** was carried out under various conditions, the reactions proved to be very sluggish and a complex mixture of products was obtained as a result of competitive CM, SM, and isomerization processes. When the corresponding alcohol **6** was subjected to a coupling reaction with the same diene **9**, the desired product **24** was obtained, accompanied by SM products and unreacted substrates. The best product yield was achieved by employing the Lipshutz^[31] procedure; ester **24** was then obtained in 38% yield and with high diastereoselectivity (Scheme 7). SM of the same substrate proceeded in the highest product yield (total yield of the mixture of diastereoisomers: 82%) in the presence of catalyst I (Scheme 7).

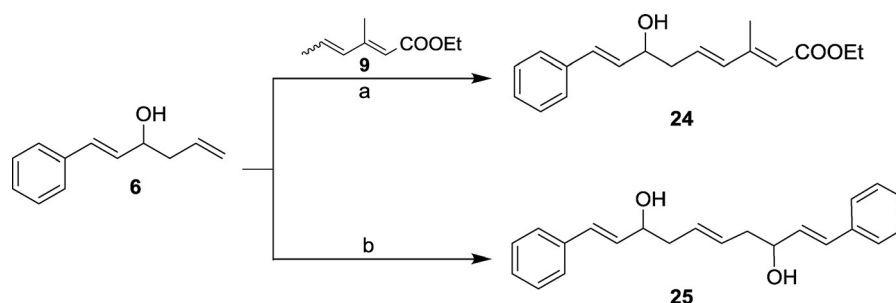
With these results in hand, we examined the application of the method to retinoid and curcuminoid syntheses. As the CM reaction of model 1-phenylprop-2-en-1-ol (**1**) with ethyl 3-methylhexa-2,4-dienoate (**9**) was much more efficient than CM of the corresponding vinyl ketone **3**, the synthesis of retinoids **10** and **28** from allylic alcohols was designed by employing the two-step procedure consisting of CM followed by oxida-

tion (Scheme 8). In both cases (substrates **1** and **26**), CM reactions gave the desired products (**14** and **27**) in high yields and with high *E* stereoselectivity. Oxidation of CM products by PDC yielded retinoids **10** and **28** in satisfactory yields.

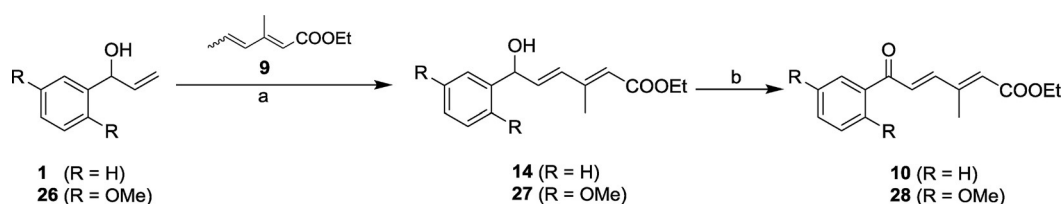
The initial studies proved that a similar strategy should also be optimal for the preparation of symmetrical curcuminoids. Allylic and homoallylic alcohols **2**, **26**, and **31** were subjected to SM reactions followed by oxidation to afford the desired analogues of curcumin (Scheme 9). The unsymmetrical curcuminoid **34** was obtained through the CM reaction between phenyl vinyl ketone (**3**) and homoallylic alcohol **31** in a good yield and with high *E* selectivity (Scheme 10).

2.2. Biological Tests

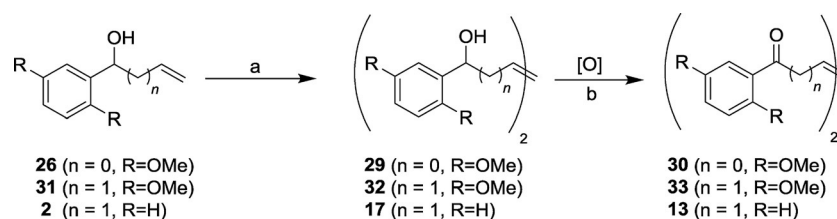
The synthesized analogues of etretinate and curcumin were screened against various tumor cells. The T-lymphoblastic leukemia CEM cell line proved to be the most sensitive to the retinoids and curcuminoids (Table 5) in the micromolar range. The most effective antiproliferative activity on all of the tested cell lines was displayed by compound **22** (IC₅₀ 0.9–2.8). However, retinoids and curcuminoids also appeared toxic toward normal human fibroblasts (BJ) at a similar concentration. The cytotoxic



