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SAR Studies on Curcumin's Pro-inflammatory Targets: Discovery of Prenylated Pyrazolocurcuminoids as Potent and Selective Novel Inhibitors of 5-Lipoxygenase

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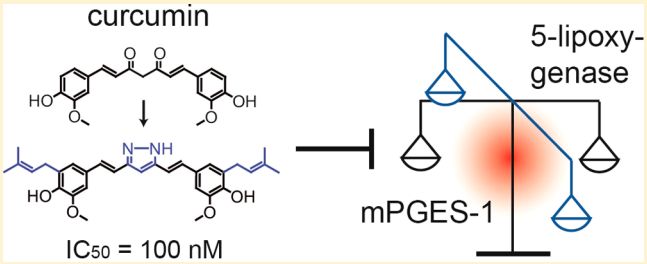
Supporting Information

ABSTRACT: The anticarcinogenic and anti-inflammatory properties of curcumin have been extensively investigated, identifying prostaglandin E_2 synthase (mPGES)-1 and 5-lipoxygenase (5-LO), key enzymes linking inflammation with cancer, as high affinity targets. A comparative structure–activity study revealed three modifications dissecting mPGES-1/5-LO inhibition, namely (i) truncation of the acidic, enolized dicarbonyl moiety and/or replacement by pyrazole, (ii) hydrogenation of the interaryl linker, and (iii) (dihydro)prenylation. The prenylated pyrazole analogue **11** selectively inhibited 5-LO, outperforming curcumin by a factor of up to 50, and impaired zymosan-induced mouse peritonitis along with reduced 5-LO product levels. Other pro-inflammatory targets of curcumin (i.e., mPGES-1, cyclooxygenases, 12/15-LOs, nuclear factor-κB, nuclear factor-erythroid 2-related factor-2, and signal transducer and activator of transcription 3) were hardly affected by **11**. The strict structural requirements for mPGES-1 and 5-LO inhibition strongly suggest that specific interactions rather than redox or membrane effects underlie the inhibition of mPGES-1 and 5-LO by curcumin.



INTRODUCTION

The diarylheptanoid curcumin (diferuloylmethane, **1**, Table 1) is a major ingredient of turmeric (*Curcuma longa* L.), and is used in folk and herbal medicine for the treatment of a host of conditions, mainly in the realm of inflammation and infection.[1] Small scale human clinical trials have suggested anti-inflammatory and anticancer properties for curcumin,[2] fostering an intense research activity on this dietary compound. There is a general agreement that curcumin exerts anticancer and anti-inflammatory effects by interfering with multiple targets. However, only few of them are sensitive to the submicromolar concentrations of this compound that can be achieved by supplementation with various formulations of curcumin.[3] Thus, curcumin activates the redox-regulated transcription factor nuclear factor-erythroid 2-related factor-2 (Nrf2, at 30 μM),[4] interferes with nuclear factor (NF)-κB, p53 and signal transducer and activator of transcription (STAT)3 signaling (NF-κB, $IC_{50} \approx 20$ μM; p53, at 10 μM; STAT3 ≈ 10–25 μM),[5−7] and inhibits histone acetyltransferases (IC_{50} = 20–40 μM),[8] cyclooxygenase (COX)-1 (IC_{50} = 25–50 μM),[9,10] and protein kinase C (IC_{50} = 15 μM).[11] A host of other targets respond to curcumin at concentrations that can be achieved only topically in the gastrointestinal tract (up to 20 μmol/kg) but that are unlikely to be reached in plasma or in organs after oral application.[11] The substantial dissection between the pharmacodynamic and the pharmacokinetic profiles of curcumin, and the lack of solid clinical documentation of activity, make it difficult to evaluate the potential of this dietary compound in medicine.[12] We and others have recently identified curcumin as a potent dual inhibitor of microsomal prostaglandin E_2 synthase (mPGES)-1 and 5-lipoxygenase (5-LO) that are key enzymes at the interface of inflammation and cancer. The submicromolar IC_{50} values (0.2–0.7 μM)[9,13] are in the range of the plasma concentrations of curcumin conjugates observed upon oral administration of various formulations[3,14] or megadosages of

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the unformulated natural product.[15] Metabolic curcumin conjugates are in general devoid of anti-inflammatory activity,[2] but mPGES-1 and 5-LO are strongly expressed in inflamed and tumor tissues,[11,12] where accumulation of free curcumin has been observed as a result of the local upregulation of deconjugating enzymes.[16] mPGES-1 and 5-LO catalyze the formation of the potent pro-inflammatory lipid mediators prostaglandin (PG)E_2 and leukotrienes (LTs) that not only promote inflammation, allergy, pain, and fever but also possess pro-tumorigenic properties.[17,18] For their biosynthesis, arachidonic acid is first released from phospholipids by phospholipases A_2 and then transformed by COX and 5-LO to PGH_2 and LTA_4, respectively.[17,19] PGH_2 is further converted by mPGES-1 to PGE_2,[17] while LTA_4 is hydrolyzed to the chemotactic LTB_4 or metabolized to cysteinyl-LTs, which induce bronchoconstriction and elevate vascular permeability.[19] Pharmacological strategies to intervene with LTs are based on antagonists of the G-protein-coupled LT receptors as well as on inhibitors of 5-LO (i.e., zileuton), which are used in asthma therapy.[20] Moreover, preclinical studies suggest anti-LT therapy being effective for the treatment of cardiovascular disease and cancer.[19,20] The high potency of the dietary diarylheptanoid curcumin to inhibit mPGES-1 and 5-LO together with its selective accumulation in inflamed and tumor tissues qualify these inducible enzymes as primary targets, providing a potential pharmacodynamic and pharmacokinetic basis for the "smart" mechanism of action of curcumin, which lacks significant toxicity and side effects unlike most anti-inflammatory agents.

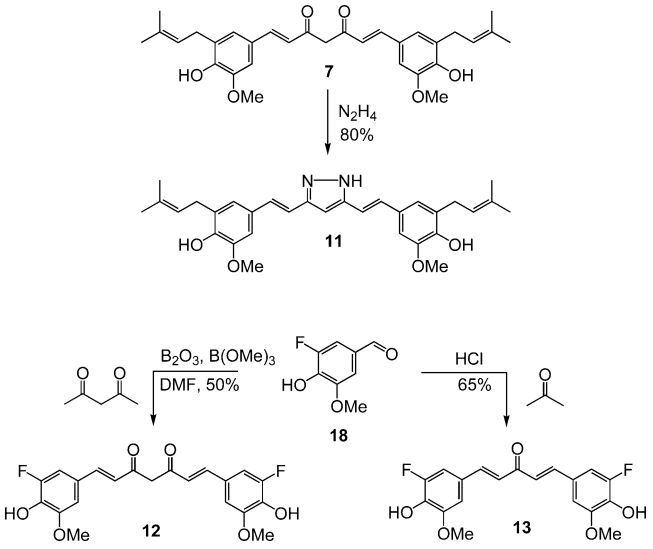
A large number of curcumin derivatives have been synthesized and assayed on diverse molecular targets with often relatively flat SARs.[21] Surprisingly, only few studies focused on eicosanoid biosynthesis, despite its critical role in the biological profile of curcumin. These studies addressed the direct inhibition of mammalian COX-1/2,[10,22] mPGES-1,[22] 5-LO,[23] and 12-LO[24] as well as the inducible (and NF-κB-dependent) expression of COX-2.[25,26] The limited number of analogues investigated and the focus on few read-out systems make it difficult to draw comprehensive predictions about the regulation of the eicosanoid profile. To address this limitation, we have investigated the effect of curcumin and a series of analogues on various enzymes of eicosanoid biosynthesis (mPGES-1, 5-, 12-, and 15-LO, COX-1, COX-2) as well as on inducible transcription factors, such as NF-κB, STAT3 and Nrf2, that are involved in inflammatory and carcinogenic processes. We present evidence that the activity of curcumin on mPGES-1 and 5-LO can be fundamentally dissected and, in the case of 5-LO, significantly improved by structural modification, suggesting that specific interactions govern the interference of curcumin with its high-affinity targets.

