86324-48-3; (S)-27, 82950-72-9; (±)-27, 82923-75-9; (S)-27 l-cinchonidine, 85683-64-3; (S,S)-28, 86324-49-4; 2-cyano-6-fluoroquinoline, 86324-50-7; 6-fluoro-2-quinolincarboxylic acid, 86324-51-8; methyl 6-fluoro-2-quinolincarboxylate, 86324-52-9; l-cinchonidine, 485-71-2; 4-propoxyaniline, 4469-80-1; ethyl chloroformate, 105-39-5; 3,4-dimethoxyaniline, 6315-89-5; 1,4-dithiacycloheptan-6-one, 34654-19-8; ethyl glycinate hydrochloride, 623-33-6; 4-fluoro-DL-phenylalanine, 51-65-0; 6-fluoroquinoline, 396-30-5; formaldehyde, 50-00-0; angiotensin converting enzyme, 9015-82-1.

Angiotensin Converting Enzyme Inhibitors: 1-Glutarylindoline-2-carboxylic Acid Derivatives

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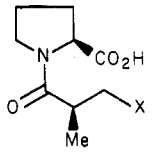
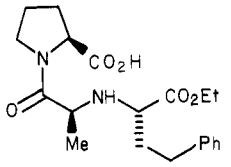
The preparation of a series of 1-glutarylindoline-2(S)-carboxylic acid derivatives, **6a–v** and **21a–c**, is described. The above compounds were tested for inhibition of angiotensin converting enzyme. The structure–activity relationship of the series is also discussed. Compound **6u**, the most potent member of the series, had an in vitro IC_{50} of 4.8×10^{-9} M. Compound **6v**, an ethyl ester of **6u**, lowered blood pressure 70 mm in spontaneous hypertensive rats at an oral dose of 30 mg/kg.

Captopril, 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline (**1a**), is an angiotensin converting enzyme (ACE)



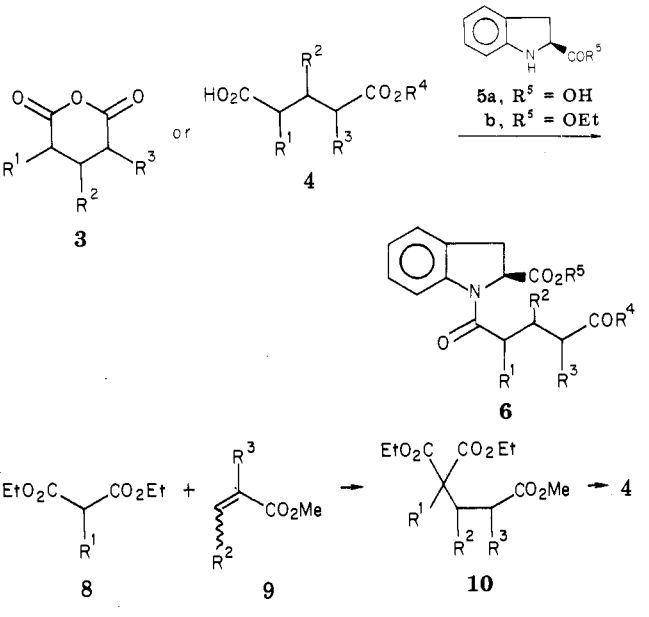
1a, X = SH
b, X = CH_2CO_2H



2

inhibitor that has been shown to be an effective antihypertensive agent in man.[1] However, the drug produces a number of side effects, most commonly, rashes and an alteration of taste.[2] These effects might be attributed to the thiol group, which binds to zinc at the enzyme active site.[3] Several effective ACE inhibitors have since been found that do not contain a thiol group.[4,5] In particular, enalapril (2), which contains a carboxylate group for binding to zinc at the active site, has been suggested to be a transition-state inhibitor that through its CO_2H, NH, and phenethyl groups achieves good binding. In the initial report on **1a** it was disclosed that replacement of the thiol in **1a** with a carboxylic acid to give **1b** substantially decreased the potency, increasing the IC_{50} from 2.3×10^{-8} to 4.9×10^{-6} M.[6] Despite this finding we set out to explore a series of substituted glutaric acid derivatives related to **1b** in which L-proline was replaced by (S)-indoline-2-carboxylic acid.[7] Herein we report the results of this work.

Scheme I

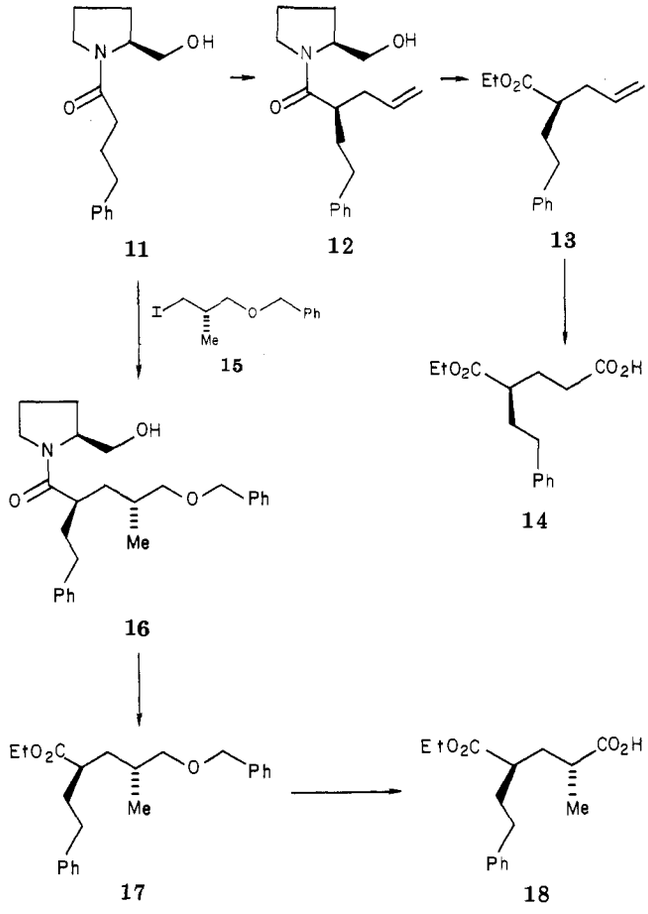


Chemistry. The desired compounds were prepared as shown in Scheme I. Reaction of glutaric anhydride derivative **3** with (S)-indoline-2-carboxylic acid (**5a**) or the corresponding ethyl ester (**5b**) resulted in amides **6** (method A). For unsymmetrical anhydrides, **5a** or **5b** added predominately to the least hindered carbonyl group, and the products were purified by fractional crystallization.