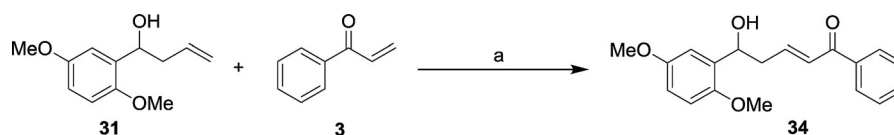
Scheme 7. CM and SM reactions of unsaturated alcohol **6**. Reagents and conditions: a) 10 mol% of catalyst II, 15 mol% of Cu₂I₂, Et₂O, reflux, 3 h, **24**: 38%, *E*/*Z* = 15:1, b) 5 mol% of catalyst I, CH₂Cl₂, reflux, 16 h, **25**: 82%.



Scheme 8. Synthesis of oxoretinoids **10** and **28**. Reagents and conditions: a) 10 mol% of catalyst III, CH₂Cl₂, reflux, 16 h, **14**: 71%, **27**: 73%, b) PDC, CH₂Cl₂, rt, 6 h, **10**: 70%, **28**: 67%.



Scheme 9. Synthesis of symmetrical curcuminoids **13**, **30**, and **33**. Reagents and conditions: a) 10 mol% of catalyst III, CH₂Cl₂, reflux, 16 h, **29**: 58%, **32**: 78%, **17**: 94%, b) **30**: 78%, *E* > 98%, [O] = MnO₂, **33**: 63%, *E* > 99%, [O] = DMP, **13**: 68%, *E* > 94%, [O] = DMP.



Scheme 10. Synthesis of unsymmetrical curcuminoid **34**. Reagents and conditions: a) 10 mol % of catalyst **III**, CH_2Cl_2 , 4, equiv of **3**, reflux, 16 h, **34**: 94%, $E > 97\%$.

Table 5. IC_{50} [μM] values obtained from the Calcein AM assays with tested cancer and normal cell lines. ^[a]					
Entry	Compound	CEM	MCF7	HeLa	BJ
Positive control	staurosporine	0.023 ± 0.064	0.023 ± 0.002	0.175 ± 0.007	0.002 ± 0.000
Retinoids					
1	10	6.9 ± 0.1	24.1 ± 5.9	> 50	28.5 ± 4.9
2	28	3.5 ± 0.0	9.0 ± 3.5	18.9 ± 7.5	8.0 ± 0.0
3	22	0.9 ± 0.1	2.8 ± 0.1	2.5 ± 0.2	1.0 ± 0.0
4	35	> 50	> 50	> 50	> 50
5	36	27.2 ± 0.7	34.2 ± 6.2	17.3 ± 0.3	14.6 ± 3.5
Curcuminoids					
6	18	35.0 ± 0.1	> 50	> 50	> 50
7	12	3.6 ± 0.1	32.4 ± 15.1	> 50	8.6 ± 0.1
8	30	5.6 ± 0.3	41.3 ± 3.0	> 50	18.0 ± 8.9
9	13	6.8 ± 0.7	34.1 ± 7.9	> 50	> 50
10	33	7.5 ± 0.2	> 50	> 50	> 50
11	19	1.8 ± 0.4	6.4 ± 0.1	12.7 ± 4.0	6.3 ± 0.2
11	34	6.5 ± 0.7	24.2 ± 0.1	40.9 ± 4.9	22.4 ± 0.6

[a] Cells were treated for 72 h with increasing serial compound concentrations. Mean values \pm SD were obtained from three independent experiments performed in triplicates.