RESULTS AND DISCUSSION

Chemistry. The fluorinated analogues **12** and **13** (Table 1) were prepared from 4-fluorovanillin (**18**)[27] by condensation with the boric complex of acetylaceton (Pabon reaction)[28] or with acetone under acidic conditions.[29] The prenylated pyrazole derivative **11** was obtained by reacting its corresponding prenylcurcuminoid (**7**)[29] with hydrazine hydrate (Scheme 1).[30] All other compounds were prepared according to literature (see Experimental Section).

Structure–Activity Relationships. In agreement with previous findings,[9,13] curcumin (**1**) was found to be an equipotent inhibitor of 5-LO and mPGES-1 in cell-free activity assays with IC_{50} values of 0.5 and 0.6 μM, respectively (Table 1). The high

Scheme 1. Synthesis of the Novel Curcuminoids 11–13



potency of curcumin against these two targets suggests the involvement of specific molecular interactions. To evaluate this hypothesis, we investigated if, and how, chemical modification could affect inhibition of mPGES-1 and 5-LO by curcumin in cell free systems. To this aim, we focused on (i) the 1,3-diketone motif (truncation and replacement with a pyrazole ring), (ii) the interaryl linker (reduction), and (iii) the aryl substitution pattern of the natural product (introduction of substituents *ortho* to the free hydroxyl). The biological profile of curcuminoids is, indeed, critically sensitive to these maneuvers,[18] and aryl fluorination and prenylation were selected for their potential to alter the pharmacokinetic properties of phenolics.[31,32] The role of curcumin's interaryl linker was evaluated by comparing the activity of curcumin with that of compounds **2**–**4**, the archetypal members of the classes of truncated (C5-) curcuminoids and tetrahydrocurcuminoids, respectively. Because of the complete tautomerization of the dicarbonyl system at physiological pH (i.e., pH 7.2 to 7.4), curcumin is a weak acid[33] like arachidonic acid and PGH_2, the natural substrates of 5-LO and mPGES-1, respectively. The monoketonic C5-curcuminoid **2** is not enolizable but was almost equipotent to curcumin in terms of inhibiting the activity of purified 5-LO, while the diketonic C5-curcuminoid **3** and tetrahydrocurcumin **4** were 5–6-fold less effective (Table 1), the latter in accordance with a previous finding.[9] However, all three modifications were detrimental for inhibition of mPGES-1, because compounds **2**–**4** did not, or only slightly, suppress the activity of this enzyme up to 10 μM (Table 1). Thus, shortening the interaryl linker of curcumin substantially dissects mPGES-1 from 5-LO inhibition, even if the enolizable 1,3-dicarbonyl system is preserved. Nonspecific antioxidant activity does not seem to be involved in the inhibition of mPGES-1 or 5-LO because all compounds were poor radical scavengers (EC_{50} > 10 μM, Table 1); ascorbic acid (**16**; used as positive control) worked as expected.

The pyrazole analogue of curcumin **5** has previously been described as potent inhibitor of LT formation by activated rat neutrophils, and 5-LO was suggested as a molecular target.[23] Replacement of the 1,3-dicarbonyl system of curcuminoids with a pyrazole ring retains the hydrogen donor and acceptor properties of the enolized 1,3-dicarbonyl system of curcumin. Because this element is critical for the interaction with mPGES-1, we speculated that pyrazole analogues of curcumin might combine

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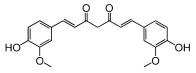
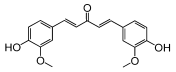
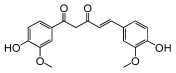
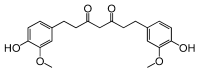
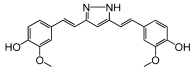
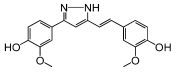
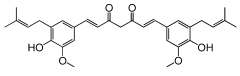
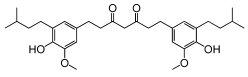
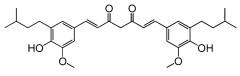
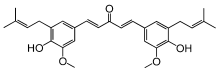
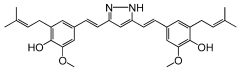
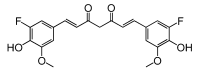
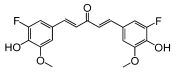
Table 1. Stability of Curcumin and Structural Analogues and Effects on mPGES-1, 5-LO, and Antioxidant Activity

	compound / structure	mPGES-1[a]	5-LO[a]		radical scavenging[a]	stability at pH 7.9[f]
			purified	neutrophils		
1		0.6±0.0	0.5±0.0	3.9±0.5	63.9±3.5[c] **	41.6±5.5***
2		ni[b]	0.8±0.1	5.2±0.4	80.7±0.3[c] ***	ni
3		72.6±6.6[c] *	2.3±0.7	9.9±0.7	80.2±2.2[c] **	72.0±10.3
4		ni	3.2±1.6	70.1±11.4[c]	62.2±0.3[c] ***	ni
5		54.8±5.2[c] **	0.07±0.01	0.7±0.0	59.6±1.7[c] ***	ni
6		62.2±9.8[c]	0.09±0.02	1.9±0.8	70.3±.2.3[c] ***	ni
7		59.6±8.6[c] *	0.7±0.1	74.3±5.3[c] *	57.9±11.2[c]	ni
8		ni	0.4±0.2	2.9±0.8	71.9±0.9[c] ***	87.4±2.4
9		71.7±2.1[c] ***	2.8±1.0	80.2±9.3	64.3±1.7[c] ***	ni
10		1.5±0.3	0.5±0.1	1.7±1.0	75.8±0.5[c] ***	ni
11		68.0±2.2[c] ***	0.1±0.0	0.8±0.1	57.3±3.3[c] ***	ni
12		8.7.±4.4	0.4±0.0	10.0±3.8	61.4±3.2[c] ***	ni
13		58.8±8.2[c] *	0.6±0.1	8.1±1.0	80.5±2.9[c] **	ni
14	BWA4C	nd	0.02±0.01	0.05±0.02	nd	nd
15	MD52	0.02±0.00	nd	nd	nd	nd
16	ascorbic acid	nd[d]	nd	nd	20.4±1.1[c] ***	nd

[a]IC_{50} values (μM) and residual activity (% of control) are given as mean ± SEM of single determinations obtained in three to four independent experiments. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$; student t-test. [b]No inhibition/no effect. [c]Residual activity at 10 μM compound concentration. [d]Not determined. [f]Residual activity at 50 μM compound concentration. [g]Residual amount of compound (% of control).

superior inhibition of 5-LO with decreased inhibition of mPGES-1. In fact, both the pyrazole analogues of curcumin (**5**) and C5-curcumin (**6**) potently interfered with purified human recombinant 5-LO, outperforming curcumin by a factor of 6 and 7, respectively (Table 1), while inhibition of mPGES-1 was considerably reduced ($IC_{50} \geq 10$ μM).

