of Minnesota. The technical assistance of Kathy Norman in the preparation of the Ac-Asp-Glu-OH analogues 2–5 is gratefully acknowledged.

Registry No. 2, 28809-18-9; 3, 128135-23-9; 4, 99624-53-0; 5, 104870-25-9; 6, 128164-09-0; 7, 128135-24-0; 8, 33981-72-5; 9, 128135-25-1; 10, 128135-26-2; 11, 128135-27-3; 12, 89092-61-5; 13, 128164-22-7; 14, 99429-23-9; 15, 104870-31-7; 16, 128135-28-4; 17, 128135-29-5; 18, 128135-30-8; 19, 104870-33-9; 20, 128135-31-9; 21, 128135-32-0; 22, 128135-33-1; 23, 128135-34-2; 24, 128135-35-3; 25, 128135-36-4; 26, 128135-37-5; 27, 128135-38-6; 28, 128135-39-7; 29, 128135-40-0; 30, 128135-41-1; 31, 100945-16-2; 32 (diastereomer 1), 128135-42-2; 32 (diastereomer 2), 128135-43-3; 33 (diastereomer 1), 128135-52-4; 33 (diastereomer 2), 128135-53-5; 34a, 128135-44-4; 34b, 128135-51-3; 35, 128135-45-5; 36 (diastereomer-1), 128164-23-8; 36 (diastereomer-2), 128164-24-9; 37 (diastereomer-1), 128135-49-9; 37 (diastereomer-2), 128135-50-2; 38, 7633-32-1; 39a, 128164-25-0; 39b, 128135-48-8; 40, 128135-46-6; 41, 128135-47-7; (*E*)-*t*-BuOCOCH=CHCOOBu-*t*, 7633-38-7; OHCCOOMe, 922-68-9; (±)-$(MeO)_2P(O)CH(NHZ)COOMe$, 100945-15-1; H-Glu(OMe)-OMe·HCl, 23150-65-4; H-Glu(OBu-*t*)-OBu-*t*·HCl, 32677-01-3; H-Glu-OH, 56-86-0; H-Glu(OBzl)-OBzl, 2768-50-5; H-Glu(OBzl)-OBzl·TsOH, 2791-84-6; (*E*)-MeOCOCH=CHCOOH, 2756-87-8; BOC-Asp(OBu-*t*)-OH, 1676-90-0; BOC-Glu(OBzl)-OH, 13574-13-5; BOC-D-Asp(OBzl)-OH, 51186-58-4; H-Asp(OBzl)-OBzl·TsOH, 2886-33-1; BOC-Asp(OBzl)-OH, 7536-58-5; succinic anhydride, 108-30-5; maleic anhydride, 108-31-6; NAALA depeptidase, 111070-04-3.

Penta- and Hexadienoic Acid Derivatives: A Novel Series of 5-Lipoxygenase Inhibitors

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The synthesis of a series of pentadienoic and hexadienoic acid derivatives is reported. These compounds were tested as inhibitors of 5-lipoxygenase (5 LO) and cyclooxygenase (CO) in vitro and as inhibitors of arachidonic acid (AA) induced ear edema in mice in vivo. Their potency is compared with that of the standard inhibitors nafazatrom, BW 755C, NDGA, KME4, quercetine, and L 652,243. The most potent compound in vivo, diethyl 2-hydroxy-5-(ethylthio)-2(*Z*),4(*Z*)-hexadienedioate (**20**) inhibited AA-induced ear edema when administered topically or orally, with an ED_{50} value of 0.01 mg/ear and 20 mg/kg, respectively. Among the standard compounds tested, L 652, 243 was the most active compound in this test with an ED_{50} value of 0.01 mg/ear and 1 mg/kg po, but unlike this compound, **20** is a selective inhibitor of 5-LO (IC_{50} = 2 μM) without any significant activity against CO (IC_{50} > 50 μM). Most of the other compounds in this series are also selective 5-LO inhibitors.

The arachidonic acid (AA) cascade is known to generate numerous mediators involved in specific diseases including inflammation and immediate and delayed hypersensitivities. Inhibition of the cyclooxygenase (CO) pathway may explain, in part, the therapeutic properties of nonsteroidal antiinflammatory drugs. Since the sulfidopeptidal leukotrienes are potent bronchoconstrictors and LTB_4 is a potent chemotactic factor on human leukocytes, orally active, specific inhibitors of 5-lipoxygenase (5-LO) may be of interest as potential therapeutic agents.

It is generally believed that 5-LO contains a catalytically important iron atom, as seems to be the case for other LO enzymes.[1] It is assumed that the first step of the reaction involves a reduction of the enzymatic Fe^{III} to Fe^{II} at the same time as the *pro-S* hydrogen atom of C_7 in AA is abstracted to give a delocalized radical which reacts with molecular oxygen[1] (Scheme I).

This biochemical background led several groups to develop rational strategies to design potential 5-LO inhibitors: structural analogues of AA[2] or of 15-HETE,[3] antioxidant compounds,[4] and Fe^{III} chelating agents such as hydroxamic acid derivatives.[5,6]

We report herein the synthesis and some biological activities of dienoic acid derivatives, structurally unrelated to any previous 5-LO inhibitors. Most of these compounds have been shown to inhibit 5-LO in vitro and some of them are active in vivo in an acute inflammation animal model.

Chemistry

The synthetic pathway for the preparation of the compounds listed in Tables I–IV is shown in Scheme II. Compound 1 as the perchlorate salt had been previously described.[7] We synthesized vinamidinium tetrafluoroborates 1–4 by reaction of the appropriate enaminones with

Scheme I

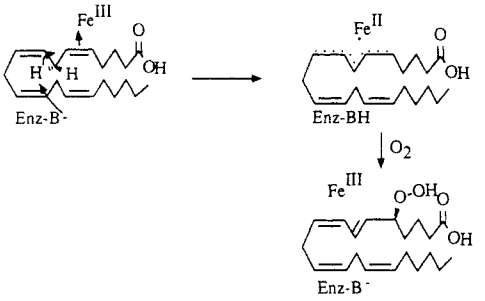

Table I. Vinamidinium Salts 1–5

compd	W	Z	mp, °C	% yield
1	BF_4^-	$CO_2C_2H_5$	102	58
2	BF_4^-	$CON(CH_3)_2$	170	79
3	BF_4^-	COC_6H_5	140	51
4	BF_4^-	C_6H_5	136	93
5	Cl^- [a]	H	190[b]	51

[a] Reference 15. [b] Literature mp 187–189 °C.

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§ Département d'Analyses.