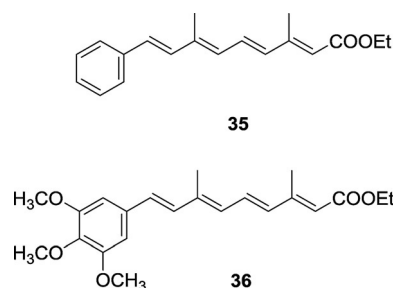


Figure 5. Phenyl and trimethoxyphenyl analogues of ethyl retinoate **35** and **36**.^[22]

activity results obtained for oxoretinoids (**10**, **22**, **28**) and the analogues of ethyl retinoate (**35** and **36**)^[22] (Figure 5) proved that the presence of a carbonyl group in the polyene chain significantly improves antitumor properties (Table 5, entries 1–5). Among the oxoretinoids, higher antiproliferative activity was observed for the compound possessing the methoxy group in the benzene ring (**28**) and a longer polyenic chain (**22**). The polyene chain length seems to be essential. In the curcuminoid series, analogues with the double bond in the chain, especially conjugated to the carbonyl group, exhibited a stronger cytotoxic effect (Table 5, compare entries 6 vs. 7 vs. 11, and 7 vs. 8, and 9 vs. 10). In contrast to the retinoids, the presence of me-

thoxy groups in the curcuminoid benzene ring decreased the cytotoxicity.

The oxygen radical absorbance capacity (ORAC) is the ability of compounds to scavenge free peroxy radicals in vitro.^[32] The synthesized compounds were found to be moderate antioxidants whose activities reached $\leq 7.9\%$ of trolox activity on an equimolar basis (Table 6). In detail, **19** was the most active radical scavenger, whereas the activities of **35**, **18**, and **28** were below the detection limit.

Table 6. Oxygen radical absorbance capacity given as a ratio between compound and trolox on an equimolar basis.^[a]

Entry	Compound	ORAC (compound/trolox)
Curcuminoids		
1	18	nd ^[b]
2	12	0.065 ± 0.004
3	30	0.02 ± 0.003
4	13	0.052 ± 0.005
5	33	0.063 ± 0.001
6	19	0.079 ± 0.002
7	34	nd
Retinoids		
8	28	nd <
9	22	0.041 ± 0.001
10	10	0.026 ± 0.001

[a] Data are expressed as mean \pm SD ($n=4$). [b] Not detected.