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Table 2. Effect of Curcumin and Structural Analogues on COX-1, COX-2, 12-LO, and 15-LO

	COX-1		COX-2[a]	neutrophils	
	enzyme[a]	platelets	enzyme	12-LO	15-LO[a]
1	ni[b]	8.1 ± 0.5[c]	ni	65.0 ± 4.4**,[a]	ni
2	ni	ni	ni	70.1 ± 3.5**,[a]	ni
3	ni	ni	ni	60.6 ± 5.5**,[a]	ni
4	ni	ni	ni	87.4 ± 10.1[a]	ni
5	ni	82.3 ± 5.3[a]	ni	7.3 ± 2.4[c]	ni
6	ni	ni	ni	7.7 ± 1.7[c]	ni
7	80.2 ± 26.1	ni	ni	ni	89.7 ± 3.3
8	82.7 ± 16.0	ni	ni	76.4 ± 4.1*,[a]	ni
9	ni	ni	ni	75.7 ± 1.9***,[a]	ni
10	78.7 ± 8.7	ni	ni	64.7 ± 9.7[a]	ni
11	ni	ni	ni	68.7 ± 3.2**,[a]	ni
12	ni	7.5 ± 0.1[c]	87.2 ± 7.8	69.7 ± 5.3*,[a]	ni
13	ni	65.2 ± 3.2**,[a]	ni	67.7 ± 5.7*,[a]	ni
indomethacin	25.0 ± 2.8***	5.9 ± 3.1***,[a]	43.0 ± 2.4***	nd[d]	nd

[a]Residual activities (% of control) at a compound concentration of 10 μM. [b]No inhibition. [c]IC_{50} values are given as mean ± SEM of single determinations obtained in three independent experiments. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$; student t-test. [d]Not determined.

To further explore the structural determinants for mPGES-1 and 5-LO inhibition and to modulate the pharmacodynamic and pharmacokinetic profile of the resulting analogues, we introduced either a fluorine- or a (dihydro)prenyl-group on the aromatic ring. Distinct fluorinated curcuminoids have been described to inhibit COX-1 and COX-2 more potently than curcumin,[25] and we speculated that fluorination of curcumin, apart from slowing its metabolism, might also affect its interaction with 5-LO and mPGES-1. Prenylated curcuminoids are unknown as natural products,[34] but prenylation is a classic strategy to improve the bioavailability of phenolics,[35] which is of particular interest due to the poor oral absorption of curcumin. Prenyl-groups are frequently found in phenolic 5-LO and/or mPGES-1 inhibitors.[36−40] Here, prenylation (**7** and **11**) was clearly detrimental for the inhibition of mPGES-1 but had little effect on the inhibition of purified 5-LO by either curcumin, C5-curcumin (**2**), or the pyrazole analogue **5** (Table 1). In contrast, prenylation unexpectedly turned the poorly active analogue C5-curcuminoid **2** into the moderate mPGES-1 inhibitor **10**, and dihydroprenylation either reduced (**9**) or improved (**8**) inhibition of purified 5-LO depending on the parent compound (curcumin or tetrahydrocurcumin **4**, Table 1). Opposing SARs were also observed when fluorine was introduced on the aromatic ring of either curcumin (yielding **12**) or C5-curcumin (**2**, yielding **13**). While the curcumin derivative **12** inhibited mPGES-1 less potently than the parent compound, both inhibition of mPGES-1 and purified 5-LO was enhanced by fluorination of C5-curcumin (**13**; Table 1). The 5-LO reference drug **14** (*N*-[(*E*)-3-(3-phenoxyphenyl)prop-2-enyl]acetohydroxamic acid; BWA4C)[41] and the mPGES-1 inhibitor **15** (2-(2-chlorophenyl)-1*H*-phenanthro[9,10-*d*]-imidazole; MD52)[42] were used as control (Table 1). Taken together, our SAR study has identified three complementary strategies to dissect mPGES-1 and 5-LO inhibition by curcumin, namely (i) truncation of the dicarbonyl system and/or its replacement with a pyrazole ring, (ii) hydrogenation of the interaryl linker, and (iii) (dihydro)prenylation.

Curcumin is not stable in aqueous solutions at physiological pH and rapidly degrades mainly into *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal as previously shown[43] and confirmed in the present study by reversed phase liquid chromatography ESI mass spectrometry (Table 1 and Supporting Information Figure S1). The stability of curcuminoids is strongly increased both by introduction of fluorine- (**12**, **13**) or (dihydro)prenyl-groups (**7**−**11**) on the aromatic ring and by disruption of the α,β-unsaturated 1,3-diketone motif through either hydration (**4**, **8**), truncation (**2**, **10**, **13**), or replacement by pyrazole (**5**, **6**, **11**) (Table 1).

Effect of Curcuminoids on Key Enzymes of Eicosanoid Formation and Selected Molecular Targets of Curcumin. Studying the interference of multitarget compounds like curcumin with specific metabolic pathways requires a comprehensive evaluation of the related target profile. For the synthesis of eicosanoids, particularly COX and LO isoenzymes have to be taken into account because curcumin was previously described as a direct but weak inhibitor of COX-1, COX-2, and 12-LO ($IC_{50} \geq 16$ μM).[10,13,24,25] In line with these studies, we found that curcumin and its derivatives (up to 10 μM) did not, or only slightly, inhibit COX-1 and -2 as well as 12- and 15-LO activities in cell-free assays, in intact human platelets (COX-1), and/or in activated human neutrophils (5-, 12-, 15-LO) (Table 2). Weak inhibition of COX-1 in intact platelets was particularly evident for the fluorinated curcumin **12** ($IC_{50} \geq 7.5$ μM), and 12-LO product formation was considerably suppressed by the pyrazole analogues **5** and **6** ($IC_{50} \geq 7.3$ μM). Conversely, prenylation of **5** to generate **11** strongly reduced the potency to inhibit 12-LO. These observations show that the chemical modification of curcumin can generate selective inhibitors of 5-LO.