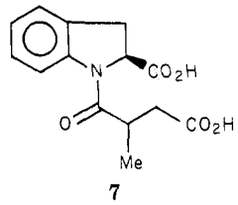
(1) (a) Bravo, E. L.; Tarazi, R. C. *Hypertension* **1979**, *1*, 39. (b) Brunner, H. R.; Gavras, H.; Waeber, B.; Kershaw, G. R.; Turini, G. A.; Vukovich, R. A.; McKinstry, D. N. *Ann. Intern. Med.* **1979**, *90*, 19. (c) Case, D. B.; Atlas, S. A.; Laragh, J. H.; Sealey, J. E.; Sullivan, P. A.; McKinstry, D. N. *Prog. Cardiovasc. Dis.* **1978**, *21*, 195. (d) Gavras, H.; Brunner, H. R.; Turini, G. A.; Kershaw, G. R.; Tifft, C. P.; Cuttelod, S.; Gavras, I.; Vukovich, R. A.; McKinstry, D. N. *N. Engl. J. Med.* **1978**, *298*, 991.
(2) Atkinson, A. B.; Robertson, J. J. S. *Lancet* **1979**, *2*, 836.
(3) Das, M.; Soffer, R. L. *J. Biol. Chem.* **1975**, *250*, 6762.
(4) Almquist, R. G.; Chao, W. R.; Ellis, M. E.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392.
(5) Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvrett, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Broeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.
(6) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.
(7) The use of this amino acid in a series of mercaptan-containing ACE inhibitors has been described: Stanton, J. L.; Gruenfeld, N.; Babiarz, J. E.; Ackerman, M. H.; Friedmann, R. C.; Yuan, A. M.; Macchia, W. *J. Med. Chem.*, following paper in this issue.

0022-2623/83/1826-1277$01.50/0

Scheme II



Alternatively, compounds **6** were prepared by coupling **5a** or **5b** with a glutaric monoacid derivative **4** or the corresponding acid chloride (method B). The method chosen was based on the desired functionality of R^4 and R^5 of products **6**. The diacid products **6** could be prepared either by opening of **3** with **5a** in pyridine or by method B, followed by alkaline hydrolysis of the corresponding diester or either monoester. Amide **6d** was obtained by ammonolysis of methyl ester **6c**. The lower homologue (**7**) of **6b** was prepared by opening 2-methylsuccinic anhydride with ethanol, coupling this intermediate with **5b**, and then hydrolyzing the product in base to **7**.

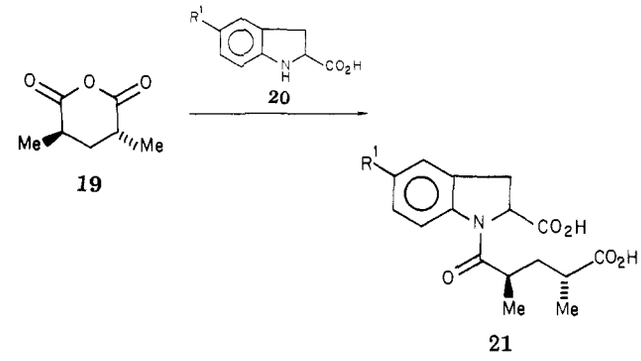


7

The starting glutaric anhydrides were commercially available or were prepared from substituted glutaric acids by treatment with acetyl chloride or acetic anhydride. The racemic substituted glutaric acids, which were not commercially available, were synthesized as illustrated in Scheme I. Thus, base-catalyzed Michael addition of malonate **8** with acrylate **9** generated triester **10**. Hydrolysis and decarboxylation of **10** afforded glutaric acid **4**.

The chiral acid required for **6e** and **6f** was prepared from pulegone by the procedure of Eisenbraun.[8] (2*R*,4*R*)-2,4-dimethylglutaric acid was obtained by resolution of 2,4-dimethylglutaric acid via its (+)-2-methylbenzylamine salt. Ethyl hydrogen (4*R*)-4-(2-phenylethyl)glutarate (**14**) was prepared by asymmetric synthesis as shown in Scheme II. Asymmetric alkylation by the method of Evans[9] was carried out with 1-(4-phenylbutyryl)-L-prolinol (**11**) and allyl bromide to yield **12**, which was converted to ester **13** in 10% ethanolic sulfuric acid. Hydrogenation of **13**, followed by hydroboration and oxidation, produced the chiral acid **14**. Ethyl hydrogen (2*R*,4*R*)-2-methyl-4-(2-phenylethyl)-glutarate (**18**) was prepared similarly. Asymmetric alkylation of **11** with (*S*)-3-(benzyloxy)-2-methylpropyl iodide (**15**)[10] afforded **16**. Treatment of **16** with ethanolic HCl generated ester **17**. Hydrogenation of **17**, followed by oxidation with pyridinium dichromate, resulted in the desired acid **18**. Compounds **6a–v** and **7** described above are listed in Table I.

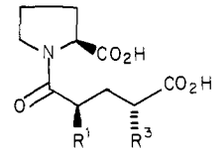
Scheme III



Several indoline derivatives with substituents on the aromatic ring were also prepared. For comparison with **6o**, (2*R*,4*R*)-2,4-dimethylglutaric anhydride (**19**) was used for each coupling with racemic 5-substituted indoline-2-carboxylic acids (**20**) to give products **21** (Scheme III).