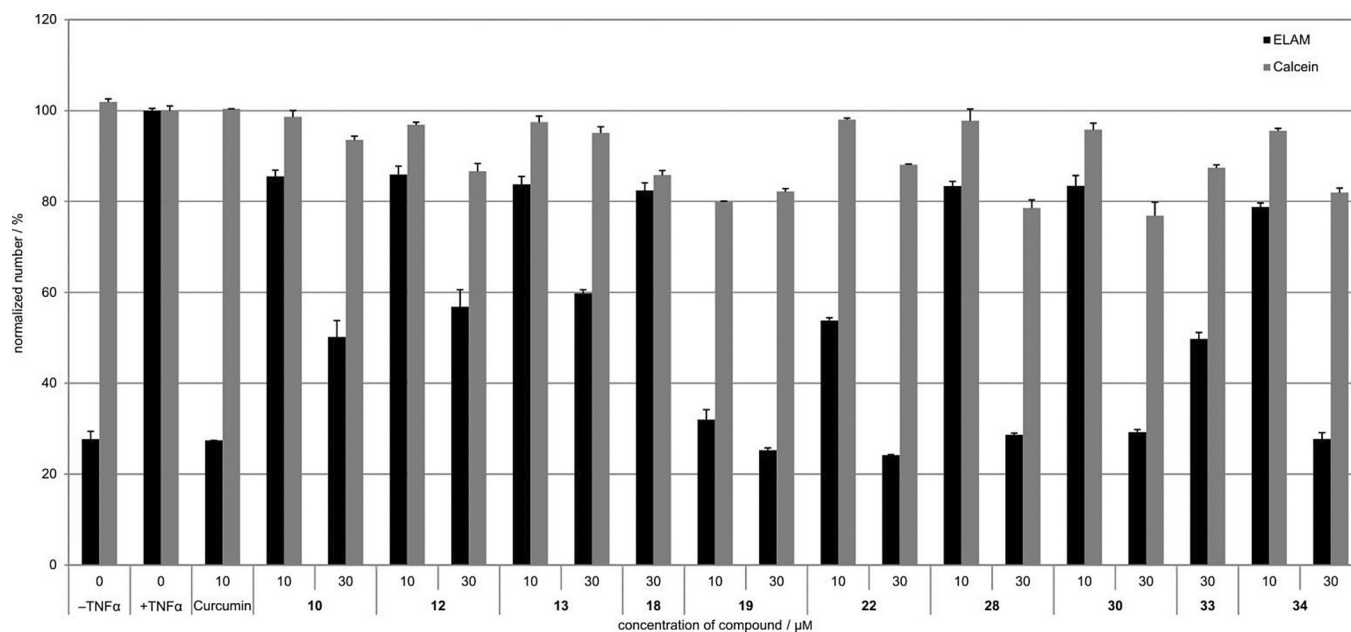


Figure 6. E-selectin (ELAM) expression (4 h) in TNF α -induced HUVECs pretreated with retinoids and curcuminoids (30 min) as compared to cytotoxicity determined by Calcein AM.

The *in vitro* anti-inflammatory properties of retinoids and curcuminoids were studied by using enzyme-linked activity assays (ELISAs) in pretreated human umbilical vein endothelial Cells (HUVECs), in which these compounds could inhibit NF- κ B. E-selectin (ELAM) expression was induced by TNF α , which is indicative of NF- κ B activation. The observed reduction of ELAM expression upon treatment of the HUVECs with 10 or 30 μ M of retinoids and curcuminoids was significant for all of the tested compounds in all concentrations in a dose-dependent manner. The most active derivatives were **22** and **19**, as compared to 10 μ M curcumin as a positive control (Figure 6). In parallel, the cytotoxicity of all compounds was investigated. These derivatives had a very slight influence on cell viability. The obtained results provide evidence that the NF- κ B pathway was targeted by the retinoids and curcuminoids.

3. Conclusions

We studied the metathesis reactions of vinyl and allyl ketones as well as that of the corresponding alcohols under various conditions to find the best route for the synthesis of oxoretinoids and curcuminoids. Our studies proved that the CM of unsaturated alcohols is much more efficient than that of unsaturated ketones. The exception was 1-phenylpenta-1,4-dien-3-ol (**5**), which showed a high tendency for isomerization under metathesis conditions. Although second-generation catalysts are known to be able to promote metathesis reactions of electron-deficient substrates such as α,β -unsaturated carbonyl compounds, in the case of the unsaturated ketone reactions we examined, the desired products were obtained with only low-to-moderate yields, albeit with high diastereoselectivity. For vinyl and allyl ketones with an additional double bond conjugated to the ketone moiety, we observed increased reac-

tivity of the internal double bond that led to unselective metathesis of such substrates. The most likely reason for this was the high electron density of the double bond, owing to conjugation with both the benzene ring and the carbonyl group. Taking into account the obtained results, a convenient synthetic route to various retinoids and curcuminoids was elaborated. The method consists of a two-step procedure starting from allylic and homoallylic alcohols with the CM reaction as the first step, followed by mild oxidation of alcohols to ketones. The desired oxoretinoids and curcuminoids were obtained in good yields and with high diastereoselectivity. The synthesized compounds were tested for antiproliferative, antioxidant, and anti-inflammatory activity *in vitro*. All examined derivatives exhibited strong cytotoxicity against leukemia cell line CEM, except for **18** and **35**. However, the tests demonstrated that they are also toxic towards the normal human fibroblasts (BJ). The antioxidant activity was weak for all of the tested compounds. In contrast, they exhibited strong anti-inflammatory activity *in vitro* without affecting cell viability.

Experimental Section

Chemistry

The melting points presented here were determined by using Toledo Mettler-MP70 apparatus. NMR spectra were recorded with Bruker Avance II 400 or Avance DPX 200 spectrometers operating at 400 and 200 MHz, respectively, using CDCl₃ solutions with TMS as the internal standard (only selected signals in the ¹H NMR spectra are reported). Infrared spectra (in chloroform solution) were recorded by using a Nicolet series II Magna-IR 550 FTIR spectrometer. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. The reaction products were isolated by column chromatography, performed using 70–230 mesh silica gel (J. T. Baker) or by

semi-preparative HPLC. The purity of final compounds was found to be $\geq 95\%$ as determined by HPLC analysis. HPLC analysis was performed with a LabAlliance apparatus comprised of pumps (III Pump series), a UV/Vis detector (525 Dual-wavelength), and an injection valve (Rheodyne Model 7725i). Analytical HPLC was carried out with a Supelco-Si column (5 mm), 0.46×25 cm, and semi-preparative HPLC with a SMT-Si column (5 mm), 1.0×25 cm.