Next, we investigated the effect of curcuminoids on previously proposed molecular targets (i.e., NF-κB and STAT3 signaling and Nrf2 activation) not directly linked to the production of eicosanoid but capable of affecting the expression of genes involved in inflammation and carcinogenesis. SARs toward NF-κB and Nrf2 (a transcription factor regulating antioxidant response genes) were previously explored for the curcumin derivatives **2**, **4**, **7**, **8**, and **10**.[29] In this context, prenylation (**7**) and hydrogenation (**4**) were detrimental for anti-NF-κB activity, while truncation to C5-curcuminoids (**2**) had an opposite effect.[29] Here, we show that these modifications influence STAT3 signaling and Nrf2 activation in the same way (Table 3). The electrophilic compound **2**[29] thus represents a potent NF-κB/STAT3 dual inhibitor with potential antitumoral activity in addition to being a potent inhibitor of 5-LO (Table 1) and activator of Nrf2 (Table 3). On the other hand, the inclusion of pyrazole (**5** and **6**) increased the potency of curcuminoids to

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Table 3. Effect of Curcumin and Structural Analogues on NF-κB, Nrf2 and STAT3 Transcriptional Activities and Cell Viability

				cell viability[a]	
	NF-κB[a]	STAT3[a]	Nrf2[b]	LNCaP	PBMC
1	20.9 ± 2.6	33.9 ± 2.3	9.9 ± 5.9	36.0 ± 3.8	34.8 ± 5.0
2	1.8 ± 1.7***	6.0 ± 2.4***	0.9 ± 0.4	10.0 ± 3.4***	34.7 ± 7.1
3	28.3 ± 3.2	35.7 ± 7.9	negative	>50	>50
4	>50	>50	negative	>50	>50
5	29.8 ± 3.5	16.2 ± 9.9**	negative	>50	>50
6	30.0 ± 8.3	18.2 ± 2.7**	negative	>50	>50
7	>50	>50	negative	>50	>50
8	33.4 ± 3.6	>50	negative	>50	>50
10	32.3 ± 2.6	22.4 ± 6.6*	negative	43.8 ± 6.4	>50
11	>50	>50	negative	>50	>50
12	>50	15.6 ± 8.4**	8.6 ± 4.1	>50	>50
13	16.8 ± 0.5**	19.4 ± 7.4**	3.1 ± 0.9	25.1 ± 4.8*	>50

[a]IC_{50} values (μM) are given as mean ± SEM of single determinations obtained in four independent experiments. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ compared to curcumin; student *t*-test. [b]EC_{50} values (μM) give the concentration of half-maximal activity compared to *tert*-butylhydroquinone (20 μM).

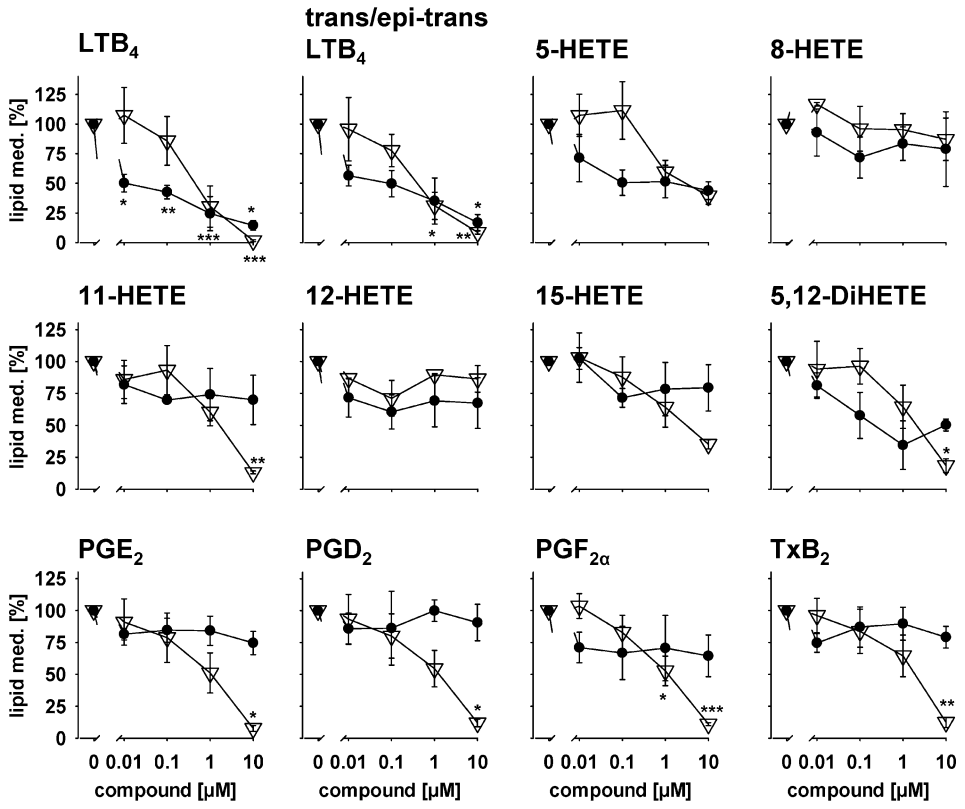

Figure 1. Effect of curcuminoids on eicosanoid formation in activated human monocytes. Lipopolysaccharide-primed monocytes were preincubated with vehicle (DMSO), curcumin ("open triangle") or **11** ("closed circle") for 15 min. Eicosanoid formation was initiated by 2.5 μM A23187 plus 20 μM arachidonic acid. Data are expressed as means ± SEM of single determinations obtained in two to three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs vehicle control; ANOVA + Tukey HSD posthoc tests.

inhibit 5-LO 5−7-fold (Table 1) but neither activated Nrf2 nor induced cytotoxicity (Table 3). The effect of **5** and **6** on NF-κB and STAT3 signaling was comparable or even significantly enhanced compared to curcumin. In contrast, the prenylated C7 curcuminoids **7** and **11** (derived from curcumin and pyrazole **5**, respectively) neither interfered with NF-κB and STAT3 signaling nor activated Nrf2 or were cytotoxic (Table 3). Although compounds targeting NF-κB and STAT3 pathways are being developed mainly for the treatment of cancer, it is expected that chronic inhibition of these pathways could induce side effects such as immunosuppression and/or loss of homeostatic renewal cells. In the same sense, Nrf2 was shown to promote the formation and chemoresistance of solid cancers,[44] thereby acting as proto-oncogene. Altogether, our results highlight compound **11** as an outstanding selective 5-LO inhibitor.

Activity of Curcuminoids on 5-LO Product Formation in Intact Cells. Compounds that inhibit 5-LO in cell-free assays are not necessarily active in intact cells.[36] Hence, we investigated the effect of curcumin and its derivatives on 5-LO product formation in stimulated human neutrophils and

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monocytes. 5-LO products include LTB_4, its all-*trans* isomers, 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) and 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-DiHETE). Although SARs were similar for inhibition of purified 5-LO and neutrophil 5-LO activity within this series of curcuminoids, suppression of cellular 5-LO activity was, in general, less marked than under cell-free conditions but still in the (sub)micromolar range (Table 1). Exceptions are (dihydro)prenylated curcumin **7** and **9** and the fluorinated curcuminoids **12** and **13**, which were considerably less potent in intact cells (Table 1). The most efficient inhibitors of 5-LO within our series were the pyrazoles **5**, **6**, and **11**, as expected from the data obtained in the cell-free assay. These compounds were 2−52-fold more potent in suppressing 5-LO product formation than curcumin both in activated neutrophils (Table 1) and monocytes (Figure 1, Supporting Information Figures S2 and S3; pyrazoles, IC_{50} = 0.013−1.9 μM; curcumin, IC_{50} = 0.6−3.8 μM). Remarkably, the prenylated C5-curcuminoid **10** was 2-fold superior to curcumin in inhibiting neutrophil 5-LO product formation despite being equipotent in the cell free assay (Table 1).