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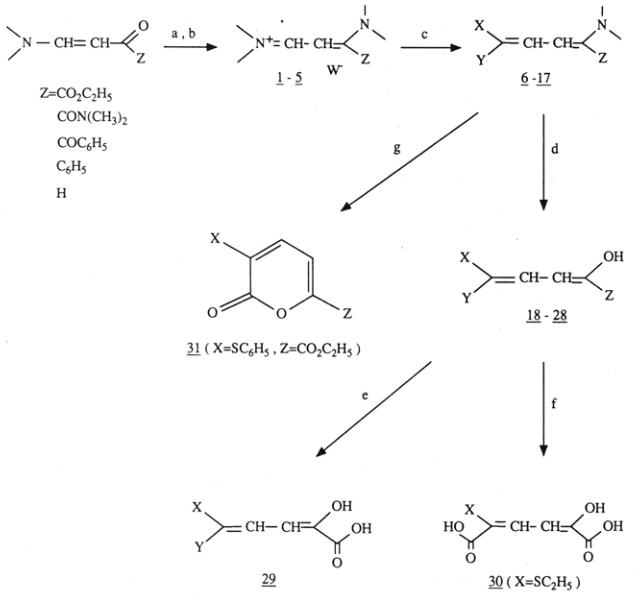
Table II. Dienamines **6–17**

compd	X	Y	Z	mp, °C	% yield
6	C_6H_5S	$CO_2C_2H_5$	$CO_2C_2H_5$	76	51
7	C_6H_5S	$CO_2C(CH_3)_3$	$CO_2C_2H_5$	oil	61
8	$C_6H_5CH_2S$	$CO_2C_2H_5$	$CO_2C_2H_5$	oil	96
9	CH_3CH_2S	$CO_2C_2H_5$	$CO_2C_2H_5$	oil	65
10	C_6H_5S	COC_6H_5	$CO_2C_2H_5$	oil	68
11	CH_3S	COC_6H_5	$CO_2C_2H_5$	78	66
12	C_6H_5	$CO_2C_2H_5$	$CO_2C_2H_5$	oil	41
13	C_6H_5CO	$CO_2C_2H_5$	$CO_2C_2H_5$	oil	92
14	C_6H_5S	$CO_2C_2H_5$	$CON(CH_3)_2$	84	63
15	C_6H_5S	$CO_2C_2H_5$	COC_6H_5	58	68
16	C_6H_5S	$CO_2C_2H_5$	C_6H_5	99	52
17	C_6H_5S	$CO_2C_2H_5$	H	128	51

Scheme II[a]



[a] Reagents: (a) $Et_3O^+BF_4^-/CH_2Cl_2$; (b) $HNMe_2/CH_2Cl_2$; (c) XCH_2Y/base (EtONa or BuLi); (d) 1 N HCl/EtOH; (e) 1 N NaOH/EtOH then 1 N HCl; (f) 1 N NaOH excess then 1 N HCl; (g) aqueous HCl/AcOH starting from **7**: X = SC_6H_5, Y = $CO_2C(CH_3)_3$, Z = $CO_2C_2H_5$.

triethyloxonium tetrafluoroborate and then dimethylamine (Table I). Methylene-activated compounds XCH_2Y (e.g. ethyl phenylthioacetate) were condensed in the presence of a base (EtONa or BuLi) with salts **1–5** to give dienamines **6–17** (Table II). Hydrolysis of the dienamines by refluxing for 5 min with 1 N HCl gave dienols **18–28** (Table III). In one case, X = SC_6H_5, Y = $CO_2C(CH_3)_3$, Z = $CO_2C_2H_5$, lactone **31** was obtained by using a mixture of acetic acid and hydrochloric acid. Dienol esters **18** and **20** were converted to the corresponding dienol acids **29** and **30** by treatment with 1 N NaOH in EtOH followed by acidification with 1 N HCl (Table IV). Obtainment of mono or diacids depends on the duration of the saponification (see the Experimental Section). Compounds **32–34** were prepared according to the literature.[8-10] Compound **35**, the lower homologue of **27**, was obtained by reaction of ethyl phenylthioacetate with benzoyl chloride in the presence of a base.

For compounds **18–30**, the enol form is generally observed in structural analysis. Concerning the stereochemistry of compounds **6–30**, their synthesis afforded just one stereoisomer but the stereochemistry cannot be assigned from spectral data except in the case of dienol **20** for which single crystal X-ray analysis[11a] was performed (Figure 1).[11b] According to this result, the stereochemistry assigned to **20** was 2*Z*, 4*Z*.

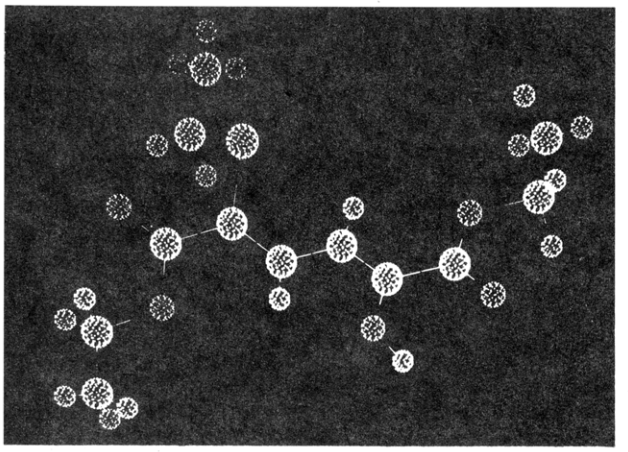
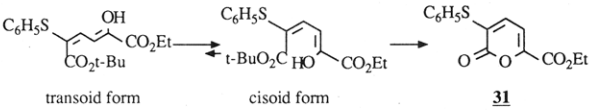


Figure 1. Crystal structure for **20**.

Scheme III



In addition, it must be noted that formation of lactone **31** from enamine **7** can only be explained by cyclization of the cisoid form of one isomer (2*Z*, 4*E*) of the intermediate dienol (Scheme III).

Results and Discussion

In Vitro Studies. Compounds **6**, **11**, and **18–35** and standard compounds were evaluated in vitro in a PMN_1 peritoneal 5-LO assay and in a rat basophil leukemia type 1 (RBL_1) 5-LO and CO assay (see the Experimental Section). The results are shown in Tables V and VI. Nafazatrom, NDGA, and quercetine are selective 5-LO inhibitors whereas BW 755C, KME4, and L 652,243, in addition to their 5-LO inhibitory activity, significantly inhibited CO in vitro.

Enamines **6** and **11** and lactone **31** were inactive. Except for enol acid derivative **29**, all the new enol compounds were inactive as CO inhibitors. By contrast, most of them exhibited in vitro a 5-LO inhibitory activity in the micromolar range with rather similar results in the PMN_1 model was compared to those of the RBL_1 model. A similar potency was observed for compounds **18** and **26** bearing $CO_2C_2H_5$ and COC_6H_5, respectively, as the Z substituent. The analogues with Z = C_6H_5 (**27**) or H (**28**) were even more active, suggesting that the nature of the Z group is not critical for activity. Surprisingly the dimethylcarbamoyl analogue **25** was inactive. Carboxylic acid **29** was less potent than the corresponding ester **28**, and the other carboxylic acid derivatives **30** and **34** were practically inactive.