Results and Discussion

The compounds were tested for in vitro inhibition of ACE, and the results also appear in Table I. Although the free diacids are required for potent binding to the enzyme site, the corresponding monoesters could be expected to be comparable in vivo if in vivo hydrolysis takes place. A comparison of **6a** and **6o** with **22a**[6] and **22b**,[5] respectively,



22a, R^1 = H; R^3 = H
b, R^1 = Me; R^3 = Me

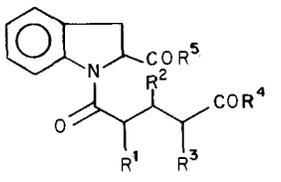
shows that replacing L-proline with (*S*)-indoline-2-carboxylic acid (**5a**) resulted in an approximately 200-fold increase in in vitro potency. This improved activity exemplifies the superiority of **5a** relative to proline in binding at the ACE active site. The lower homologue **7** was found to have reduced potency, which was also observed in the proline series.[6] The effect of substitution on the indole moiety was found to be insignificant (compare **21a–c** with **6o**). The effect of α-, β-, and γ-methyl substitution on the

(8) Eisenbraun, E. J.; McElvain, S. M. *J. Am. Chem. Soc.* **1955**, 77, 3383.

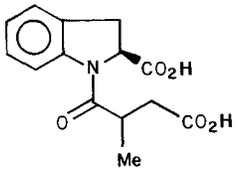
(9) Evans, D. A.; Takacs, J. M. *Tetrahedron Lett.* **1980**, 4233.

(10) Prepared from (*S*)-(+)-hydroxyisobutyric acid, a generous gift from Dr. Noal Cohen, Hoffmann-LaRoche, following known methods: Branca, Q.; Fischli, A. *Helv. Chim. Acta* **1977**, *60*, 925. Scheffold, R.; Saladin, E. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 229.

Table I. 1-Glutarylindoline-2-carboxylic Acid Derivatives



6a–v



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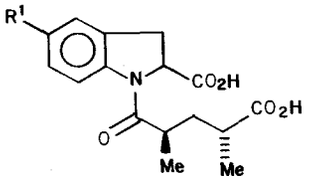

21a–c

compd	R^1	R^2	R^3	R^4	R^5	mp,[a] °C	$[\alpha]_D$, deg	method	ACE IC_{50},[b] nM	AI % inhibn,[b,c] mg/kg iv	SHR max Δ BP,[b,d] mm (mg/kg po)
6a	H	H	H	OH	OH	175–177	−97.8 (*c* 1.0, EtOH)	A	260	78 (10)	−37 (30)
6b	Me	H	H	OH	OH	72–74	−73.2 (*c* 1.0, EtOH)	B	64	84 (1.0)	−61 (50)
6c	Me	H	H	OMe	OH	97–99		B	6 900	45 (0.1)	−61 (50)
6d	Me	H	H	NH_2	OH	192–194		B	16 000	71 (10)	
6e	(*R*)-Me	H	H	OH	OH	125–127	−118.5 (*c* 0.2, EtOH)	B	44	94 (1.0)	−35 (30)
6f	(*R*)-Me	H	H	OEt	OH	133–135	−120.5 (*c* 0.2, EtOH)	B	11 000	27 (0.1)	−66 (50)
6g	*i*-Pr	H	H	OH	OH	184–186	−81 (*c* 0.2, EtOH)	B	50	77 (1.0)	
6h	H	Me	H	OH	OH	125–127	−88.9 (*c* 1.0, EtOH)	A	37 000	20 (10)	
6i	H	Me_2	H	OH	OH	132–134	−151 (*c* 0.2, EtOH)	A	290 000		
6j	H	H	Me	OH	OH	172–174	−94 (*c* 1.0, EtOH)	A	920	41 (10)	
6k	H	H	Me	OH	OEt	104–106	−92.7 (*c* 1.0, EtOH)	A	170 000	11 (10)	
6l[e]	Me	H	Me	OH	OEt	132–134	−66.7 (*c* 1.0, EtOH)	A	8 800	84 (10)	−33 (50)
6m[i]	Me	H	Me	OH	OH	58–60	−83.2 (*c* 1.0, EtOH)	A	190	76 (1.0)	
6n[j]	Me	H	Me	OH	OH	70–72	−76.1 (*c* 1.0, EtOH)	A	54	84 (1.0)	
6o	(*R*)-Me	H	(*R*)-Me	OH	OH	132–134	−144 (*c* 1.0, EtOH)	A	28	45 (0.1)	−39 (50)
6p	(*R*)-Me	H	(*R*)-Me	OEt	OH	128–130	−162.8 (*c* 0.91, EtOH)	B	5 500	52 (0.1)	−75 (50)
6q	(*R*)-Me	H	(*R*)-Me	OH	OEt	110–112	−156.5 (*c* 0.2, EtOH)	B	28 000	16 (0.3)	
6r	H	H	CH_2CH_2Ph	OH	OH	136–138	−69.1 (*c* 1.0, EtOH)	B	39	75 (10)	−46 (50)
6s	H	H	(*R*)-CH_2CH_2Ph	OH	OH	132–134	−79.1 (*c* 0.81, $CHCl_3$)	B	33		
6t	H	H	(*R*)-CH_2CH_2Ph	OEt	OH	100–102	−83.4 (*c* 1.1, $CHCl_3$)	B	4 300	17 (1.0)	
6u	(*R*)-Me	H	(*R*)-CH_2CH_2Ph	OH	OH	136–138	−62 (*c* 0.25, $CHCl_3$)	B	4.8	60 (0.1)	
6v	(*R*)-Me	H	(*R*)-CH_2CH_2Ph	OEt	OH	96–98	−117.5 (*c* 0.88, $CHCl_3$)	B	11 000	89 (0.4)	−70 (30)
7						83–85		B	300	27 (1.0)	
21a[e]	Cl					185–187	−26.5 (*c* 0.2, $CHCl_3$)	A	440	32 (2.0)	
21b[e]	Me					198–200	+0.9 (*c* 0.2, $CHCl_3$)	A	490	40 (2.0)	
21c[e]	OMe					150–152	−130 (*c* 0.2, $CHCl_3$)	A	110	56 (1.0)	
22a									70 000[f]		
22b									4 800[g]		
captopril (1a)									15[h]	90 (1.0)	−55 (30)
										70 (0.3)	−45 (10)

