Non-Steroidal Glucocorticoid-like Substances: Receptor Binding and in Vivo Activity

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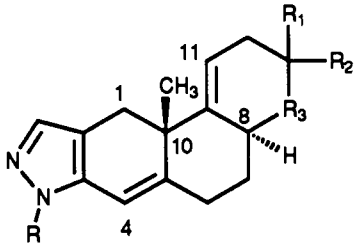
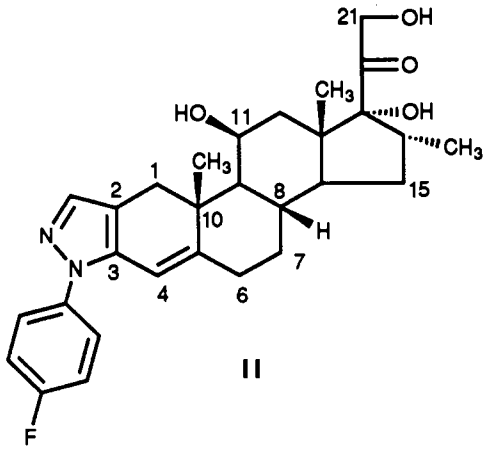
Compounds of general structure **I**, prepared by a Diels–Alder reaction with diene **3**, are relatives of the known potent glucocorticoid **II** but possess a markedly modified C- and D-ring environment. Despite these structural changes, **4**, **5**, **9**, **10**, **12a**, **13**, and **14** bound to the glucocorticoid receptor with an affinity which approximated that of the reference standard, 6-α-methylprednisolone. Four of these compounds not only exhibited antiinflammatory activity in the α-tocopherol pouch test but also exhibited marked adrenal suppression and other typical glucocorticoid properties at doses in the same range as the effective antiinflammatory doses.

The antiinflammatory steroids are effective and often life-saving drugs but still suffer from a constellation of serious side effects, the diminution of any one of which would result in an important therapeutic advance. Most of the early work in corticosteroid research was restricted to modifications of each position of the steroidal skeleton. These efforts resulted in the discovery of a number of activity-enhancing and activity-modifying substituents,[1] as illustrated by the attachment of [3,2-*c*]-2′-aryl pyrazole derivatives to the A-ring of the corticosteroid. This resulted in compounds which were ~2000 times more potent than hydrocrotisone in the rat.[2,3] The 4-fluorophenylpyrazole **II** structural modification has emerged as the most powerful activity-enhancing group with respect to antiinflammatory activity[3] from a large number of chemical modifications of the glucocorticoids.



I



II

In a program aimed at finding novel compounds while retaining a major portion of the steroid nucleus for glucocorticoid-like activity, we have synthesized compounds represented by the general structure **I**. The advantage of these hybrid structures **I** is the possible dissociation of antiinflammatory activity and the associated side effects when compared to other compounds containing the intact steroidal skeleton. Identification of such an antiinflammatory steroid or non-steroidal compound which did not depress adrenal function would represent a significant structure–activity relationship breakthrough.

We were interested in developing a Diels–Alder approach from a readily available steroid synthon **1**[4] which would yield the compounds represented by the general structure **I**. This would also allow us to synthesize steroid relatives in which the C- and D-ring environment could be varied widely. To increase the likelihood of achieving activity similar to that of traditional steroidal glucocorticoids, we have also incorporated the activity-enhancing group, 4-fluorophenyl pyrazole.

It should be noted that earlier efforts to prepare non-steroidal glucocorticoid-like compounds resulted in either inactive compounds[5-9] or substances with weak activity.[10-14] The topical activity (antiinflammatory and glucocorticoid) of the present compounds has been reported earlier.[15]

Chemistry. The target compounds were synthesized by the route shown in Schemes I–III. The key step was the Diels–Alder condensation with both hetero- and carbodienophiles. The N-substituted pyrimidines **12–18** were prepared by alkylation of **11**. Assignment of regiochemistry of the indantrione (ninhydrin) **4** and **5** and pyrimidinetetrone (alloxan) **6**, **7**, and **11** adducts is based on their ^{1}H NMR spectra which exhibited one allylic proton α to oxygen. The corresponding regioisomer would possess two such protons. It was not possible to determine the regiochemistry of the adducts **8–10** by this method. The assigned regiochemistry of adducts **8–10** is in accord with theoretical considerations and precedent,[1] that the C-8 hydrogen atom and the C-10 methyl group[16] were *trans*.