Replacement of the phenylthio group by benzylthio (**19**), ethylthio (**20**), or phenyl (**23**) had little effect on potency in compound **18**. By contrast replacement of the phenylthio group of **18** by benzoyl (**24**), hydrogen (**32**), or

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Table III. Dienols 18–28

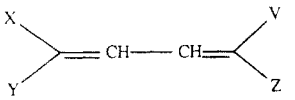
compd	X	Y	Z	recryst solv	mp, °C	% yield	formula	anal.
18	C_6H_5S	$CO_2C_2H_5$	$CO_2C_2H_5$	$(iPr)_2O$	98	98	$C_{16}H_{18}O_5S$	CHS
19	$C_6H_5CH_2S$	$CO_2C_2H_5$	$CO_2C_2H_5$	*a*	*b*	68	$C_{17}H_{20}O_5S$	CHS
20	CH_3CH_2S	$CO_2C_2H_5$	$CO_2C_2H_5$	cyclohexane/ACOEt	65	57	$C_{12}H_{18}O_5S$	CHS
21	C_6H_5S	COC_6H_5	$CO_2C_2H_5$	*i*-PrOH	130	70	$C_{20}H_{18}O_4S$	CHS
22	CH_3S	COC_6H_5	$CO_2C_2H_5$	$(i\text{-Pr})_2O$	86	86	$C_{15}H_{16}O_4S$	CHS
23	C_6H_5	$CO_2C_2H_5$	$CO_2C_2H_5$	cyclohexane	107	43	$C_{16}H_{18}O_5$	CH
24	C_6H_5CO	$CO_2C_2H_5$	$CO_2C_2H_5$	cyclohexane/$(i\text{-Pr})_2O$	85	18	$C_{17}H_{18}O_6$	CH
25	C_6H_5S	$CO_2C_2H_5$	$CON(CH_3)_2$	$(i\text{-Pr})_2O$	88	74	$C_{16}H_{19}NO_4S$	CHNS
26	C_6H_5S	$CO_2C_2H_5$	COC_6H_5	cyclohexane	110	78	$C_{20}H_{18}O_4S$	CHS
27	C_6H_5S	$CO_2C_2H_5$	C_6H_5	*a*	oil	80	$C_{19}H_{18}O_3S$	CHS
28	C_6H_5S	$CO_2C_2H_5$	H	cyclohexane/ACOEt	120	33	$C_{13}H_{14}O_3S$	CHS

[a] Purification by flash chromatography. [b] Melting point <40 °C.

Table IV. Dienol Acids 29 and 30

compd	X	Y	recryst solv	mp, °C	% yield	formula	anal.
29	C_6H_5S	$CO_2C_2H_5$	$(i\text{-Pr})_2O/CH_3CN$	172	59	$C_{12}H_{10}O_5S$	CHS
30	CH_3CH_2S	CO_2H	dioxane	235	38	$C_8H_{10}O_5S$	CHS

Table V. In Vitro Inhibition of CO and 5-LO and in Vivo Inhibition of AA-Induced Ear Edema



compd	X	Y	Z	V	IC_{50}, μM, RBL_1[a] PGD_2	IC_{50}, μM, RBL_1[a] 5-HETE	IC_{50}, μM, PMN_1:[a] LTB_4	AA edema ED_{50} topic mg/ear	AA edema ED_{50} po mg/kg
6	C_6H_5S	$CO_2C_2H_5$	$CO_2C_2H_5$	$N(CH_3)_2$	>50	>50	>50	>2	–
11	CH_3S	COC_6H_5	$CO_2C_2H_5$	$N(CH_3)_2$	>50	>50	>50	–	–
18	C_6H_5S	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	2	2	0.7	60
19	$C_6H_5CH_2S$	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	3.5	0.8	>2	–
20	CH_3CH_2S	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	2	2	0.01	20
21	C_6H_5S	COC_6H_5	$CO_2C_2H_5$	OH	>50	–	5	>2	–
22	CH_3S	COC_6H_5	$CO_2C_2H_5$	OH	>50	2.5	2	1	50
23	C_6H_5	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	1.1	0.8	>2	–
24	C_6H_5CO	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	>50	50	>2	–
25	C_6H_5S	$CO_2C_2H_5$	$CON(CH_3)_2$	OH	>50	>50	>5	>0.1	>50
26	C_6H_5S	$CO_2C_2H_5$	COC_6H_5	OH	12	2	3	>2	>50
27	C_6H_5S	$CO_2C_2H_5$	C_6H_5	OH	22	1	0.8	0.1	>100
28	C_6H_5S	$CO_2C_2H_5$	H	OH	>50	0.9	1	2	–
29	C_6H_5S	$CO_2C_2H_5$	CO_2H	OH	7	5	15	0.5	30
30	CH_3CH_2S	CO_2H	CO_2H	OH	>50	50	>50	>0.5	–
31	C_6H_5S	*b*	$CO_2C_2H_5$	–	>50	50	>50	>2	>100
32[c]	H	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	–	>50	–	–
33[d]	OH	$CO_2C_2H_5$	$CO_2C_2H_5$	OH	>50	>50	>50	>2	–
34[e]	C_6H_5	H	CO_2H	OH	>50	50	50	>5	–
35	$C_6H_5SCH(CO_2C_2H_5)(COC_6H_5)$			–	15	1	2	1	50

[a] IC_{50} values are the mean of two to six determinations (see the Experimental Section). [b] **31** is the lactonized compound. [c] See ref 8. [d] See ref 9. [e] See ref 10.

Table VI. Comparison of the Inhibitory Activities of **20** with Some Reference Inhibitors

compd	IC_{50}, μM, RBL_1 PGD_2	IC_{50}, μM, RBL_1 5-HETE	IC_{50}, μM, PMN_1: LTB_4	AA edema ED_{50} topic mg/ear	AA edema ED_{50} po mg/kg
nafazatrom	>50	2.5	10	2	>100
BW 755 C	6	2	13	0.08	75
NDGA	>50	0.5	0.3	0.1	>100
KME 4	5	0.6	5	0.3	100
quercetine	>50	0.4	0.5	1	>100
L 652,243	1	10	5	0.01	1
20	>50	2	2	0.01	20

hydroxyl (**33**) eliminated activity.

In any case the uncertainty of the stereochemistry of the compounds makes any structure–activity relationship questionable.

In Vivo Studies. The compounds listed in Table V and standard compounds (Table VI) were also evaluated against ear edema produced in mice by AA,[12] an inflammation model involving both CO and 5-LO products but known to be very sensitive to 5-LO inhibitors.[13] Not surprisingly, topical administration onto the site of inflammation was the more effective route. Very few compounds were orally active.

All the standard compounds tested were topically active, the most potent being L 652,243 (ED_{50} = 0.01 mg/ear). This compound was also the most active when administered orally (ED_{50} = 1 mg/kg) whereas the other compounds exhibited low activity (BW 755C: ED_{50} = 75 mg/kg) or were inactive (ED_{50} > 100 mg/kg for nafazatrom, NDGA, KME4, and quercetine.)

On the whole, the dienoic acid derivatives were moderately potent except **20**, which was topically as active as

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L 652,243 but was less active when administered orally (ED_{50} = 20 mg/kg).