[a] All compounds had satisfactory C, H, and N elemental analyses and exhibited IR and NMR spectra consistent with the structure. [b] See ref 7 for details of procedure. [c] Tabulated results indicate percent inhibition of angiotensin I pressor response 15 min after intravenous administration of test compound. [d] Tabulated results indicate maximal change in blood pressure recorded during the 4-day test period. [e] Dicyclohexylamine salt. [f] Reference 6. [g] Reference 5. [h] Literature[6] IC_{50} = 23 nM. [i] Erythro. [j] Threo.

glutaryl group parallels the structure–activity profile reported for the corresponding proline series.[6] Thus, α-substitution on the glutaric acid side chain was found to increase potency about fivefold (compare **6a** with **6e,g**). The effect of β-substitution on the glutaryl group lowered the activity (see **6h,i**). The effect of a γ-methyl group was small (**6a** vs. **6j**), but a γ-phenethyl group was quite beneficial (**6a** vs. **6r**). The γ-phenethyl group not only duplicates the analogous substituent in enalapril[5] but also places a phenyl group in the same position as one in the natural substrate, the phenylalanine at the 8-position of angiotensin I (AI). The optimal α and γ substituents were combined in a single glutaryl group employing the preferred geometry analogous to that found for **1a** and **2**, as well as in AI. The resulting compound, **6u**, had an $IC_{50} = 4.8 \times 10^{-9}$ M and was the most potent member of the series, having about 3 times the in vitro potency of captopril (**1a**). In addition **6u**, whose glutaryl group is a carbon isostere of the side chain of **2**, achieves its good potency without an NH in the side chain. This contrasts with the report of **2** describing the importance of the NH in such "transition-state" inhibitors.[5] Thus, the combined contributions to binding of the substituents of **6u**, especially of the terminal amino acid (*S*)-indoline-2-carboxylic acid, compensate for the lack of an NH relative to **2**, as well as for the comparatively weaker binding of the carboxylic acid group relative to the mercapto group of **1a**.

Most of the compounds tested in vitro were also screened for in vivo ACE activity and antihypertensive activity. Key results that clarify the structure–activity picture are included in Table I.[11] The intravenous AI screen was used as the primary in vivo test to measure intrinsic activity and to provide a qualitative measure of the relative activity of the test compounds. The tabulated values are representative of the inhibition for the 1-h test period. The 4-day spontaneous hypertensive rat (SHR) screen was used to determine the relative oral efficacy of the test compounds. The tabulated values indicate the relative difference in activity seen during the 4-day test period. It was generally found that a diacid was significantly less active orally than when given intravenously, possibly due to poor absorption. In addition, when the diacids were replaced with monoacids containing esters of the glutaryl group, improved oral efficacy ensued.[12] (For example, compare **6o** with **6p**.) Interestingly, having an ethyl ester on the indoline resulted in a reduction of in vivo activity, indicating that this ester is not very effectively hydrolyzed in vivo (see **6r**). Several monoesters showed particularly good oral activity in the SHR screen. Comparable to the reference compound captopril (**1a**), compounds **6f** and **6p** lowered blood pressure up to 66 and 75 mm, respectively, at 50 mg/kg po. Compound **6v**, the ethyl ester of **6u**, was found to be the most potent compound in vivo, lowering blood pressure 70 mm at 30 mg/kg po.

The findings presented here describe potent ACE inhibitors not containing a mercapto group. Instead of relying on strong binding to zinc at the active site, these glutaric acid derivatives achieve potent binding through increased interaction with several lipophilic regions of the active site. Thus, the structure of the most active compound in vitro, **6u**, provides additional insight into the binding requirements of the ACE active site.

Experimental Section

Proton NMR spectra were determined on a Varian EM-390 spectrometer with Me_4Si as the internal standard. Infrared spectra were recorded on a Perkin-Elmer Model 457 or 137 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The reported compounds were prepared by methods identical with those described below. The intermediate products were used directly without further purification.

1-(4-Carboxy-3,3-dimethylbutyryl)indoline-2(*S*)-carboxylic Acid (6i). Method A. To (*S*)-indoline-2-carboxylic acid hydrochloride (4.0 g, 0.020 mol) and triethylamine (2.02 g, 0.020 mmol) in 40 mL of toluene was added 3,3-dimethylglutaric anhydride (2.84 g, 0.020 mol). The reaction was stirred at 80 °C for 4 h and then rotary evaporated. The residue was partitioned between 25 mL of 1 N HCl and 25 mL of methylene chloride. The aqueous layer was washed with 2 × 25 mL of methylene chloride. The combined organic portions were dried ($MgSO_4$) and rotary evaporated to give 3.17 g of crude product. Recrystallization from ether–hexane gave **6i** (1.3 g, 21%): mp 132–134 °C; $[\alpha]_D$ −151° (*c* 0.2, EtOH); NMR (Me_2SO-d_6) δ 1.19 (6 H, s), 2.55 (4 H, m), 3.39 (2 H, m), 5.19 (1 H, d, *J* = 8 Hz), 7.18 (3 H, m), 8.25 (1 H, d, *J* = 8 Hz), 12.2 (2 H, br s); IR (Nujol) 3050, 2980–2450, 1710, 1665, 1462, 1230, 999 cm^{-1}. Anal. ($C_{16}H_{19}NO_5$) C, H, N.