Effect of Curcuminoids on the Eicosanoid Profile of Activated Human Monocytes. Structural modification of curcumin might not only influence the interaction with existing targets but also create novel ones, especially because eicosanoid formation is a complex process involving multiple isoenzymes, regulatory loops, and compensatory mechanisms.[45] To recognize additional high-affinity targets of pyrazolocurcuminoids, the most potent 5-LO inhibitors of this series, we monitored the spectrum of eicosanoids produced by activated human monocytes using ultraperformance liquid chromatography-coupled ESI tandem mass spectrometry (UPLC-MS/MS). At low physiologically relevant concentrations, curcumin preferentially suppressed the formation of 5-LO products, but also the formation of PGE_2, $PGF_{2\alpha}$, PGD_2, thromboxane (Tx)B_2, 11-HETE, and 15-HETE was markedly reduced (IC_{50} =1.2−5.4 μM; Figure 1). The generation of PGE_2 (and other prostanoids) was also inhibited by the nonprenylated pyrazole analogues **5** and **6** (Supporting Information Figures S2 and S3), which is surprising as they exhibit clearly impaired inhibitory potency against mPGES-1 (Table 1). In contrast, the prenylated pyrazole **11** exclusively interfered with the synthesis of 5-LO products (IC_{50} = 0.013−0.4 μM) up to 10 μM (Figure 1), suggesting that prenyl substitution is critical for the pronounced selectivity of **11**. Specific inhibitors of 5-LO (**14**, BWA4C), COX-1/2 (indomethacin), and mPGES-1 (**15**, MD52) were used as control and inhibited eicosanoid biosynthesis as expected (Supporting Information Figure S4).

Efficiency of the Prenylated Pyrazolocurcuminoid 11 in Zymosan-Induced Peritonitis in Mice. The prenylated pyrazolocurcuminoid **11** is the most selective inhibitor of 5-LO in cellular systems within this series. Thus, we investigated its effectiveness in an in vivo model of acute inflammation, namely zymosan-induced mouse peritonitis, which is critically dependent on the formation of LTs and other lipid mediators.[46] Compound **11** (10 mg/kg, ip) suppressed the levels of cysteinyl-LTs (LTC_4 derived from resident peritoneal macrophages in the early phase[47]) and LTB_4 (derived predominantly from infiltrating neutrophils in the late phase[47]) as well as cell infiltration and exudate myeloperoxidase (MPO; a neutrophil marker) activity comparable or even superior to the well-recognized LT synthesis inhibitor **17** (3-(3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-isopropyl-1*H*-indol-2-yl)-2,2-dimethylpropanoic acid; MK-886, 1 mg/kg, ip)[48] (Table 4).

Taken together, despite its pleiotropic bioactivity profile, curcumin can selectively modulate eicosanoid biosynthesis at physiologically relevant concentrations with distinct SARs revealing prenylated pyrazol derivatives, exemplified by **11**, as selective 5-LO inhibitors with promising pharmacological profile.

Table 4. Effect of Compound 11 on Zymosan-Induced Peritonitis in Mice

treatment[a]	cysteinyl-LTs (LTC_4)[b] [ng/mL]	LTB_4[c] [pg/mL]	cell infiltration[c] [10^6/mL]	MPO activity[c] [U/mL]
vehicle	123 ± 10	682 ± 112	7.64 ± 0.51	1.23 ± 0.13
11	10 ± 1	267 ± 92	1.91 ± 0.35	0.75 ± 0.18
	92%[d]	*61%**	*75%****	*39%*
17 (MK-886)	nd[e]	258 ± 48	4.54 ± 0.33	0.72 ± 0.13
	nd	*63%***	*41%****	*41%**

[a]Male mice (n = 5−11 for each experimental group, with age of 8−9 weeks) were treated ip with 10 mg/kg compound **11**, 1 mg/kg compound **17**, or vehicle (2% DMSO) 30 min before zymosan injection. [b]Analysis was performed 30 min after zymosan injection. [c]Analysis was performed 4 h after zymosan injection. Values are given as mean ± SEM of single determinations per mouse. [d]Italics: percent inhibition of vehicle control. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$; student t-test. [e]Not determined.

CONCLUSION

Our study provides evidence that curcumin interacts with its high-affinity targets mPGES-1 and 5-LO in a specific manner, while a flat scenario of SARs was observed for the low affinity targets COX-1/2 and 12/15-LOs. Inhibition of mPGES-1 was critically dependent on the 1,3-diketone motif, possibly because an acidic enol proton is required. More structural variations were tolerated for inhibition of 5-LO (as in **2**, **7**, **8**, **10**, **12**, and **13**), with the nonenolizable pyrazole analogues (**5**, **6**, and **11**) showing remarkably enhanced inhibitory activity. Furthermore, the effect of (dihydro)prenylation, as in **7**−**11**, was strongly dependent on the length and the nature of the aryl−aryl linker, with significant differences in terms of mPGES-1 and 5-LO inhibition. Pyrazole derivatives of curcumin have previously been reported as potent inhibitors of LT biosynthesis in rat basophil leukocytes (IC_{50} = 1 μM) and in a rat model of acute inflammation.[23] In principle, this activity could be due to a direct inhibition of 5-LO, an impaired activation of 5-LO, a reduced substrate supply to 5-LO, or a combination of these effects. Our data clearly show that these compounds are direct inhibitors of 5-LO, essentially devoid of significant activity on mPGES-1 and, when prenylated, with a remarkable selectivity for 5-LO within eicosanoid biosynthesis and among pro-inflammatory targets of curcumin. More than 100 different targets have been identified for curcumin so far, including druggable transcription factors, receptors, and enzymes.[15] The (partially redundant) interference of curcumin with multiple cellular networks might explain its "cure-all" efficacy in cellular and (pre)clinical studies. However, a growing number of negative clinical trials[49] suggest that this pleiotropic profile could be difficult to translate clinically, not only because of the limited bioavailability of curcumin but also due to the potential cacophonic response associated with its interaction with such a large array of targets. By showing that specific interactions, and not simply membrane effects or antioxidant activities, underlie the capacity of curcumin to directly modulate the production of inflammatory eicosanoids, we suggest that this compound

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might represent a privileged structure for the selective modulation of distinct druggable macromolecules as well as for obtaining more potent and target-focused leads (such as the novel prenylated pyrazole derivative **11**).

EXPERIMENTAL SECTION

Solvents and Reagents. Curcumin was purchased from Sigma-Aldrich (Deisenhofen, Germany). Compounds **2**,[35] **3**,[50] **4**,[29] **5**,[30] **6**,[30] **7**,[29] **8**,[29] **9**,[29] and **10**[29] were prepared according to literature. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were measured on a Jeol Eclipse 300 spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$, δH = 7.26; acetone-d_6, δC = 77.0). Low- and high-resolution ESI-MS spectra were obtained on a LTQ OrbitrapXL mass spectrometer (Thermo Scientific). Silica gel 60 (70−230 mesh) was purchased from Macherey-Nagel (Düren, Germany). Reactions were monitored by TLC on Merck 60 F254 (0.25 mm) plates that were visualized by UV inspection and/or staining with 5% H_2SO_4 in ethanol and heating. Organic phases were dried with Na_2SO_4 before evaporation. The purity (95% or higher) of all final products evaluated for reactivity and bioactivity was assessed by analytical HPLC (isocratic elution, 70% acetonitrile/30% water containing 1% formic acid), with UV detection at 240 nm on a JASCO Herculite apparatus equipped with a Phenomenex Luna C18 column.