From the data it is clear that in the dienoic acid derivative series, 5-LO inhibition in the micromolar range does not guarantee high in vivo activity in the AA-induced edema model, even when drugs are administrated topically to the site of inflammation. For example, compounds **23**, **26**, and **28** exhibited in vitro a 5-LO inhibitory activity in the micromolar range, as did **20**, but they were at least 200 times less active than **20** in vivo when administered topically. As yet there is no apparent explanation for this discrepancy.

Conclusion

In conclusions, a series of new pentadienoic acids have been synthesized. Many of them inhibited 5-LO at micromolar concentration. These active compounds were evaluated against ear edema produced in mice by AA. Compound **20** was shown to be active in this model when administered topically or orally, with ED_{50} values of 0.01 mg/ear and 20 mg/kg, respectively. This compound has been selected for in-depth pharmacological studies.

Experimental Section

Melting points were determined on a Köfler apparatus and were uncorrected. ^1H NMR spectra were recorded on a Brücker WM 250 (250 MHz), a Brücker WP 200 (200 MHz), or a Brücker AM 400 (400 MHz). IR spectra were taken on a Perkin-Elmer Model 938G or 580B. Mass spectra were recorded on a Finnigan 3300 spectrometer. Where elemental analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values. In some cases, crude products were purified by flash column chromatography on silica gel (0.04–0.063 mm, supplied by Merck) before recrystallization. Compounds **32–34** were prepared according to the literature procedure.[8-10]

Preparation of the Vinamidinium Salts 1–4 (Procedure 1). *N*-[3-(Dimethylamino)-3-(ethoxycarbonyl)-2-propenylidene]-*N*-methylmethanaminium Tetrafluoroborate (1). To 86.2 g (0.46 mol) of triethyloxonium tetrafluoroborate in 200 mL of dichloromethane, cooled at 10 °C under argon, was slowly added 67.5 g (0.4 mol) of ethyl 4-(dimethylamino)-2-oxo-3-butenoate.[7] The reaction mixture was stirred at room temperature for 5 h. To this solution was added 26.5 mL (0.4 mol) of dimethylamine in 50 mL of dichloromethane at 15 °C, followed by 200 mL of anhydrous diethyl ether. The reaction mixture was left for 15 h at 5 °C. The resulting precipitate was collected by filtration, washed three times with 50 mL of diethyl ether, and evaporated to dryness under reduced pressure at 20 °C to give 66.4 g (58%) of **1** as a white solid (mp 102 °C): NMR ($CDCl_3$, 200 MHz) δ 1.38 (t, J = 7 Hz, 3 H, CH_3), 3.21 (s, 6 H, $N(CH_3)_2$), 3.22 and 3.39 (2 s, 2 × 3 H, $=N^+(CH_3)_2$), 4.5 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.32 (d, J = 12 Hz, 1 H, CH=), 7.41 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 1740 (C=O), 1640, and 1590 (C=O), 1240 (CO), 1060, 535, and 520 cm^{-1} (BF_4^-).

Compounds **2–4** were prepared in a similar manner.

***N*-[3-(Dimethylamino)-3-(*N*,*N*-dimethylcarbamoyl)-2-propenylidene]-*N*-methylmethanaminium tetrafluoroborate (2)** was produced in 79% yield as a yellow solid (recrystallization from EtOH, mp 170 °C) from *N*,*N*-dimethyl-4-(dimethylamino)-2-oxo-3-butenamide. This compound was prepared according to standard procedure[14] starting from *N*,*N*-dimethyl-2-oxopropanamide[15] and *N*,*N*-dimethylformamide diethyl acetal (82%, mp 170 °C): NMR ($CDCl_3$, 200 MHz) δ 3–3.10–3.16 3.24–3.33 (5 s, 3 H, 3 H, 6 H, 3 H, 3 H, $N(CH_3)_2$), 5.21 (d, J = 12 Hz, 1 H, CH=), 7.27 (d, J = 12 Hz, 1 H, CH=).

***N*-[3-(Dimethylamino)-3-benzoyl-2-propenylidene]-*N*-methylmethanaminium tetrafluoroborate (3)** was produced in 51% yield as a yellow solid (precipitation from ethyl acetate, mp 140 °C) from 2-[2-(dimethylamino)-1-ethenyl]-1-phenyl-1,2-ethanedione. This compound was prepared as above (27%, mp 88 °C) starting from 1-phenyl-1,2-ethanedione: NMR ($CDCl_3$, 200 MHz) δ 3.07 and 3.21 (2 s, 2 × 3 H, $(CH_3)_2$), 3.25 and 3.42 (2 s, 2 × 3 H, $=N^+(CH_3)_2$), 5.52 (d, J = 12 Hz, 1 H, CH=), 7.0 (d, J = 12 Hz, 1 H, CH=), 7.6 (t, 2 H, Ar), 7.72 (t, 1 H, Ar), 7.97 (d, 2 H, Ar).

***N*-[3-(Dimethylamino)-3-phenyl-2-propenylidene]-*N*-methylmethanaminium tetrafluoroborate (4)** was produced in 93% yield as a hygroscopic yellow solid (recrystallization from 2-propanol, mp 136 °C) starting from 2-(dimethylamino)-1-ethenyl phenyl ketone:[16] NMR ($CDCl_3$, 250 MHz) 2.92 and 3.07 (2 s, 2 × 3 H, $N(CH_3)_2$), 3.11 and 3.35 (2 s, 2 × 3 H, $=N^+(CH_3)_2$), 5.45 (d, J = 12 Hz, 1 H, =CH), 6.63 (d, J = 12 Hz, 1 H, =CH), 7.20 and 7.48 (2 m, 5 H, Ar).

***N*-[3-(Dimethylamino)-2-propenylidene]-*N*-methylmethanaminium chloride (5)** was produced in 51% yield as an orange solid (recrystallization from acetone, mp 190 °C) according to the literature procedure[17] (mp 188–189 °C).

Preparation of Dienamines 6–17 (Procedure 2). Diethyl 2-(Dimethylamino)-5-(phenylthio)-2,4-hexadienedioate (6). To 6.9 g (35 mmol) of ethyl phenylthioacetate and 10 g (35 mmol) of **1** in 60 mL of EtOH was added dropwise 21 mL of a 2 M sodium ethoxide solution in EtOH while the temperature was kept below 20 °C. The reaction mixture was stirred at room temperature for 140 min. After evaporation to dryness under reduced pressure, the residue was taken up with 50 mL of ethyl acetate, dried on $MgSO_4$, and concentrated. Purification by flash chromatography on silica gel eluting with 8:2 cyclohexane/ethyl acetate and recrystallization with diisopropyl ether afforded 6.2 g (51%) of **6** as a yellow solid (mp 76 °C): NMR ($CDCl_3$, 250 MHz) δ 1.14 (t, J = 7 Hz, 3 H, CH_3), 1.46 (t, J = 7 Hz, 3 H, CH_3), 2.9 (s, 6 H, $N(CH_3)_2$), 4.13 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.43 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.79 (d, J = 12 Hz, 1 H, CH=), 7.05–7.25 (m, 5 H, Ar), 7.92 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 1740 (C=O), 1680 (C=O), 1590–1550 (C=C), 1480–1450 (Ar), 850–820 (CH=) cm^{-1}; MS m/z 349 (M^+), 240 ($M^+ - C_6H_5S$). Anal. ($C_{18}H_{23}NO_4S$) C, H, N, S.