1-(4-Carboxy-2-isopropylbutyryl)indoline-2(*S*)-carboxylic Acid (6g). Method B. A solution of 2-isopropylglutaric acid[13] (14.0 g, 80.4 mmol) in 80 mL of acetyl chloride was stirred at 50 °C for 2 h and then rotary evaporated to give 2-isopropylglutaric anhydride (12.5 g, 99%): NMR ($CDCl_3$) δ 1.06 (6 H, t, *J* = 7 Hz), 1.91 (2 H, m), 2.22–3.05 (4 H, m).

A solution of the above anhydride (4.0 g, 25.6 mmol) in 20 mL of EtOH was refluxed 3 h and then rotary evaporated to yield 4-(ethoxycarbonyl)-2-isopropylbutyric acid (5.0 g, 96%): NMR ($CDCl_3$) δ 0.95 (6 H, d, *J* = 8 Hz), 1.21 (3 H, t, *J* = 7 Hz), 1.71–2.48 (6 H, m), 4.16 (2 H, q, *J* = 7 Hz).

To the above ester (5.0 g, 24.7 mmol), ethyl (*S*)-indoline-2-carboxylate hydrochloride (5.62 g, 24.7 mmol), and triethylamine (2.5 g, 24.7 mmol) in 80 mL of methylene chloride at room temperature was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (5.21 g, 27.2 mmol). The reaction was stirred overnight at room temperature and then washed with 40 mL of H_2O, 30 mL of 1 N HCl, and 30 mL of saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and rotary evaporated to give ethyl 1-[4-(ethoxycarbonyl)-2-isopropylbutyryl]indoline-2(*S*)-carboxylate (6.5 g, 70%), as a mixture of diastereomers: NMR ($CDCl_3$) δ 0.95 (6 H, m), 1.19 (6 H, m), 1.78–2.45 (6 H, m), 3.35 (2 H, d, *J* = 8 Hz), 4.18 (4 H, m), 5.12 (1 H, m), 6.75 (1 H, m), 7.17 (3 H, m).

To a solution of the above diester (6.5 g, 17.3 mmol) in 50 mL of MeOH was added 52 mL of 1 N aqueous NaOH. The reaction was stirred for 2 h at room temperature, and then the MeOH was rotary evaporated. The aqueous residue was acidified with 12 N HCl and extracted with 3 × 25 mL of methylene chloride. The combined organic portions were dried (Na_2SO_4) and rotary evaporated. The residue was crystallized from diethyl ether to yield **6g** (5.5 g, 60%), a mixture of diastereomers: mp 184–186 °C; $[\alpha]_D$ −81° (*c* 0.2, EtOH); NMR (Me_2SO-d_6) δ 0.95 (6 H, t, *J* = 7 Hz), 1.52–2.4 (6 H, m), 3.05–3.78 (2 H, m), 5.05 (1 H, dd, *J* = 9 and 1.5 Hz), 7.17 (3 H, m), 8.21 (1 H, d, *J* = 8 Hz), 12.5 2 H, s); IR (Nujol) 3150–2500, 1725, 1715, 1665, 1481, 1220, 1158, 938 cm^{-1}. Anal. ($C_{17}H_{21}NO_5$) C, H, N.

1-[(2*R*,4*R*)-4-(Ethoxycarbonyl)-2,4-dimethylbutyryl]-indoline-2(*S*)-carboxylic Acid (6p). To a solution of a meso, *dl* mixture of 2,4-dimethylglutaric acid (4.0 kg, 25 mol) in 10 L of 2-propanol was added triethylamine (1.27 kg, 12.5 mol) over a few minutes. The reaction temperatures rose to approximately 45 °C. Then (+)-α-methylbenzylamine (1.54 kg, 12.5 mol) was added, causing the temperature to rise to about 55 °C. The

(11) Details of the pharmacological profile will be reported elsewhere.

(12) Oral activity of other compounds have previously been shown to be improved through esterification: (a) Vickers, S.; Duncan, C. A.; White, S. D.; Breault, G. D.; Royda, R. B.; De Schepper, P. J.; Tempero, K. F. *Drug Metab. Dispos.* **1978**, *6*, 640. (b) Gross, D. M.; Sweet, C. S.; Ulm, E. H.; Borklund, E. P.; Morris, A. A.; Weitz, D.; Bohn, D. L.; Wenger, H. C.; Vassil, T. C.; Stone, C. A. *J. Pharmacol. Exp. Ther.* **1981**, *216*. 552.

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solution was seeded with (−)-2,4-dimethylglutaric acid (+)-α-methylbenzylamine salt and stirred for 3 days at room temperature. The product was collected by filtration, washed with 2-propanol (2 × 750 mL), and recrystallized from 5 L of 2-propanol to give 834 g (47%) of salt: mp 155–158 °C; $[\alpha]_D$ −19.6° (c 2, MeOH).

This salt (834 g, 2.96 mol) was dissolved in 290 mL of concentrated HCl, and the solution was concentrated in vacuo at 60 °C. The residue was extracted with 3 × 2.5 L of ether. The combined ether extracts were dried ($MgSO_4$) and rotary evaporated to give 440 g (92%) of (2R,4R)-2,4-dimethylglutaric acid: mp 75–78 °C; $[\alpha]_D$ −35.8° (c 2, EtOH); NMR ($CDCl_3$) δ 1.25 (6 H, d, J = 7 Hz), 1.88 (2 H, t, J = 7 Hz), 2.67 (2 H, sextet, J = 7 Hz), 12.0 (2 H, s).

The above acid (200 g, 1.25 mol) and acetyl chloride (356 mL, 5.0 mol) were stirred at 45 °C for 3 h. The reaction was filtered and rotary evaporated to yield 180 g (100%) of (2R,4R)-2,4-dimethylglutaric anhydride: mp 42–44 °C; $[\alpha]_D$ +56.5° (c 1.04, $CHCl_3$); NMR ($CDCl_3$) δ 1.33 (6 H, d, J = 7 Hz), 1.88 (2 H, t, J = 7 Hz), 2.90 (2 H, sextet, J = 7 Hz).