Example Procedure for Retinoid Synthesis

The solution of 1-phenylprop-2-en-1-ol (**1**, 20 mg; obtained from benzaldehyde through a Grignard reaction with vinylmagnesium bromide in a routine manner) in dry dichloromethane (0.5 mL) was added drop-wise to a solution of ethyl (2*E*,4*E*/*Z*)-3-methylhexa-2,4-dienoate (**9**, 5 equiv, 115 mg, 0.11 mL) and a Hoveyda–Grubbs second-generation catalyst (**III**, 10 mol%, 9 mg) in dry dichloromethane (0.5 mL). The reaction mixture was stirred at 40 °C under an argon atmosphere for 16 h. Then, the mixture was concentrated in vacuo and purified directly by using silica gel column chromatography.

CM product **14** [^1H NMR (400 MHz, CDCl_3): $\delta = 1.30$ (t, $J = 7.1$ Hz, 3H), 2.06 (bs, 1H), 2.28 (s, 3H), 4.18 (q, $J = 7.1$ Hz, 2H), 5.35 (d, $J = 4.0$, 1H), 5.82 (s, 1H), 6.26 (dd, $J = 15.6$, 5.9 Hz, 1H), 6.41 (d, $J = 15.6$ Hz, 1H), 7.33 (m, 1H), 7.39 ppm (m, 4H)]; [^{13}C NMR (100 MHz, CDCl_3): $\delta = 13.9$ (CH_3), 14.3 (CH_3), 59.8 (CH_2), 74.7 (CH), 120.2 (CH), 126.3 ($2 \times \text{CH}$), 128.1 (CH), 128.7 ($2 \times \text{CH}$), 133.5 (CH), 137.0 (CH), 142.3 (C), 151.2 (C), 166.9 ppm (C)]; IR (CHCl_3): $\tilde{\nu} = 3599$, 1705, 1614, 1239, 1161 cm^{-1} ; MS EI: [M^+] m/z (%): 246 (8), 228 (6), 171 (20), 158 (24), 105 (100), 77.0 (48)] was eluted with hexane/ethyl acetate (85:15) in 71% yield (26 mg). In the next step, the solution of alcohol **14** (20 mg) in dry dichloromethane (5 mL) was added to PDC (3 equiv, 90 mg) under argon at room temperature. The reaction mixture was stirred for 6 h until all starting material was consumed. The mixture was filtered through a small pad of Celite and silica gel with hexane/ethyl acetate (95:5), and the filtrate was evaporated under reduced pressure to give the desired product **10** in 70% (14 mg), which needed no further purification.

Ethyl (2*E*,4*E*)-3-methyl-6-oxo-6-phenylhexa-2,4-dienoate (**10**): [^1H NMR (400 MHz, CDCl_3): $\delta = 1.33$ (t, $J = 7.2$ Hz, 3H), 2.42 (s, 3H), 4.23 (q, $J = 7.2$ Hz, 2H), 6.15 (s, 1H), 7.28 (d, $J = 15.5$ Hz, 1H), 7.41 (d, $J = 15.5$ Hz, 1H), 7.52 (m, 2H), 7.61 (m, 1H), 7.98 ppm (m, 2H)]; [^{13}C NMR (100 MHz, CDCl_3): $\delta = 13.8$ (CH_3), 14.2 (CH_3), 60.3 (CH_2), 126.5 (CH), 126.6 (CH), 128.5 ($2 \times \text{CH}$), 128.7 ($2 \times \text{CH}$), 133.1 (CH), 137.8 (C), 146.9 (CH), 149.2 (C), 166.2 (C), 190.2 ppm (C)]; IR (CHCl_3): $\tilde{\nu} = 1710$, 1664, 1610, 1234, 1165 cm^{-1} ; MS EI: [M^+] m/z (%): 244.2 (16), 171.2 (72), 139.1 (31), 105 (100), 77.0 (56)]; HRMS EI calculated for $\text{C}_{15}\text{H}_{16}\text{O}_3$: 244.1099, found: 244.1106.