Compounds **7–17** were prepared in a similar manner.

1-Ethyl 6-*tert*-butyl 2-(dimethylamino)-5-(phenylthio)-2,4-hexadienedioate (7) was produced in 61% yield as a yellow oil (after flash chromatography on silica gel with dichloromethane as eluent), from **1**, *tert*-butyl phenylthioacetate, and *n*-BuLi as base: NMR ($CDCl_3$, 250 MHz) δ 1.34 (s, 9 H, $CO_2C(CH_3)_3$) 1.45 (t, J = 7 Hz, 3 H, CH_3), 2.9 (s, 6 H, $N(CH_3)_2$), 4.45 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.82 (d, J = 12 Hz, 1 H, CH=), 7.05–7.30 (m, 5 H, Ar), 7.81 (d, J = 12 Hz, 1 H, CH=).

Diethyl 2-(dimethylamino)-5-(benzylthio)-2,4-hexadienedioate (8) was produced in 96% yield as an orange oil starting from ethyl benzylthioacetate: NMR ($CDCl_3$, 250 MHz) δ 1.31 (t, J = 7 Hz, 3 H, CH_3), 1.42 (t, J = 7 Hz, 3 H, CH_3), 2.82 (s, 6 H, $N(CH_3)_2$), 3.86 (s, 2 H, SCH_2), 4.21 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.39 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.61 (d, J = 12 Hz, 1 H, CH=), 7.15–7.35 (m, 5 H, Ar), 7.68 (d, J = 12 Hz, 1 H, CH=).

Diethyl 2-(dimethylamino)-5-(ethylthio)-2,4-hexadienedioate (9) was produced in 65% yield as a red oil (after flash chromatography on silica gel with 7:3 cyclohexane/ethyl acetate as eluent), starting from ethyl ethylthioacetate: NMR ($CDCl_3$, 200 MHz) δ 1.14 (t, J = 7 Hz, 3 H, CH_3), 1.25 (t, J = 7 Hz, 3 H, CH_3), 1.38 (t, J = 7 Hz, 3 H, CH_3), 2.65 (q, J = 7 Hz, 2 H, SCH_2), 2.87 (s, 6 H, $N(CH_3)_2$), 4.15 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.35 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.83 (d, J = 12 Hz, 1 H, CH=), 7.70 (d, J = 12 Hz, 1 H, CH=).

Ethyl 2-(dimethylamino)-6-phenyl-6-oxo-5-(phenylthio)-2,4-hexadienoate (10) was produced in 68% yield as an orange oil (after flash chromatography on silica gel with 1:1 cyclohexane/ethyl acetate as eluent), starting from α-phenylthioacetophenone: NMR ($CDCl_3$, 200 MHz) δ 1.12 (t, J = 7 Hz, 3 H, CH_3), 2.90 (s, 6 H, $N(CH_3)_2$), 4.13 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.88 (d, J = 12 Hz, 1 H, CH=), 7.05–7.45 (m, 8 H, Ar), 7.50 (d, J = 12 Hz, 1 H, CH=), 7.57 (dd, 2 H, Ar).

Ethyl 2-(dimethylamino)-6-phenyl-6-oxo-5-(methylthio)-2,4-hexadienoate (11) was produced in 66% yield (re-

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crystallization from diisopropyl ether, mp 78 °C) starting from α-methylthioacetophenone: NMR ($CDCl_3$, 250 MHz) δ 1.05 (t, J = 7 Hz, 3 H, CH_3), 2.28 (s, 3 H, SCH_3), 2.93 (s, 6 H, $N(CH_3)_2$), 4.07 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.9 (d, J = 12 Hz, 1 H, CH=), 7.13 (d, J = 12 Hz, 1 H, CH=), 7.30–7.50 (m, 3 H, Ar), 7.58 (d, 2 H, Ar); MS m/z 319 (M^+), 275 ($M^+ - N(CH_3)_2$), 246 ($M^+ - CO_2C_2H_5$). Anal. ($C_{17}H_{21}NO_3S$) C, H, N, S.

Diethyl 2-(dimethylamino)-5-phenyl-2,4-hexadienedioate (12) was produced in 41% yield as an orange oil (after flash chromatography on silica gel with 8:2 cyclohexane/ethyl acetate as eluent), starting from ethyl phenylacetate: NMR ($CDCl_3$, 200 MHz) δ 1.23 (t, J = 7 Hz, 3 H, CH_3), 1.45 (t, J = 7 Hz, 3 H, CH_3), 2.71 (s, 6 H, $N(CH_3)_2$), 4.16 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.44 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.15 (d, J = 12 Hz, 1 H, CH=), 7.1–7.4 (m, 5 H, Ar), 7.55 (d, J = 12 Hz, 1 H, CH=).

Diethyl 5-benzoyl-2-(dimethylamino)-2,4-hexadienedioate (13) was produced in 92% yield as brown oil starting from ethyl benzoylacetate: IR ($CHCl_3$) 1730 (C=O), 1690 (C=O), 1640 (C=O), 1570 (C=C) cm^{-1}; MS m/z 345 (M^+), 272 ($M^+ - CO_2C_2H_5$).

Ethyl 5-(*N*,*N*-dimethylcarbamoyl)-5-(dimethylamino)-2-(phenylthio)-2,4-pentadienoate (14) was produced in 63% yield as a yellow solid (mp 84 °C, after flash chromatography on silica gel with 8:2 ethyl acetate/cyclohexane as eluent), from **2** and ethyl phenylthioacetate: NMR ($CDCl_3$, 250 MHz) δ 1.14 (t, J = 7 Hz, 3 H, CH_3), 2.92 (s, 6 H, $N(CH_3)_2$), 2.98 and 3.14 (2 s, 2 × 3 H, $CON(CH_3)_2$), 4.12 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.68 (d, J = 12 Hz, 1 H, CH=), 7.0–7.25 (m, 5 H, Ar), 7.8 (d, J = 12 Hz, 1 H, CH=).

Ethyl 5-benzoyl-5-(dimethylamino)-2-(phenylthio)-2,4-pentadienoate (15) was produced in 68% yield as yellow solid (mp 58 °C, after flash chromatography on silica gel with 8:2 cyclohexane/ethyl acetate as eluent), from **3** and ethyl phenylthioacetate. NMR ($CDCl_3$, 250 MHz) δ 1.05 (t, J = 7 Hz, 3 H, CH_3), 2.92 (s, 6 H, $N(CH_3)_2$), 4.05 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.96 (d, J = 12 Hz, 1 H, CH=), 7.05–7.3 (m, 5 H, Ar), 7.5–7.80 (m, 3 H, Ar), 7.71 (d, J = 12 Hz, 1 H, CH=), 7.98 (d, 2 H, Ar).