The above anhydride (178 g, 1.25 mol) in 360 mL of EtOH was refluxed overnight and then rotary evaporated to afford 240 g of oil. This oil was dissolved in 200 mL of ether and extracted with 5 × 200 mL of saturated aqueous $NaHCO_3$. The combined aqueous portions were cooled to 0 °C and acidified to pH 2 with concentrated HCl (110 mL). The solution was saturated with NaCl and extracted with 5 × 200 mL of ethyl acetate. The ethyl acetate extracts were dried (Na_2SO_4) and rotary evaporated to give 175 g (74%) of (2R,4R)-4-(ethoxycarbonyl)-2,4-dimethylbutyric acid: $[\alpha]_D$ −44.1° (c 1.4, EtOH); NMR ($CDCl_3$) δ 1.22 (6 H, t, J = 7 Hz), 1.26 (3 H, t, J = 7 Hz), 1.78 (2 H, t, J = 7 Hz), 2.55 (2 H, m), 4.15 (2 H, q, J = 7 Hz), 11.85 (1 H, s).

To the above acid (175 g, 0.93 mol) at 0 °C was added oxalyl chloride (245 mL, 27.8 mol) over about 20 min. The mixture was stirred at room temperature. The excess oxalyl chloride was removed by rotary evaporation to yield 190 g (100%) of (2R,4R)-4-(ethoxycarbonyl)-2,4-dimethylbutyryl chloride: $[\alpha]_D$ −30.1° (c 0.92, $CHCl_3$); NMR ($CDCl_3$) δ 1.29 (9 H, m), 1.81 (2 H, m), 2.51 (1 H, sextet, J = 7 Hz), 2.89 (1 H, sextet, J = 7 Hz), 4.15 (2 H, q, J = 7 Hz).

To (S)-indoline-2-carboxylic acid hydrochloride (201 g, 1.0 mol) in 790 mL of pyridine at 0 °C was added the above acid chloride (190 g, 0.92 mol) over 20 min. Then the reaction was stirred at room temperature for 3 h. The reaction was rotary evaporated. The residue at 0 °C was acidified with 200 mL of 12 N HCl and then extracted with 3 × 200 mL of methylene chloride. The combined organic extracts were washed with 200 mL of 3 N HCl and 200 mL of H_2O, dried ($MgSO_4$), and rotary evaporated to give 335 g (99%) of crude solid product. This product was stirred in 1.1 L of diethyl ether for 2 h and filtered. The product was washed with 4 × 250 mL of ether. After the product was dried, 244 g (73%) of **6p** was obtained: mp 128–130 °C; $[\alpha]_D$ −162.8° (c 0.91, EtOH); NMR (Me_2SO-d_6) δ 1.15 (9 H, m), 1.73 (2 H, m), 2.51 (2 H, m), 3.35 (2 H, m), 4.16 (2 H, q, J = 7 Hz), 5.15 (1 H, dd, J = 9 and 1 Hz), 7.25 (3 H, m), 8.25 (1 H, d, J = 8 Hz), 11.5 (1 H, br s); IR (Nujol) 3200–2550, 1740, 1718, 1625, 1588, 1457, 1373, 1199 cm^{-1}. Anal. ($C_{18}H_{23}NO_5$) C, H, N.

Ethyl 1-[(2R,4R)-4-(Ethoxycarbonyl)-2-methyl-4-(2-phenylethyl)butyryl]indoline-2(S)-carboxylate (6v). To L-prolinol (2.0 g, 20 mmol) in 50 mL of methylene chloride was added 25 mL of 1 N aqueous NaOH. The reaction was cooled to 0 °C, and 4-phenylbutryl chloride (4.0 g, 22 mol) was added. The reaction was stirred for 4 h at 0 °C and then 1 h at room temperature. The reaction was diluted with 50 mL of methylene chloride, and the layers were separated. The organic layer was washed with 30 mL of H_2O, dried (Na_2SO_4), and rotary evaporated to yield 11 (4.4 g, 90%): $[\alpha]_D$ −40.3° (c 1, MeOH); IR (CH_2Cl_2) 3280, 1605 cm^{-1}.

The above amide 11 (4.4 g, 7.1 mmol) in 2 mL of THF was added dropwise to a solution of lithium diisopropylamide (15.6 mmol) in 50 mL of THF at 0 °C under N_2. After 30 min at 0 °C, (S)-(+)-3-(benzyloxy)-2-methylpropyl iodide (2.03 g, 7.0 mmol) was added dropwise in 2 mL of THF. The reaction was stirred for 5 h at 0 °C, 15 h at −15 °C, and then quenched at 0 °C with excess saturated aqueous NH_4Cl. The reaction mixture was diluted with 30 mL of ether, and the layers were separated. The organic phase was washed with 15 mL of 1 N HCl, 15 mL of brine, and 15 mL of saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and rotary evaporated to yield N-[(2R,4R)-5-(benzyloxy)-4-methyl-2-(2-phenylethyl)pentanoyl]-L-prolinol (16; 2.3 g, 80%): NMR ($CDCl_3$) δ 0.89 (3 H, d, J = 6 Hz), 4.45 (2 H, s), 7.20 (5 H, s), 7.30 (5 H, s); IR (CH_2Cl_2) 1606 cm^{-1}.

A solution of the above amide 16 (2.0 g, 4.9 mmol) in 50 mL of 1 N ethanolic HCl was refluxed 15 h and then rotary evaporated. The residue was purified by column chromatography using 60 g of silica gel eluting with 2:1 pentane–ether to yield ethyl (2R,4R)-5-(benzyloxy)-4-methyl-2-(2-phenylethyl)pentanonate (17; 0.65 g, 36%): $[\alpha]_D$ +2.85° (c 1, EtOH); NMR ($CDCl_3$) δ 0.92 (3 H, d, J = 6 Hz), 1.28 (3 H, t, J = 10 Hz), 1.40–1.90 (8 H, m), 3.32 (2 H, d, J = 5.5 Hz), 4.16 (2 H, q, J = 10 Hz), 4.50 (2 H, s), 7.22 (5 H, s), 7.33 (5 H, s); IR (neat) 1723 cm^{-1}.