Compound **28** was also prepared by using this method. Compound **22** was obtained in a CM reaction of 1-phenylpenta-1,4-dien-3-one (**7**) and ethyl 3-methylhexa-2,4-dienoate (**9**) in the presence of catalyst **VI** (10 mol%); details of the procedure together with analytical data are provided in the Supporting Information.

Example Procedure for Symmetrical Curcuminoid Synthesis

The solution of unsaturated alcohol (**31**, 30 mg) in dry dichloromethane (1 mL) was added to the Hoveyda–Grubbs second-generation catalyst (**III**, 5 mol%, 5 mg) in dry dichloromethane (0.5 mL). The reaction mixture was stirred at 40 °C under an argon atmosphere for 16 h. Then, the mixture was concentrated in vacuo and

the crude product was purified directly by using silica gel column chromatography.

Dimer **32** [^1H NMR (400 MHz, CDCl_3): $\delta = 2.52$ (m, 4H), 3.78 (s, 6H), 3.81 (s, 6H), 4.92 (m, 2H), 5.57 (m, 2H), 6.78 (m, 4H), 6.95 ppm (m, 2H)]; [^{13}C NMR (100 MHz, CDCl_3): $\delta = 40.90$ (CH_2), 55.7 ($2 \times \text{CH}_3$), 40.92 (CH_2), 55.76 (CH_3), 55.83 (CH_3), 69.2 (CH), 69.3 (CH), 111.5 ($2 \times \text{CH}$), 112.5 ($2 \times \text{CH}$), 112.8 ($2 \times \text{CH}$), 129.9 (CH), 130.0 (CH), 150.4 ($2 \times \text{C}$), 153.7 ($2 < \text{M} \times > \text{C}$), 153.8 ppm ($2 \times \text{C}$)]; IR (CHCl_3): $\tilde{\nu} = 3601$, 3546, 3010, 1591, 1498, 1277, 1047, 811 cm^{-1} ; MS ESI [MNa^+] m/z : 411.0] was eluted with hexane/ethyl acetate (6:4) in 78% yield (22 mg). In the next step, the solution of Dess–Martin periodinane (3 equiv, 66 mg) in dichloromethane (6 mL) was added to the solution of alcohol **32** (20 mg), cooled to 0 °C, in dichloromethane (3 mL). The reaction mixture was stirred at 0 °C. After completion of the reaction (TLC control, 0.5 h), the reaction mixture was passed through a small pad of Celite and silica gel, and eluted with a mixture of hexane/ethyl acetate (8:2). The filtrate was evaporated under reduced pressure to give the desired product **33** in 99% yield (19 mg, isomer $E > 94\%$), which needed no further purification.

(3*E*)-1,6-Di(2,5-dimethoxyphenyl)hex-3-ena-1,4-dione (**33**): [^1H NMR (400 MHz, CDCl_3): 3.72 (s, 6H), 3.73 (m, 4H), 3.79 (s, 6H), 5.76 (m, 2H), 6.82 (d, $J = 9.0$, 2H), 6.95 (dd, $J = 9.0$, 3.2, 2H), 7.18 ppm (d, $J = 3.2$, 2H)]; [^{13}C NMR (100 MHz, CDCl_3): $\delta = 47.5$ (CH_2), 53.4 (CH_2), 55.8 ($2 \times \text{CH}_3$), 56.0 ($2 \times \text{CH}_3$), 113.1 ($2 \times \text{CH}$), 114.1 ($2 \times \text{CH}$), 120.1 ($2 \times \text{CH}$), 126.7 ($2 \times \text{CH}$), 128.2 ($2 \times \text{C}$), 153.1 ($2 \times \text{C}$), 153.5 ($2 \times \text{C}$), 200.1 ppm ($2 \times \text{C}$)]; IR (CHCl_3): $\tilde{\nu} = 1672$, 1609 1497 cm^{-1} ; MS ESI: 407.0 (MNa^+), 791.0 (2MNa^+); HRMS ESI calculated for $\text{C}_{22}\text{H}_{24}\text{O}_6\text{Na}$: 407.1471, found: 407.1473.

Compounds **12**, **13**, and **30** were also prepared by using this method; the details together with the analytical data of products are provided in the Supporting Information.