Ethyl 5-(dimethylamino)-5-phenyl-2-(phenylthio)-2,4-pentadienoate (16) was produced in 52% yield as yellow solid (mp 99 °C, after flash chromatography on silica gel with dichloromethane as eluent), from **5** and ethyl phenylthioacetate: NMR ($CDCl_3$, 200 MHz) δ 1.04 (t, J = 7 Hz, 3 H, CH_3), 2.84 (s, 6 H, $N(CH_3)_2$), 4.03 (q, J = 7 Hz, 2 H, CO_2CH_2), 5.95 (d, J = 12 Hz, 1 H, CH=), 7.0–7.5 (m, 10 H, Ar), 7.61 (d, J = 12 Hz, 1 H, CH=).

Ethyl 5-(dimethylamino)-2-(phenylthio)-2,4-pentadienoate (17) was produced in 51% yield as yellow solid (recrystallization from EtOH, mp 128 °C) starting from **5** and ethyl phenylthioacetate: NMR ($CDCl_3$, 200 MHz) δ 1.18 (t, J = 7 Hz, 3 H, CH_3), 2.92 (br, 6 H, $N(CH_3)_2$), 4.17 (q, 2 H, CO_2CH_2), 5.65 (t, J = 12 Hz, 1 H, CH=), 6.91 (d, J = 12 Hz, 1 H, CH=), 7.0–7.3 (m, 5 H, Ar), 8.01 (d, J = 12 Hz, 1 H, CH=).

Preparation of the Dienols 18–28 (Procedure 3). Diethyl 2-Hydroxy-5-(phenylthio)-2,4-hexadienedioate (18). To 6 g (17 mmol) of **6** in 40 mL of EtOH, at 40 °C, were added 25 mL of 1 N HCl. The reaction mixture was cooled to room temperature and stirred during 30 min. After evaporation to dryness under reduced pressure, the residue was taken up with 100 mL of water and the organic layer was extracted three times with 50 mL of ethyl acetate, dried over $MgSO_4$, and concentrated. Purification by recrystallization from diisopropyl ether afforded 5.4 g (98%) of **18** as a yellow solid (mp 98 °C): NMR ($CDCl_3$, 250 MHz) δ 1.09 (t, J = 7 Hz, 3 H, CH_3), 1.38 (t, J = 7 Hz, 3 H, CH_3), 4.12 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.35 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.63 (br, 1 H, OH), 6.89 (d, J = 12 Hz, 1 H, CH=), 7.10–7.3 (m, 5 H, Ar), 8.13 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 3340 (OH), 1710 + 1690 (C=O), 1635 + 1570 (C=C), 1240 (C–O) cm^{-1}; MS m/z 322 (M^+), m/z 213 ($M^+ - C_6H_5S$). Anal. ($C_{16}H_{18}O_5S$) C, H, S.

Compounds **19–28** were prepared in a similar manner.

Diethyl 2-hydroxy-5-(benzylthio)-2,4-hexadienedioate (19) was produced in 68% yield as an orange oil (after flash chromatography on silica gel with 7:3 ethyl acetate/cyclohexane as eluent), from **8** and 1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.38 (t, J = 7 Hz, 3 H, CH_3), 1.4 (t, J = 7 Hz, 3 H, CH_3), 4.04 (s, 2 H, SCH_2), 4.3 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.35 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.46 (d, J = 1 Hz, 1 H, OH), 6.65 (dd, J = 12 and 1 Hz, 1 H, CH=), 7.15–7.4 (m, 5 H, Ar), 7.92 (d, J = 12 Hz, 1 H, CH=); IR ($CHBr_3$) 3460 (OH), 1690 (C=O), 1640 + 1565 (C=O), 1230 (C–C) cm^{-1}; MS m/z 336 (M^+), 213 ($M^+ - C_6H_5CH_2S$). Anal. ($C_{17}H_{20}O_5S$) C, H, S.

Diethyl 2-hydroxy-5-(ethylthio)-2,4-hexadienedioate (20) was produced in 57% yield as a yellow solid (recrystallization from cyclohexane, mp 65 °C), from **9**: NMR $CDCl_3$, 200 MHz) δ 1.21 (t, J = 7 Hz, 3 H, CH_3CH_2S), 1.35 (t, J = 7 Hz, 3 H, CH_3), 1.4 (t, J = 7 Hz, 3 H, CH_3), 2.84 (q, J = 7 Hz, 2 H, SCH_2), 4.3 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.36 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.45 (br, 1 H, OH), 6.86 (d, J = 12 Hz, 1 H, CH=), 7.95 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 3390 (OH), 1705 + 1685 (C=O), 1630 + 1555 (C=C), 1235 + 1215 (C–O) cm^{-1}; MS m/z 274 (M^+), 213 ($M^+ - SC_2H_5$), 200 ($M^+ - HCO_2C_2H_5$). Anal. ($C_{12}H_{18}O_5S$) C, H, S.

Ethyl 2-hydroxy-6-phenyl-6-oxo-5-(phenylthio)-2,4-hexadienoate (21) was produced in 70% yield as yellow solid (recrystallization from 2-propanol, mp 130 °C), from **10** and 1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.39 (t, J = 7 Hz, 3 H, CH_3), 4.38 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.5 (d, J = 1 Hz, 1 H, OH), 6.96 (dd, J = 12 and 1 Hz, 1 H, CH=), 7.10–7.30 (m, SH, ArS), 7.3–7.60 (m, 3 H, Ar), 7.55 (d, J = 12 Hz, 1 H, CH=), 7.71 (d, 2 H, Ar); IR (KBr) 3380 (OH), 1695 (C=O), 1650 (C=O), 1630 + 1560 (C=C), 1245 (C–O) cm^{-1}. Anal. ($C_{20}H_{18}O_4S$) C, H, S.

Ethyl 2-hydroxy-6-phenyl-6-oxo-5-(methylthio)-2,4-hexadienoate (22) was produced in 86% yield as a yellow solid (recrystallization from diisopropyl ether, mp 86 °C), from **11** and 1 N HCl: NMR ($CDCl_3$, 250 MHz) δ 1.41 (t, J = 7 Hz, 3 H, CH_3), 2.27 (s, 3 H, SCH_3), 4.38 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.29 (s, 1 H, OH), 6.84 (d, J = 12 Hz, 1 H, CH=), 7.16 (d, J = 12 Hz, 1 H, CH=), 7.4–7.65 (m, 3 H, Ar), 7.85 (dt, 2 H, Ar); IR (KBr) 3380 (OH), 1710 (CO), 1650 (CO), 1630 + 1555 (C=C), 1245 (C–O) cm^{-1}; MS m/z 218 nM$^+$), 174 ($M^+ - CO_2$), 157 ($M^+ - SC_2H_5$). Anal. ($C_{15}H_{16}O_4S$) C, H, S.