All test compounds were dissolved in DMSO, stored in the dark at −20 °C, and freezing/thawing cycles were kept to a minimum. The mPGES-1 inhibitor **15** (MD52) was kindly provided by Dr. M. Schubert-Zsilavecz (University of Frankfurt, Germany). Materials used: DMEM/high glucose (4.5 g/L) medium, penicillin, streptomycin, trypsin/EDTA solution, PAA (Coelbe, Germany); PGH_2, Larodan (Malmö, Sweden); 11β-PGE_2, PGB_1, human recombinant COX-2, ovine COX-1, Cayman Chemical (Ann Arbor, MI). Solvents, **14** (BWA4C) and all other chemicals were obtained from Sigma-Aldrich (Deisenhofen, Germany) unless stated otherwise.

4,4′-(1*E*,1′*E*)-2,2′-(1*H*-Pyrazole-3,5-diyl)bis(ethene-2,1-diyl)-bis(2-methoxy-6-(3-methylbut-2-enyl)phenol) (11). To a stirred solution of **7** (200 mg, 0.39 mmol, 1 equiv) in AcOH (1 mL), hydrazine monohydrate (44 μL, 0.897 mmol, 2.3 equiv) was added. The reaction was stirred overnight at 60 °C, and then quenched by addition of brine and extraction with EtOAc. After evaporation, the residue was triturated with ether to afford 157 mg (80%) of an orange foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (2H, d, J = 16.4 Hz), 7.00 (4H, br m), 6.82 (2H, d, J = 16.3 Hz), 6.54 (1H, s), 5.33 (2H, t, J = 5.8 Hz), 3.90 (3H, s), 3.89 (3H, s), 3.35 (4H, d, J = 7.0 Hz), 2.10 (6H, br s), 2.09 (6H, br s). ^{13}C NMR (acetone-d_6, 75 MHz) δ 146.7, 144.0, 133.0, 132.1, 131.3, 128.1, 127.7, 122.2, 122.1, 121.6, 117.7, 114.5, 110.2, 105.9, 56.1, 28.1, 25.9, 17.9. (+) ESI-MS m/z 523 $[M + Na]^+$. HR-ESI-MS m/z 523.2784 (calcd for $C_{31}H_{36}N_2O_4Na$ 523.2573).

5,5′-Difluorocurcumin (12). To a stirred solution of 3-fluoro-4-hydroxy-5-methoxybenzaldehyde (540 mg, 3.2 mmol, 1 equiv) in dry DMF (3.3 mL), B_2O_3 (225 mg, 3.2 mmol, 1 equiv), 2,4-pentandione (173 μL, 1.6 mmol, 0.5 equiv) and $B(OCH_3)_3$ (355 μL, 3.2 mmol, 1 equiv) were sequentially added. The reaction was stirred at 90 °C, and n-butylamine (71 μL) was added over a period of 2.5 h. After 1 h, the reaction was cooled to 60 °C, and 5% AcOH (8 mL) was added. The mixture was stirred at 60 °C for 3 h, during which a precipitate was formed. After filtration and washing with water, the crude product was purified by gas column chromatography on silica gel (petrol ether/EtOAc 7:3 as eluent) to afford 5,5′-difluorocurcumin (**12**) as an orange solid (324 mg, 50%); mp 195 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (2H, d, J = 15.7 Hz), 7.00 (2H, dd, J = 10.8, 1.8 Hz,), 6.85 (2H, br t, J = 1.8 Hz), 6.46 (2H, d, J = 15.7 Hz), 5.79 (s, 1H), 3.96 (s, 6H). ^{13}C NMR (acetone-d_6, 75 MHz) δ 183.5, 153.0, 149.8, 149.6, 149.6, 139.8, 139.8, 137.1, 136.9, 126.4, 126.2, 122.9, 109.1, 108.8, 107.5, 101.5, 56.0. (+) ESI-MS m/z 427 $[M + Na]^+$. HR-ESI-MS m/z 427.0951 (calcd for $C_{21}H_{18}F_2O_6Na$ 427.0969).

(1*E*,4*E*)-1,5-Bis(3-fluoro-4-hydroxy-5-methoxyphenyl)penta-1,4-dien-3-one (13). To a stirred solution of 3-fluoro-4-hydroxy-5-methoxybenzaldehyde (400 mg, 2.35 mmol, 1 equiv) in EtOH (2.5 mL), acetone (85 μL, 1.17 mmol, 0.5 equiv), and HCl (37%) (235 μL, 100 μL/mmol) were sequentially added. The reaction was stirred at 40 °C overnight and quenched with the addition of brine and extraction with EtOAc. The crude product was purified by gas column chromatography on silica gel (petrol ether/EtOAc 6:4 as eluent) to afford **13** as an orange solid (275 mg, 65%); mp 190 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.60 (2H, d, J = 15.6 Hz), 7.06 (2H, dt, J = 10.8, 1.8 Hz), 6.90 (2H, d, J = 15.6 Hz), 6.91 (2H, d, J = 1,8 Hz), 3.97 (6H, s). ^{13}C NMR (acetone-d_6, 75 MHz) δ 187.7, 152.9, 149.8, 149.6, 149.6, 139.8, 139.8, 137.2, 137.0, 126.3, 126.2, 124.5, 109.3, 109.0, 107.6, 56.1. (+) ESI-MS m/z 385 $[M + Na]^+$. HR-ESI-MS m/z 385.0879 (calcd for $C_{19}H_{16}F_2O_5Na$, 385.0863).

Blood Cell Isolation and Cultivation of Cell Lines. Human platelets, neutrophils, and monocytes were freshly isolated from peripheral blood obtained at the Institute for Transfusion Medicine of the University Hospital Jena (Germany) as described.[51] Venous blood from healthy adult donors was subjected to centrifugation (4000g/20 min/20 °C) to prepare leukocyte concentrates. Cells were promptly isolated by dextran sedimentation and centrifugation on Nycoprep cushions (PAA, Coelbe, Germany). The pellet was resuspended, erythrocytes were lysed under hypotonic conditions, and neutrophils were recovered by centrifugation. For the isolation of platelets, the supernatant obtained after centrifugation on Nycoprep cushions (platelet-rich plasma) was mixed with PBS pH 5.9 (3:2 v/v) and centrifuged (2100g, 15 min). The pellet was washed with PBS pH 5.9/0.9% NaCl (1:1, v/v). Monocytes were isolated from the peripheral blood mononuclear cell (PBMC) fraction by adherence to culture flasks (Greiner, Nuertingen, Germany). Washed PBMCs were seeded at 2 × 10^7 cells/mL in RPMI 1640 medium containing L-glutamine (2 mM) and penicillin/streptomycin (100 U/mL and 100 μg/mL, respectively; monocyte medium). Adherent cells were collected after 1.5 h at 37 °C and 5% CO_2. The purity of the monocyte preparation was >85% as defined by forward- and side-light scatter properties and detection of the CD14 surface molecule by flow cytometry (BD FACS Calibur, Heidelberg, Germany). Washed cells were finally suspended in PBS pH 7.4 plus 1 mM $CaCl_2$ (platelets) containing 1 mg/mL glucose (neutrophils) or monocyte medium supplemented with FCS (5%, v/v; monocytes). The experimental protocol was approved by the ethics committee of the University of Jena.