Example Procedure for Unsymmetrical Curcuminoid Synthesis

The solution of unsaturated alcohol **31** (12 mg) in dry dichloromethane (0.5 mL) was added drop-wise to the solution of phenyl vinyl ketone **3** (4 equiv, 30 mg) and the Hoveyda–Grubbs second-generation catalyst (**III**, 10 mol%, 4 mg) in dry dichloromethane (0.5 mL). The reaction mixture was stirred at 40 °C under argon atmosphere for 16 h. Then, the mixture was concentrated in vacuo and purified directly by using silica gel column chromatography. Product **34** was eluted with hexane/ethyl acetate (7:3) in 94% yield (16 mg, $E > 97\%$).

(2*E*)-5-(2,5-Dimethoxyphenyl)-5-hydroxy-1-phenylpent-2-en-1-one (**34**): [^1H NMR (400 MHz, CDCl_3): $\delta = 2.71$ (m, 1H), 2.79 (m, 2H), 3.78 (s, 3H), 3.82 (s, 3H), 5.10 (m, 1H), 6.81 (m, 2H), 6.91 (m, 1H), 6.97 (d, $J = 2.8$, 1H), 7.07 (dt, $J = 15.4$, 7.2; 1H), 7.46 (m, 2H), 7.55 (m, 1H), 7.89 ppm (m, 2H)]; [^{13}C NMR (100 MHz, CDCl_3): $\delta = 40.8$ (CH_2), 55.7 (CH_3), 55.8 (CH_3), 69.5 (CH), 11.5 (CH), 112.91 (CH), 112.93 (CH), 128.3 (CH), 128 ($2 \times \text{CH}$), 128.6 ($2 \times \text{CH}$), 132.4 (C), 132.6 (CH), 137.8 (C), 145.8 (CH), 150.4 (C), 153.8 (C), 190.9 ppm (C)]; IR (CHCl_3): $\tilde{\nu} = 3600$, 3009, 1670, 1622, 1228, 1047 cm^{-1} ; MS EI [M^+] (%): 311.9 (8), 293.9 (9), 166.9 (100), 145.9 (70), 105 (47)]; HRMS EI calculated for $\text{C}_{19}\text{H}_{20}\text{O}_4$: 312.1362, found: 312.1358.

Compound **19** was also prepared by this method; the details together with the analytical data of product are provided in the Supporting Information.

Biological Tests

Cell Culture

Stock solutions (10 mmolL⁻¹) of retinoids and curcuminoids were prepared by dissolving an appropriate quantity of each substance in dimethyl sulfoxide (DMSO). Dulbecco's modified Eagle's medium (DMEM, RPMI 1640 medium), fetal bovine serum (FBS), L-glutamine, penicillin, and streptomycin were purchased from Sigma (MO, USA). Calcein AM was obtained from Molecular Probes (Life Technologies, CA, USA).

The screening cell lines (T-lymphoblastic leukemia CEM cell line, breast carcinoma cell line MCF7, cervical carcinoma cell line HeLa and BJ human fibroblasts) were obtained from the American Type Culture Collection (Manassas, VA, USA). The CEM cell line was cultured in RPMI 1640 medium and the others in DMEM medium (Sigma, MO, USA); both media were supplemented with 10% fetal bovine serum, 2 mmolL⁻¹ L-glutamine, 10000 U penicillin, and 10 mg mL⁻¹ streptomycin. The cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO₂ in a humid environment. Cells were sub-cultured twice or three times a week by using the standard trypsinization procedure.

HUVECs were cultured in ECGM medium (endothelial cell growth medium, Provitro, Berlin, Germany), supplemented with 10% fetal bovine serum (Sigma Aldrich, Munich, Germany). Cells were maintained under standard cell culture conditions at 37 °C and 5% CO₂ in a humid environment. The cells were sub-cultured twice or three times a week by using the standard trypsinization procedure. The HUVECs were a kind gift from Prof. Jitka Ulrichová (Medical Faculty, Palacky University, Olomouc).