Diethyl 2-hydroxy-5-phenyl-2,4-hexadienedioate (23) was produced in 43% yield as a beige solid (recrystallization from cyclohexane, mp 107 °C), from **12** and 1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.29 (t, J = 7 Hz, 3 H, CH_3), 1.32 (t, J = 7 Hz, 3 H, CH_3), 4.28 (q, J = 7 Hz, 4 H, CO_2CH_2), 6.22 (d, J = 12 Hz, 1 H, CH=), 6.46 (br, 1 H, OH), 7.20–7.5 (m, 5 H, Ar), 7.92 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 3400 (OH), 1700 (C=C), 1630 (C=C), 1250 + 1230 (C–O) cm^{-1}; MS m/z 290 (M^+), m/z 244 ($M^+ - C_2H_5OH$), 217 ($M^+ - CO_2C_2H_5$). Anal. ($C_{16}H_{18}O_5$) C, H.

Diethyl 5-benzoyl-2-hydroxy-2,4-hexadienedioate (24) was produced in 18% yield as yellow solid (recrystallization from 1:1 cyclohexane/diisopropyl ether, mp 85 °C), from **13** and 1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.12 (t, J = 7 Hz, 3 H, CH_3), 1.26 (t, J = 7 Hz, 3 H, CH_3), 4.17 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.26 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.20 (d, J = 12 Hz, 1 H, CH=), 6.65 (br, 1 H, OH), 7.4–7.7 (m, 3 H, Ar), 7.89 (dt, 2 H, Ar), 8.04 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 3460 (OH), 1710 (C=O), 1680 (C=O), 1630 (C=C), 1235 (C–O) cm^{-1}. Anal. ($C_{17}H_{18}O_6$) C, H.

Ethyl 5-(*N*,*N*-dimethylcarbamoyl)-5-hydroxy-2-(phenylthio)-2,4-pentadienoate (25) was produced in 74% yield as a white powder (recrystallization from diisopropyl ether, mp 88 °C), from **14** and 1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.09 (t, J = 7 Hz, 3 H, CH_3), 2.98 and 3.0 (2 s, 6 H, $N(CH_3)_2$), 4.05 (d, J = 7 Hz, 2 H, $COCH_2$), 4.10 (q, J = 7 Hz, CO_2CH_2), 7.1–7.4 (m, 5 H, Ar), 7.48 (t, J = 7 Hz, 1 H, CH=); IR (KBr) 3100 + 2700 (OH), 1700 (C=O), 1615 (C=O), 1640 + 1560 (C=C), 1240 (C–O) cm^{-1}; MS m/z 321 (M^+), 248 ($M^+ - HCON(CH_3)_2$), 212 ($M^+ - C_6H_5S$). Anal. ($C_{16}H_{19}NO_4S$) C, H, N, S.

Ethyl 5-benzoyl-5-hydroxy-2-(phenylthio)-2,4-pentadienoate (26) was produced in 78% yield as a yellow solid (recrystallization from cyclohexane, mp 110 °C), from **15** and 1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.13 (t, J = 7 Hz, 3 H, CH_3), 4.17 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.75 (dd, J = 12 and 1.5 Hz, 1 H, CH=), 7.15–7.35 (m, 5 H, Ar), 7.34 (d, J = 1.5 Hz, 1 H, OH), 7.4–7.6 (m, 5 H, Ar), 8.25 (d, J = 12 Hz, 1 H, CH=); IR (KBr), 3370 (OH), 1690 (C=O), 1635 (C=O, C=C), 1240 (C–O) cm^{-1}; MS m/z 354 (M^+), 249 ($M^+ - C_6H_5CO$), 245 ($M^+ - C_6H_5S$). Anal. ($C_{20}H_{18}O_4S$) C, H, S.

Ethyl 5-oxo-5-phenyl-2-(phenylthio)-2-pentenoate (27) was produced in 80% yield as an orange oil (after flash chromatography on silica gel with dichloromethane as eluent), from **16** and

1 N HCl: NMR ($CDCl_3$, 200 MHz) δ 1.13 (t, J = 7 Hz, 3 H, CH_3), 4.14 (q, J = 7 Hz, 2 H, CO_2CH_2), 4.24 (d, J = 7 Hz, 2 H, CH_2CO), 7.1–7.3 (m, 5 H, Ar), 7.4–7.7 (m, 3 H, Ar), 7.73 (t, J = 7 Hz, 1 H, CH=), 8.0 (d, J = 7.5 Hz, 2 H); IR ($CDCl_3$), 1710 (C=O), 1685 (C=O), 1600 + 1580 (C=C, Ar), 1250 (C–O) cm^{-1}; MS m/z 326 (M^+), 217 ($M^+ - SC_6H_5$). Anal. ($C_{19}H_{18}O_3S$) C, H, S.

Ethyl 5-hydroxy-2-(phenylthio)-2,4-pentadienoate (28) was produced in 33% yield as a yellow solid (recrystallization from 1:1 cyclohexane/ethyl acetate, mp 120 °C), from **17** and 1 N HCl: NMR (DMSO, 200 MHz) δ 1.13 (t, J = 7 Hz, 3 H, CH_3), 4.11 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.1 (t, J = 12 Hz, 1 H, CH=), 7.05–7.4 (m, 6 H, Ar, OH), 7.58 (d, J = 12 Hz, 1 H, CH=), 8.03 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 3150 (OH), 1680 (C=O), 1620 + 1570 (C=C), 1250 (C–O) cm^{-1}; MS m/z 250 (M^+), 204 ($M^+ - C_2H_5OH$), 176 ($M^+ - HCO_2Et$). Anal. ($C_{13}H_{14}O_3S$) C, H, S.

Ethyl 3-(phenylthio)-2-oxo-3,5-pyran-6-carboxylate (31) was produced in 62% yield as a beige solid (recrystallization from cyclohexane, mp 100 °C), from **7** and 1 equiv of AcOH and of 1 N HCl: NMR ($CDCl_3$, 400 MHz) δ 1.38 (t, J = 7 Hz, 3 H, CH_3), 4.36 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.51 (d, J = 6.5 Hz, 1 H, CH=), 6.96 (d, J = 6.5 Hz, 1 H, CH=), 7.45–7.65 (m, 5 H, Ar); IR ($CHBr_3$) 1730–1710 (C=O), 1625 + 1520 (C=C), 1290 (C–O) cm^{-1}. Anal. ($C_{14}H_{12}O_4S$) C, H, O.