It was possible to isolate the *cis* isomer **12b** as a minor product from the Diels–Alder reaction which has been catalyzed by anhydrous $SnCl_4$ (**12a** was the major product). The structural assignments of **12a** and **12b** were made on

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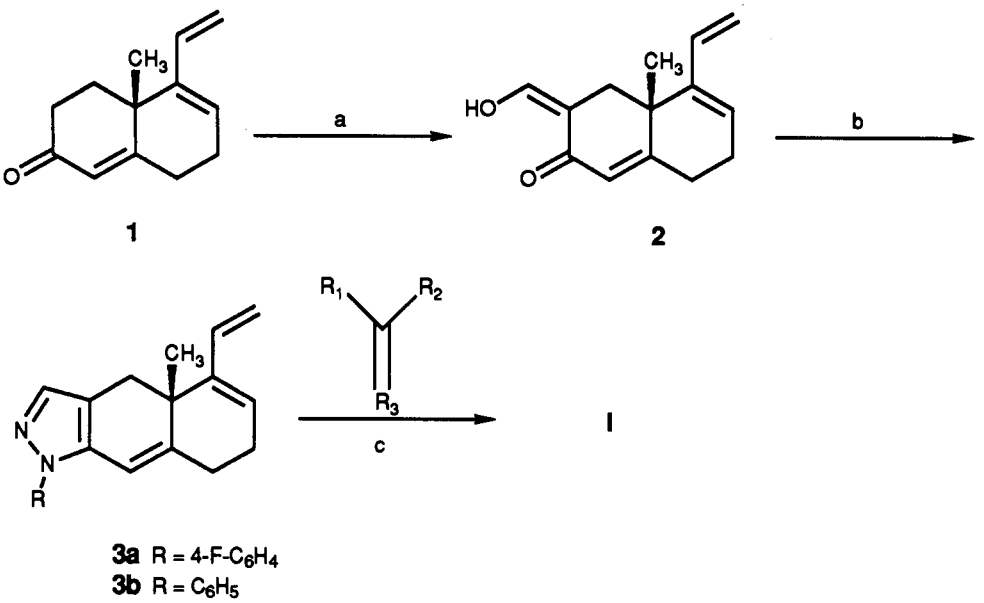
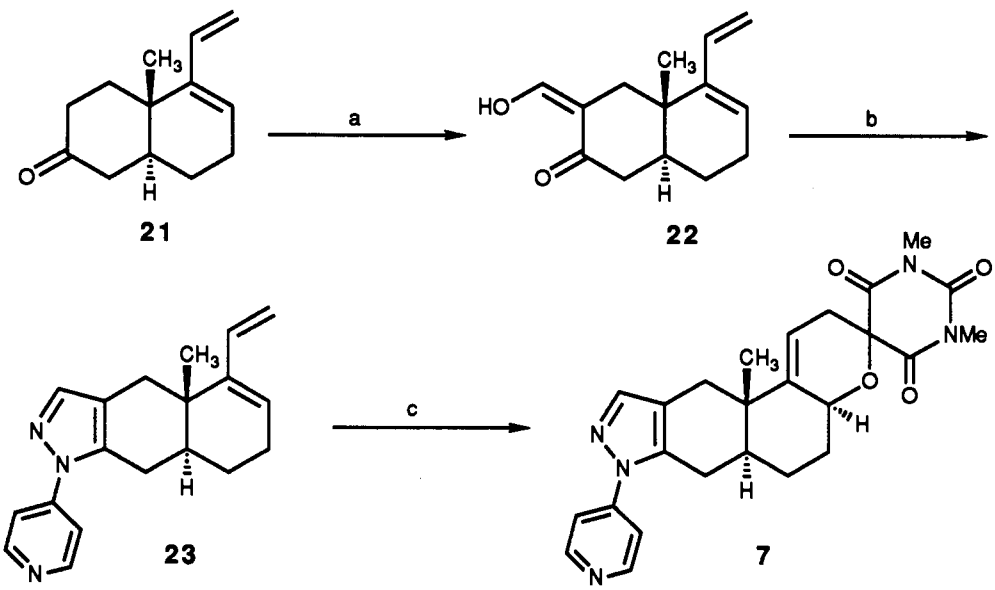
Scheme I[a]



3a R = 4-F-C_6H_4
3b R = C_6H_5

[a] Reagents: (a) HCO_2Me, NaOMe, THF; (b) HOAc, $RNHNH_2$; (c) Δ, xylenes.

Scheme II[a]



[a] Reagents: (a) HCO_2Me, NaOMe, Et_2O; (b) HOAc, KOAc, 4-pyridylNHNH$_2$; (c) *N,N*-dimethylalloxan, Δ, xylenes.