Calcein AM Assay

Suspensions of the tested cell lines (ca. 1.0 × 10⁵ cells mL⁻¹) were placed in 96-well microtiter plates; after 24 h of stabilization (time zero), the tested compounds were added (in three 20 μL aliquots) in serially diluted concentrations in DMSO. Control cultures were treated with DMSO alone, and the final concentration of DMSO in the incubation mixtures never exceeded 0.6%. The tested compounds were typically evaluated at six three-fold dilutions, and the highest final concentration was generally 50 μM. After 72 h incubation, 100 μL of Calcein AM solution (Molecular Probes, Invitrogen, CA, USA) was added, and incubation was continued for a further 1 h. Fluorescence of viable cells was then quantified by using a Fluoroskan Ascent instrument (LabSystems, Finland). The percentage of surviving cells in each well was calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from the control wells and multiplying it by 100. These ratios were then used to construct dose-response curves, from which IC₅₀ values, that is, the concentrations of respective compounds that were lethal to 50% of the tumor cells, were calculated. The results obtained for selected compounds are shown in Table 5.

Determination of Oxygen Radical Absorbance Capacity

The ORAC was determined according to Ou et al.^[32] Briefly, 100 μL of 500 nm fluorescein and 25 μL of diluted solutions of the tested compounds were pipetted into each working well of a (96-well) microplate, pre-incubated at 37 °C. Then, 25 μL of 250 μM AAPH was added and the microplate was shaken for 5 s in a fluorimeter, Infinite 200 (Tecan, Mannedorf, Switzerland). Fluorescence (Ex.

485 nm, Em. 510 nm) was read every 2 min for 60 min. The net area under the curve was used to calculate the ORAC, which was expressed as a ratio between the tested compound and trolox on an equimolar basis.

Anti-inflammatory Activity In Vitro

CD62E (E-selectin, ELAM) Induction Assays

Each well of the 96-well plate was coated with collagen G for 15 min at 37 °C. The outer wells (A1-A12, H1-H12, 1-H1, and A12-H12) contained only 200 μL per well medium and served as an evaporation barrier. A total of 1 × 10⁴ of HUVECs was seeded in each of the other wells in 200 μL medium and grown for 48 h to optimal confluence. Increasing concentrations of compounds were then added to the HUVEC-containing wells in triplicate, and the cells were incubated for 30 min, after which 10 ng mL⁻¹ TNFα was added per well to stimulate NF-κB, and thus ELAM. After further 4 h incubation, levels of ELAM in each of the HUVEC-containing wells were determined by using ELISAs, as described below.

Cell-Surface ELISA ELAM

Cells were washed once with phosphate-buffered saline (PBS) and fixed with 0.1% glutaraldehyde (Sigma-Aldrich, Munich, Germany) for 15 min at room temperature. Then, the cells were washed three times with 200 μL PBS/0.05% Tween 20 per well, blocked with 200 μL 5% BSA/PBS per well for 1 h, and washed again three times with 200 μL PBS/0.05% Tween 20 per well. Then, the anti-ELAM antibody (clone BBA-1, R&D Systems, Minneapolis, MN, USA), diluted 1:5000 in 0.1% BSA/PBS (100 μL per well) was added for 1 h at room temperature and washed five times with 200 μL PBS/0.05% Tween 20 per well. Subsequently, goat anti-mouse HRP antibody (Sigma-Aldrich, Munich, Germany), diluted 1:10000 in 0.1% BSA/PBS (100 μL per well), was applied and the cells were incubated for 1 h in the dark at room temperature and, after decanting, washed five times with 200 μL, PBS/0.05% Tween 20 per well. HRP activity of the cells in each of the wells was estimated by using fast-OPD (o-phenylenediaminedihydrochloride) (Sigma-Aldrich, Munich, Germany) assay, as described,^[33] and absorbance was measured at OD_{492nm} in a vertical spectrophotometer.

Cytotoxicity Testing

For the ELAM expression assay, the toxicity of the tested compounds was assessed in the HUVECs by Calcein AM (Molecular Probes, Invitrogen, Karlsruhe, Germany) cytotoxicity assays in 96-well microtiter plates.^[34] 20 μL portions of each of the compound concentrations were added in triplicate to the cells, which were then incubated at 37 °C in an atmosphere containing 5% CO₂ for 4 h, after which Calcein AM solution was added for 1 h according to the manufacturer's instructions. The fluorescence of viable cells was quantified by using a Fluoroskan Ascent reader (Lab-systems, Finland) and cytotoxic concentrations were calculated on the basis of triplicate experiments.

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Keywords: anti-inflammatory activity · antitumor agents · curcuminoids · metathesis · retinoids

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