Human lung adenocarcinoma epithelial A549 cells were cultured in DMEM/high glucose (4.5 g/L) medium supplemented with heat-inactivated FCS (10%, v/v), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37 °C and 5% CO_2. Confluent cells were detached using 1× trypsin/EDTA and reseeded at 2 × 10^6 cells in 20 mL medium in 175 cm^2 flasks.

HaCaT-ARE-luc and LNCaP (human prostate carcinoma) cell lines were cultured in DMEM containing 10% FCS, L-glutamine (2 mM), penicillin (50 U/mL), and streptomycin (100 μg/mL). 5.1 cells were cultured in RPMI 1640 containing 10% FCS, penicillin (50 U/mL), and streptomycin (100 μg/mL).

Preparation of mPGES-1. A549 cells were treated with interleukin-1β (2 ng/mL) at 37 °C and 5% CO_2 for 48 h to induce mPGES-1. Harvested cells were frozen in liquid nitrogen and then taken up in ice-cold homogenization buffer (0.1 M potassium phosphate buffer pH 7.4, 1 mM phenylmethanesulphonyl fluoride, 60 μg/mL soybean trypsin inhibitor, 1 μg/mL leupeptin, 2.5 mM glutathione, and 250 mM sucrose). After incubation for 15 min, cells were sonicated on ice (3 × 20 s) and centrifuged at 10000g for 10 min. The microsomal fraction (pellet) was obtained by centrifugation of the supernatant at 174000g for 1 h at 4 °C. The pellet was resuspended in homogenization buffer and diluted in potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione to a concentration of 25−50 μg total protein/mL.

Determination of mPGES-1 Activity. mPGES-1 activity in microsomes of A549 cells was determined as described.[52] Microsomes of interleukin-1β-treated A549 cells, used as source of mPGES-1, were pre-incubated with the test compounds (dissolved in DMSO) for 15 min at 4 °C. The reaction was started by addition of PGH_2 (20 μM, final concentration; reaction volume, 100 μL) and stopped after 1 min by addition of stop solution (100 μL; 40 mM $FeCl_2$, 80 mM citric acid, and

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10 μM of 11β-PGE_2 as internal standard). PGE_2 was extracted and analyzed by reversed phase-HPLC as described.[52]

Expression and Purification of Human Recombinant 5-LO. *Escherichia coli* Bl21 (DE3) cells were transformed with pT3−5LO plasmid, lysed in 50 mM triethanolamine/HCl pH 8.0 plus EDTA (5 mM), soybean trypsin inhibitor (60 μg/mL), phenylmethanesulphonyl fluoride (1 mM), dithiothreitol (1 mM), and lysozyme (1 mg/mL) and then sonified (3 × 15 s). The homogenate was centrifuged at 10000*g* for 15 min, and the remaining supernatant at 40000*g* for 70 min at 4 °C. 5-LO in the supernatant was partially purified by affinity chromatography on an ATP-agarose column as described.[53] Semipurified 5-LO was diluted in PBS containing EDTA (1 mM) and ATP (1 mM) and immediately used for activity assays.

Activity Assay for Human Recombinant 5-LO. Human recombinant 5-LO was preincubated with the test compounds for 10 min at 4 °C and prewarmed for 30 s at 37 °C. 5-LO product formation was initiated by addition of 2 mM $CaCl_2$ and 20 μM arachidonic acid. After 10 min at 37 °C, the reaction was terminated by addition of 1 mL of ice-cold methanol. Formed 5-LO metabolites (all-*trans* isomers of LTB_4 and 5-H(P)ETE) were analyzed by RP-HPLC as described.[40]

Determination of Radical Scavenging Activity. Compounds were dissolved in ethanol and combined with an equal volume of 100 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) in ethanol (final volume: 200 μL). After incubation for 30 min, absorbance was measured at 520 nm using a Multiskan Spectrum microplate reader (Thermo Scientific) as described.[46]

Determination of the Stability of Curcuminoids in Aqueous Solution. Curcuminoids (2 μM) in 10 mM aqueous ammonium bicarbonate pH 7.9 were incubated for 30 min at 37 °C. Degradation was stopped by acidification to pH 3.3 with an equal volume of ice-cold methanol plus 0.14% formic acid. Curcuminoids and curcumin's main degradation product *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal were immediately quantified by reversed phase liquid chromatography-coupled mass spectrometry.

Determination of 5-LO Product Formation in Neutrophils. Freshly isolated neutrophils (1×10^7/mL) were preincubated with the test compounds for 15 min at 37 °C. Then, 5-LO product formation was started by addition of 2.5 μM Ca^{2+}-ionophore A23187 plus 20 μM arachidonic acid. The reaction was stopped after 10 min at 37 °C with 1 mL of methanol. Major 5-LO metabolites (LTB_4 and its all-*trans* isomers and 5-H(P)ETE) were extracted and analyzed by HPLC as described.[54] Cysteinyl-LTs C_4, D_4, and E_4 and oxidation products of LTB_4 were not determined.

Determination of COX-1 Activity in Washed Platelets. Freshly isolated platelets (10^8/mL) were preincubated with the test compounds for 5 min at room temperature and prewarmed at 37 °C for 1 min. Formation of 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid (12-HHT; which is nonenzymatically formed from COX-derived PGH_2) was started by addition of 5 μM arachidonic acid. After incubation for 5 min at 37 °C, 12-HHT was extracted and analyzed by RP-HPLC as described.[51]

Activity Assays of Isolated COX-1 and -2. Purified ovine COX-1 (50 units) or human recombinant COX-2 (20 units) were preincubated with the test compounds in reaction buffer (1 mL; 100 mM Tris buffer pH 8, 5 mM glutathione, 5 μM hemoglobin, and 100 μM EDTA) for 5 min at 4 °C followed by 1 min at 37 °C. Arachidonic acid (COX-1:5 μM; COX-2:2 μM) was added to initiate COX product formation. COX-derived 12-HHT was extracted after 5 min and analyzed by HPLC as described.[51]

Determination of NF-κB, STAT3, and Nrf2 Transcriptional Activities. Anti-NF-κB activity was investigated in 5.1 cells, a validated in vitro model to study TNFα-induced NF-κB activation.[55] 5.1 cells were stimulated with TNFα (20 ng/mL) in the presence or absence of the compounds for 6 h. To study the activity of the compounds on the Nrf2 pathway, we generated the HaCaT-ARE-Luc cell line. Nqo1 Antioxidant Response Element (ARE)-Luc reporter plasmid and pPGK-Puro plasmid were cotransfected into HaCaT cells. After antibiotic selection, the cells were cloned by limiting dilution, and several positive clones responding to *tert*-butylhydroquinone were pooled to generate the HaCaT-ARE-Luc cell line. Cells were stimulated with the compounds for 6 h. To study STAT3 signaling, LNCaP cells were transiently transfected with the pTATA-TK-Luc plasmid that contains four copies of the STAT-binding site. Transient transfections were performed with Rotifect (Carl Roth GmbH, Karlsruhe, Germany) according to the manufacturer's instructions, and 24 h after transfection, cells were preincubated with increasing concentrations of the compounds and then treated with interleukin-6 (10 ng/mL) for another 12 h. After stimulation of the transfected cell lines, cells were washed twice with PBS pH 7.4 and lysed in 25 mM Tris-phosphate pH 7.8, 8 mM $MgCl_2$, 1 mM DTT, 1% Triton X-100, and 7% glycerol for 15 min at room temperature in a horizontal shaker. Following centrifugation, the supernatant was used to measure luciferase activity using an Autolumat LB 9510 (Berthold) following the instructions of the luciferase assay kit (Promega, Madison, WI, USA).