A solution of 17 (0.60 g, 1.7 mmol) in 50 mL of EtOH was hydrogenated at 40 psi for 3 h at room temperature in the presence of 500 mg of 5% palladium on carbon. The catalyst was removed by filtration through Celite, and the filtrate was rotary evaporated to yield ethyl (2R,4R)-5-hydroxy-4-methyl-2-(2-phenylethyl)-pentanoate (0.41 g, 91%): NMR ($CDCl_3$) δ 0.91 (3 H, d, J = 6 Hz), 1.29 (3 H, t, J = 7 Hz), 1.40–2.9 (8 H, m), 3.30–3.80 (2 H, m), 4.19 (2 H, q, J = 7 Hz), 7.24 (5 H, s); IR (CH_2Cl_2) 3607, 1722 cm^{-1}.

To the above alcohol (0.35 g, 1.33 mmol) in 15 mL of DMF at room temperature was added pyridinium dichromate (2.5 g, 6.60 mmol). The reaction was stirred for 15 h at room temperature, poured into 150 mL of H_2O, and extracted with 4 × 40 mL of ether. The combined ether extracts were washed with 3 × 20 mL of a 1:1 solution of $NaHCO_3/K_2CO_3$ (pH 10.5). The basic aqueous portions were acidified to pH 2 with concentrated H_2SO_4 and extracted with 4 × 20 mL of ether. The organic portions were washed with 20 mL of brine, dried ($MgSO_4$), and rotary evaporated to give 18 (0.28 g, 76%): $[\alpha]_D$ −4.91° (c 1, EtOH); NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 7 Hz), 1.29 (3 H, t, J = 7 Hz), 1.6–2.2 (4 H, m), 2.2–2.9 (4 H, m), 4.18 (2 H, q, J = 7 Hz), 7.23 (5 H, s), 11.1 (1 H, s); IR ($CDCl_3$) 1720, 1706 cm^{-1}.

To 18 (0.91 g, 3.3 mmol) in 30 mL of methylene chloride was added oxalyl chloride (1.37 g, 10.8 mmol). The reaction was stirred for 2.5 h at room temperature and then rotary evaporated to yield (2R,4R)-4-(ethoxycarbonyl)-2-methyl-4-(2-phenylethyl)butyryl chloride (0.95 g, 97%), used directly in the following reaction.

To a solution of the above acid chloride (0.95 g, 3.3 mmol) in 40 mL of pyridine was added (S)-indoline-2-carboxylic acid hydrochloride (**5a**; 0.91 g, 5.0 mmol). The reaction was stirred for 3 h at room temperature and then rotary evaporated. The residue in 120 mL of methylene chloride was washed with 4 × 25 mL of 3 N HCl and 25 mL of brine, dried ($MgSO_4$), and rotary evaporated. The product was crystallized from ethyl acetate–pentane to yield **6v** (1.2 g, 85%): mp 96–98 °C; $[\alpha]_D$ −117.5° (c 0.88, $CHCl_3$); NMR ($CDCl_3$) δ 0.95 (3 H, t, J = 7 Hz), 1.05–1.40 (5 H, m), 1.50–2.75 (4 H, m), 2.95–4.15 (6 H, m), 5.15 (1 H, m), 7.20 (9 H, m), 10.70 (1 H, s); IR (Nujol) 3400–2710, 1725, 1715, 1655, 1592, 1480, 1175 cm^{-1}. Anal. ($C_{25}H_{29}NO_5$) C, H, N.

1-[(2R,4R)-4-Carboxy-2-methyl-4-(2-phenylethyl)-butyryl]indoline-2(S)-carboxylic Acid (6u). To a solution of **6v** (0.28 g, 0.66 mmol) in 3 mL of MeOH was added 2 mL of 1 N aqueous LiOH. The reaction was stirred for 6 h at 55 °C and then evaporated. The residue in 30 mL of H_2O was washed with 15 mL of ether, acidified to pH 2 with 1 N aqueous HCl, and extracted with 3 × 25 mL of methylene chloride. The combined methylene chloride portions were dried ($MgSO_4$) and rotary evaporated. The residue was crystallized from pentane to give **6u** (0.21 g, 80%): mp 137–139 °C; $[\alpha]_D$ −62° (c 0.25, $CHCl_3$); NMR ($CDCl_3$) δ 1.21 (3 H, d, J = 7 Hz), 1.55–2.2 (4 H, m), 2.65 (4 H, m), 3.45 (2 H, m), 4.98 (1 H, dd, J = 9 and 1 Hz), 7.17 (8 H, m), 8.25 (1 H, d, J = 8 Hz), 10.75 (2 H, s); IR (Nujol) 3400–2750, 1719, 1712, 1659, 1455, 1194 cm^{-1}. Anal. ($C_{23}H_{25}NO_5$) C, H, N.