Preparation of Dienol Acids 29 and 30 (Procedure 4). 5-(Ethoxycarbonyl)-2-hydroxy-5-(phenylthio)-2,4-pentadienecarboxylic Acid (29). To 3.4 g (11.6 mmol) of **18** in 40 mL of EtOH was added 62.2 mL of 1 N NaOH and the temperature was kept below 20 °C. The reaction mixture was stirred for 135 min at room temperature and acidified with 1 N HCl to pH 1–2. After evaporation to dryness under reduced pressure, the residue was taken up with 40 mL of water and the organic phase was extracted twice with 40 mL of ethyl acetate, dried over $MgSO_4$, and concentrated. Purification by recrystallization with 1:1 diisopropyl ether/acetonitrile afforded 2 g (59%) of **29** as yellow crystals (mp 172 °C): NMR ($CDCl_3$, 250 MHz) δ 1.06 (t, J = 7 Hz, 3 H, CH_3), 4.07 (q, J = 7 Hz, 2 H, CO_2CH_2), 6.65 (d, 1 H, OH), 7.10–7.4 (m, 6 H, Ar, OH), 8.15 (d, J = 12 Hz, 1 H, CH=); IR (KBr), 3340 (OH), 3100 + 2200 (OH), 1690 + 1680 (C=O), 1625 + 1570 (C=C), 1240 (C–C) cm^{-1}; MS m/z 294 (M^+), 185 ($M^+ - SC_6H_5$). Anal. ($C_{14}H_{14}O_5S$) C, H, S.

Compound **30** was prepared in a similar manner.

2-Hydroxy-5-(ethylthio)-2,4-hexadienedioic acid (30) was produced in 38% yield as a yellow solid (recrystallization from dioxane, mp 235 °C) from **20** and after agitation from 6 h with 1 N sodium hydroxide at room temperature: NMR (DMSO, 250 MHz) δ 1.10 (br, 3 H, CH_3), 2.78 (br, 2 H, SCH_2), 6.63 (d, J = 12 Hz, 1 H, CH=), 7.85 (d, J = 12 Hz, 1 H, CH=); IR (KBr) 3435 (OH), 3350 + 2200 (OH), 1680 (C=O), 1635 + 1555 (C=C), 1255 (C–O) cm^{-1}. MS m/z 218 (M^+), 174 ($M^+ - CO_2$). Anal. ($C_8H_{10}O_5S$) C, H, S.

Ethyl 2-(Phenylthio)-2-benzoylacetate (35). To 22.6 mL (107 mmol) of hexamethyldisilazane and 105 mL of THF under argon and cooled to −78 °C was slowly added 67 mL (107 mmol) of 1.6 M *n*-BuLi in hexane. The solution was stirred for 15 min and 21 g of ethyl phenylthioacetate (107 mmol) in 105 mL of THF was added while the temperature was kept below 0 °C. This mixture was cooled to −78 °C and added to a solution of 14.1 g (100 mmol) of benzoyl chloride in 105 mL of THF. After stirring for an additional 30 min at 0 °C, the mixture was poured into an aqueous solution of NH_4Cl and extracted twice with 200 mL of diethyl ether. The organic phase was dried over $MgSO_4$ and evaporated under vacuum. The residue was taken up with 200 mL of cyclohexane and the precipitate (benzoic acid) was filtered. The filtrate was evaporated in vacuum. THF (100 mL) and 70 mL of 5 N NaOH were added. The organic phase was acidified with 6 N HCl and extracted with 200 mL of dichloromethane. The organic phase was dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by flash chromatography on silica gel eluting with 7:3 cyclohexane/ethyl acetate to yield 3 g (10%) of **35** as a pale yellow oil: NMR ($CDCl_3$, 200 MHz; equilibrium between keto and enol forms) δ 1.17 (2 t, J = 7 Hz, 2 × 3 H, 2 CH_3), 4.20 and 4.27 (2 q, J = 7 Hz, 2 × 2 H, 2 CO_2CH_2), 5.35 (s, 0.75 H, SCH), 7.1–7.7 (m, 8.5 Hz, Ar), 7.96 (dd, 1.5 H, Ar), 14.2 (s, 0.25 H, OH); IR ($CHBr_3$) 1730 (C=O), 1625 (C=O), 1560 (C=O), 1260 (C–O) cm^{-1}. MS m/z 300. Anal. ($C_{17}H_{17}O_3S$) C, H, S.

Biological Evaluation. PMN_1 Peritoneal 5-LO Assay. Peritoneal PMN_1 were obtained from male Wistar rats (200–300 g) that had received an intraperitoneal (ip) injection of 1.2% caseinate solution (10 mL). After 18 h, rats were killed by CO_2 asphyxia, and peritoneal cells were harvested by peritoneal lavage using Ca^{2+}– and Mg^{2+}–free Hanks' balanced salt solution (HBSS). The peritoneal exudate was centrifuged at 400*g* for 10 min. The lavaged fluid was removed, and the cell pellets were resuspended in HBSS. The PMN_1, after purification on Ficoll, were resuspended in HBSS containing Ca^{2+} and Mg^{2+} at a concentration of 2.5×10^6 cells/mL. To 1-mL fractions of cell suspension were added test drugs, or vehicle, and they were incubated at 37 °C for 10 min.

The reaction was stopped by centrifugation at 400*g* for 10 min. Then, the supernatants were assayed for LTB_4 by using a radioimmunoassay technique (LTB_4: Amersham kit ref). IC_{50} values were calculated by linear-regression analysis (average of three to six different experiments).[18]

Rat Basophil Leukemia Type 1 (RBL_1) 5-LO and CO Assay. Cyclooxygenase and 5-lipoxygenase enzymes were assayed as a 1600*g* supernatant from sonicated RBL_1 cells by the modified method of Steinhoff et al.[19] and Jakschik et al.[20] The 1600*g* supernatant was diluted with the homogenization buffer to the equivalent of $1–3 \times 10^7$ cells/mL and made 2.5 mM with respect to $CaCl_2$. Aliquots of 0.2 mL were dispersed into tubes and incubated either with 2.5 μL of dimethylformamide (DMF) or the tested compound in DMF at the desired concentration and incubated at 37 °C for 2 min. [^{14}C]AA was then added to give a final concentration of 7 MM and 0.25 μCi per tube, and the reaction was continued at 37 °C for 8 min. The reaction was terminated by the addition of 1 mL of MeOH and 25 μL of 2 N formic acid and cooling on ice. The mixture was extracted with 3 mL of $CHCl_3$. The concentrated organic phase was then analyzed by TLC on silica gel using as eluent the following solvent system: ethyl acetate/(2,2,4-trimethylpentyl)acetic acid/distilled water (11:5:2:10; v/v). Activity was measured as the percentage of the total radioactivity found in [^{14}C]-5-HETE and [^{14}C]PGD_2. IC_{50} values were calculated by linear-regression analysis using five or more concentrations for at least two determinations, causing significant inhibition compared to the controls.

Ear Edema Tests. The ear edema test was performed according to Crummey et al.[12] Briefly, 1 mg of AA dissolved in 20 μL of acetone was applied to the inner surface of the left ear. For topical drug application, each compound to be tested was applied 30 min before AA administration. Control animals received solvent on the opposite ear. For oral-route testing the drugs were administered 1 h before the agent.

After 1 h, the animals were sacrified by cervical dislocation and both ears were cut off at the hair line and weighed. Ear edema induced by AA was estimated by subtracting the weight of the right ear (vehicle control) from the left ear (treated ear). Percentage inhibition by the tested compound was calculated as = 100– [(swelling in the presence of compound divided by swelling in the absence of compound) times 100]. ED_{50} values correspond to the doses of compounds reducing 50% the ear edema in response to AA inflammation.

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