Cytotoxicity Assays. LNCaP cells (10^4 cells/well) and human PBMC (2×10^5/well) were seeded in 96-well plates (LNCaP, 200 μL; monocytes, 100 μL) and incubated with the compounds for 24 h. MTT (5 mg/mL; LNCaP, 100 μL; monocytes, 20 μL) was added to each well, and cells were incubated for 4 h at 37 °C and 5% CO_2 in darkness. The formazan product was solubilized with SDS (10%, w/v in 20 mM HCl) for monocytes and after removal of the supernatant by addition of DMSO (100 μL) for LNCaP cells. Finally, the absorbance was measured at 550 nm using a TriStar LB 941 Microplate Reader (Berthold Technologies, GmbH & Co. KG) and at 570 nm using a Multiskan Spectrum microplate reader (Thermo Scientific), respectively. The absorbance of untreated cells was taken as 100% viability to calculate cytotoxic IC_{50} values.

Analysis of the Eicosanoid Profile of Activated Monocytes. Freshly isolated monocytes (5×10^6/6 well plate) were stimulated with lipopolysaccharide (1 μg/mL) for 24 h at 37 °C and 5% CO_2. Cells were washed, preincubated with the test compounds for 15 min, and treated with 2.5 μM Ca^{2+}-ionophore A23187 plus 20 μM arachidonic acid for 30 min. Then, the reaction was stopped and eicosanoids extracted as described.[56]

Reversed Phase Liquid Chromatography and Mass Spectrometry. Eicosanoids were separated on an Acquity UPLC BEH C18 column (1.7 μm, 2.1 mm × 50 mm, Waters, Milford, MA) using an Acquity UPLC system (Waters, Milford, MA, USA) as previously described.[56] In brief, chromatography was performed at a flow rate of 0.8 mL/min and a column temperature of 45 °C. The solvents for the mobile phase were acetonitrile (A) and water/acetonitrile (90/10; B), both acidified with 0.07% (v/v) formic acid. Isocratic elution at A/B = 30/70 was performed for 2 min and followed by a linear gradient to A/B = 70/30 within 5 min. In variation, curcuminoids and *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal were analyzed at a flow rate of 0.7 mL/min using a gradient from A/B = 30/70 to 80/20 within 3 min and 100% B for 1 min. The retention times are summarized in Supporting Information Table S1.

The chromatography system was coupled to a QTRAP 5500 mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with an electrospray ionization source. For eicosanoid analysis, parameters were adjusted as described.[56] Identification of eicosanoids was based on the detection of specific fragment ions through multiple reaction monitoring. Monitored transitions and their collision energies are given in Supporting Information Table S2. The transition first mentioned ("transition 1") was used for quantification. Curcuminoids and *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal were analyzed by full scans in the negative (**1**−**8**, **10**−**13**) and/or positive ion mode (**1**−**3**, **6**, **9**, **11**). The ion spray voltage was set to 4500 V in the negative and 5500 V in the positive ion mode, the heated capillary temperature to 500 °C, the sheath gas and auxiliary gas pressure to 50−60 psi, and the declustering potential to 50 V in the negative and 190 V in the positive ion mode. Automatic peak integration was performed with Analyst 1.6 software (AB Sciex, Darmstadt, Germany) using IntelliQuan default settings. For eicosanoid analysis, data were normalized on the internal standard PGB_1 and are given as relative intensities. The reported method was optimized to compare eicosanoid profiles between samples and not for absolute quantification.

Zymosan-Induced Peritonitis in Mice. Male CD-1 mice (33−39 g, Charles River Laboratories, Calco, Italy) were housed in a controlled

environment (21 ± 2 °C) and provided with standard rodent chow and water. Animals were allowed to acclimate for 4 days prior to experiments and were subjected to a 12 h light−12 h dark schedule. Experiments were conducted during the light phase. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purpose (Ministerial Decree 116/92) as well as with the European Economic Community Regulations (Official Journal of E.C. L 358/1 12/18/1986). The animal studies were approved by the local ethical committee of the University of Naples Federico II on 27th of February 2014 (2014/0018760).

Mouse peritonitis was induced and parameters analyzed as previously described.[46] In brief, compounds **11** (10 mg/kg), **17** (1 mg/kg), or vehicle (0.5 mL of 0.9% saline solution containing 2% DMSO) were given ip 30 min before zymosan ip injection (0.5 mL of a 2 mg/mL suspension in 0.9% w/v saline). Mice were killed by inhalation of CO_2 at the indicated time points followed by a peritoneal lavage with 3 mL of cold PBS pH 7.4. Exudates were collected, and cells were counted with a light microscope in a Burker's chamber after vital trypan blue staining. After centrifugation of the exudates (18000*g*, 5 min, 4 °C), the amounts of cysteinyl-LTs and LTB_4 were analyzed in the supernatant by enzyme immunoassay (Enzo Life Sciences GmbH, Lörrach, Germany) according to manufacturer's instructions. For determining MPO activity, the pelleted cells were disrupted, the homogenate centrifuged, and the supernatant (20 μL) mixed with 0.167 mg/mL of *o*-dianisidine and 0.0005% hydrogen peroxide in PBS pH 7.4 (200 μL) as described.[46] The change of absorbance was monitored in kinetic mode using an iMark microplate absorbance reader (Biorad). Levels of MPO were determined from a calibration curve using human neutrophil MPO as reference standard and are expressed as units MPO per mL (U/mL).

Statistics. Data are expressed as mean ± standard error (SEM). Statistical evaluation of the data was performed by one-way ANOVAs for independent or correlated samples followed by Tukey HSD posthoc tests or by Student's *t* test for paired and correlated samples. *P* values <0.05 were considered statistically significant. All statistical calculations were performed using GraphPad InStat 3.10 (GraphPad Software Inc., La Jolla, CA, USA). IC_{50} values were determined by graphical analysis using SigmaPlot 12.0 (Systat Software Inc., San Jose, USA).

■ ASSOCIATED CONTENT

Supporting Information

Retention times of curcuminoids, conditions for UPLC-MS/MS analysis of eicosanoids, stability of curcumin as well as effect of compound **5** and **6**, and control inhibitors on the eicosanoid profile of activated human monocytes. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

COX, cyclooxygenase; H(P)ETE, hydro(pero)xy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid; 12-HHT, 12(*S*)-hydroxy-5-*cis*-8,10-*trans*-heptadecatrienoic acid; LO, lipoxygenase; LT, leukotriene; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-erythroid 2-related factor-2; mPGES, microsomal prostaglandin E_2 synthase; PG, prostaglandin; PBMC, peripheral blood mononuclear cells; STAT3, signal transducer and activator of transcription 3

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