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Registry No. 4 (R^1, R^2 = H; R^3 = *i*-Pr; R^4 = OH), 32806-63-6; 4 (R^2, R^3 = H; R^1 = *i*-Pr; R^4 = OEt), 82924-31-0; *meso*-4 (R^1, R^3 = CH_3; R^2 = H; R^4 = OEt), 3891-70-1; *dl*-4 (R^1, R^3 = CH_3; R^2, R^4 = H), 3891-69-8; (2*R*,4*R*)-4 (R^1, R^3 = CH_3; R^2 = H; R^4 = OH) (+)-α-methylbenzylamine salt, 86309-43-5; (2*R*,4*R*)-4 (R^1, R^3 = CH_3; R^2 = H; R^4 = OH), 24018-75-5; (2*R*,4*R*)-4 (R^1, R^3 = CH_3; R^2 = H; R^4 = OEt), 82924-08-1; **5a**·HCl, 82923-76-0; **5b**·HCl, 79854-42-5; **6a**, 82923-77-1; **6b**, 82923-90-8; **6c**, 82923-79-3; **6d**, 82924-01-4; **6e**, 82924-13-8; **6f**, 82924-06-9; **6g** (isomer 1), 86309-39-9; **6g** (isomer 2), 86309-42-4; **6h**, 82923-92-0; **6i**, 82924-33-2; **6j**, 82923-88-4; **6k**, 82923-87-3; **6l**, 82923-94-2; **6l** (base), 82923-93-1; **6m**, 82923-95-3; **6o**, 82950-75-2; **6p**, 82924-03-6; **6q**, 82950-74-1; **6r**, 82923-85-1; **6s**, 82924-29-6; **6t**, 82924-28-5; **6u**, 82950-76-3; **6v**, 82924-14-9; **6** (R^1 = *i*-Pr; R^2, R^3 = H; R^4, R^5 = Et) (isomer 1), 86309-40-2; **6** (R^1 = *i*-Pr; R^2, R^3 = H; R^4, R^5 = Et) (isomer 2), 86309-41-3; **7**, 82923-86-2; **11**, 82924-16-1; **15**, 72297-80-4; **16**, 86309-44-6; **17**, 82924-18-3; **18**, 82924-20-7; **19**, 82950-73-0; **21a**, 86390-74-1; **21a** (base), 86362-11-0; **21b**, 86390-75-2; **21b** (base), 86362-12-1; **21c**, 86390-76-3; **21c** (base), 86362-13-2; (2*R*,4*R*)-4-(ethoxycarbonyl)-2,4-dimethylbutyryl chloride, 82924-02-5; 4-phenylbutyryl chloride, 18496-54-3; ethyl (2*R*,4*R*)-5-hydroxy-4-methyl-2-(2-phenylethyl)pentanoate, 82924-19-4; (2*R*,4*R*)-4-(ethoxycarbonyl)-2-methyl-4-(2-phenylethyl)butyryl chloride, 82924-21-8; 3,3-dimethylglutaric anhydride, 4160-82-1; 2-isopropylglutaric anhydride, 57280-77-0; L-prolinol, 23356-96-9; angiotensin converting enzyme, 9015-82-1.

Aromatic Retinoic Acid Analogues. 2. Synthesis and Pharmacological Activity

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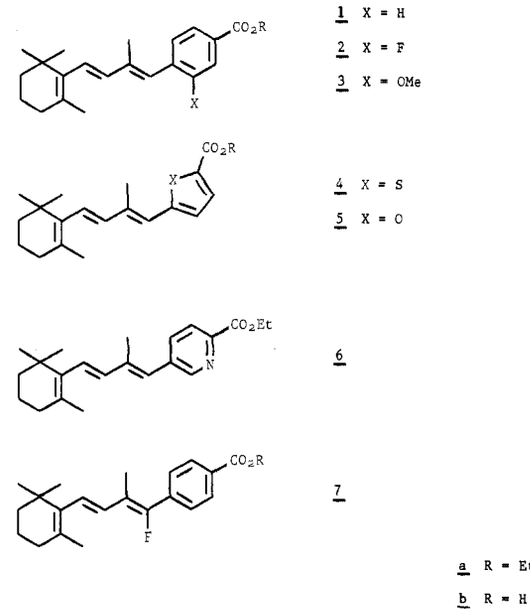
Aromatic analogues of (*E*)-1-(4-carboxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butadiene (**1b**) and its ethyl ester (**1a**) were synthesized as potential chemopreventive agents for the treatment of epithelial cancer and such skin diseases as psoriasis and cystic acne. The phenyl ring of **1** was replaced by 2-fluorophenyl, 2-methoxyphenyl, thienyl, furanyl, and pyridyl groups. The 1-fluorobutadiene analogue of **1** was also synthesized. With exception for the furanyl analogue, these compounds demonstrated good activity in reversing keratinization in hamster tracheal organ culture and in inhibiting the induction of ornithine decarboxylase in mouse epidermis by a tumor promoter.

Retinoids have pharmaceutical importance because of their potential value as chemopreventive agents in the treatment of epithelial cancer, psoriasis, and cystic acne.[1] We recently reported the synthesis of aryl triene **1b** and its ethyl ester **1a**.[2] These compounds displayed significant activity in two bioassays: (1) the reversal of keratinization in hamster tracheal organ culture (TOC assay) and (2) the inhibition of the induction of ornithine decarboxylase in mouse dorsal epidermis by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (ODC assay). Positive activity in these assays has been shown to correlate with the ability of a retinoid to reverse the process of neoplastic transformation in vivo.[3,4] We now report the synthesis and biological testing results for aromatic ring modified analogues of **1**.

Synthesis. The proton at the 2-position of the phenyl ring was replaced by either a fluoro or a methoxy group to determine the effect of an electron-withdrawing or -donating group, respectively, on biological activity (compounds **2** and **3**). The phenyl ring was replaced by a thienyl, furanyl, or pyridyl ring to determine the effects of ring polarity and aromaticity on activity (compounds **4–6**). In addition, the proton at the 10_R-position[5] of **1** (position 1 of the butadiene chain) was replaced by fluorine (compound **7**). This substitution was made because Pawson and co-workers reported that 10_R-fluoro analogues of the 4-methoxy-2,3,6-trimethylphenyl retinoids have enhanced activity.[6] The structures of analogues **2** to **7** are shown in Chart I.

Analogues **2** to **6** were readily prepared by the reaction sequences outlined in Scheme I. Because 9_R double-bond isomers in this type of series are unusually difficult to separate, the synthetic scheme was designed so that this bond was introduced stereospecifically by employing the

Chart I



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