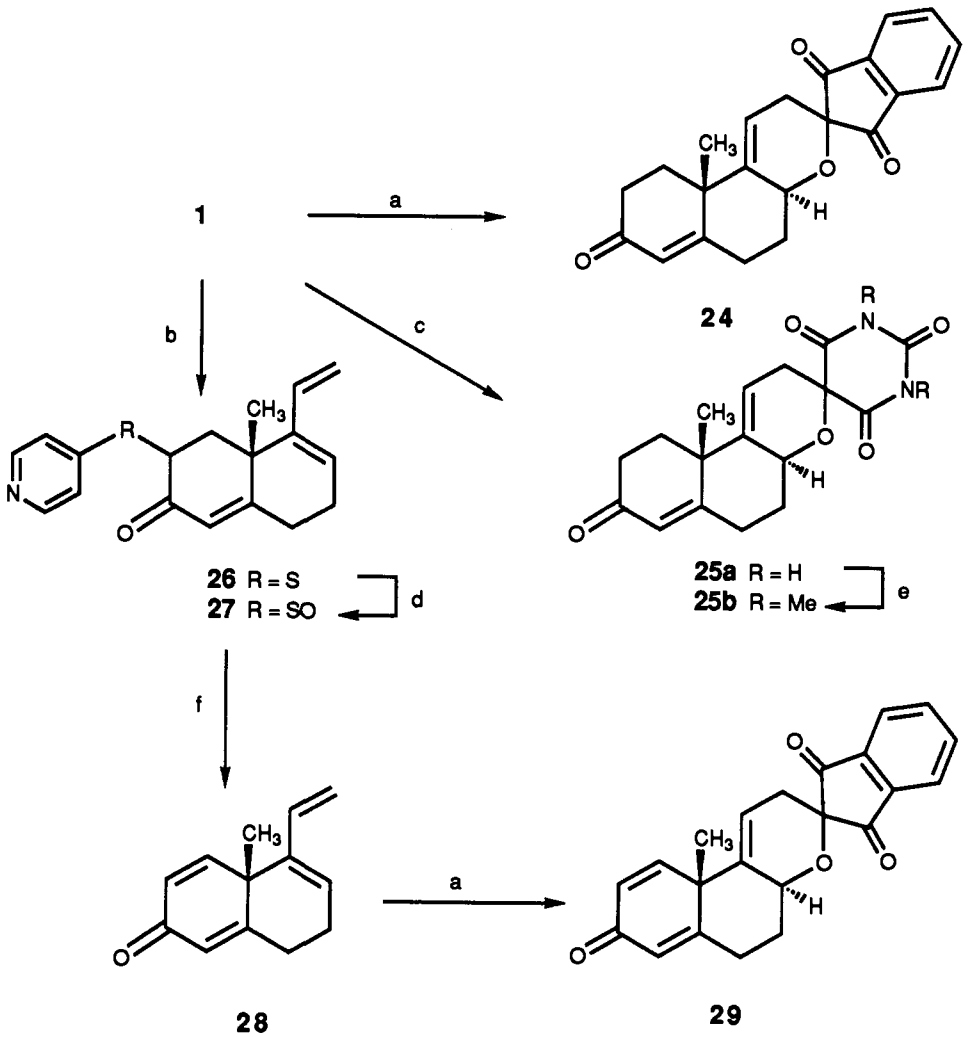
the basis of ^1H NMR and NOE experiments. Irradiation of C-10 methyl group resulted in 10–15% enhancement of C-11 proton[16] signal intensity for both the compounds. However, no enhancement of C-8 proton signal was observed for **12a**. In contrast **12b** showed an 11% enhancement of C-8 proton thus providing the stereochemical assignment of the above two and related compounds.

The 4,5-dihydro-5-α derivative[16] **7** was prepared as shown in Scheme II from the diene **21**. The enones **24** and **25**, the dienone **29**, and for comparison the $\Delta^{9,11}$ steroidal pyrazole **32** were prepared as outlined in Schemes III and IV. The physical properties of the compounds **I** are summarized in Table I.

Biological Results and Discussion. The results of the biological evaluation of the compounds **I** are presented in Table II. Binding of a compound to the glucocorticoid receptor constitutes the primary event in the action of a glucocorticoid agonist. Depression of thymus weight, depression of adrenal weight, depression of body weight gain without a change in food consumption, and antiinflammatory activity constitute significant aspects of the in vivo profile of a glucocorticoid in the rat. Depression of adrenal weight is an indirect measure of depression of pituitary function, and depression of body weight gain is an indirect measure of catabolic activity. Thymus involution is a direct consequence of glucocorticoid action on the thymus gland.

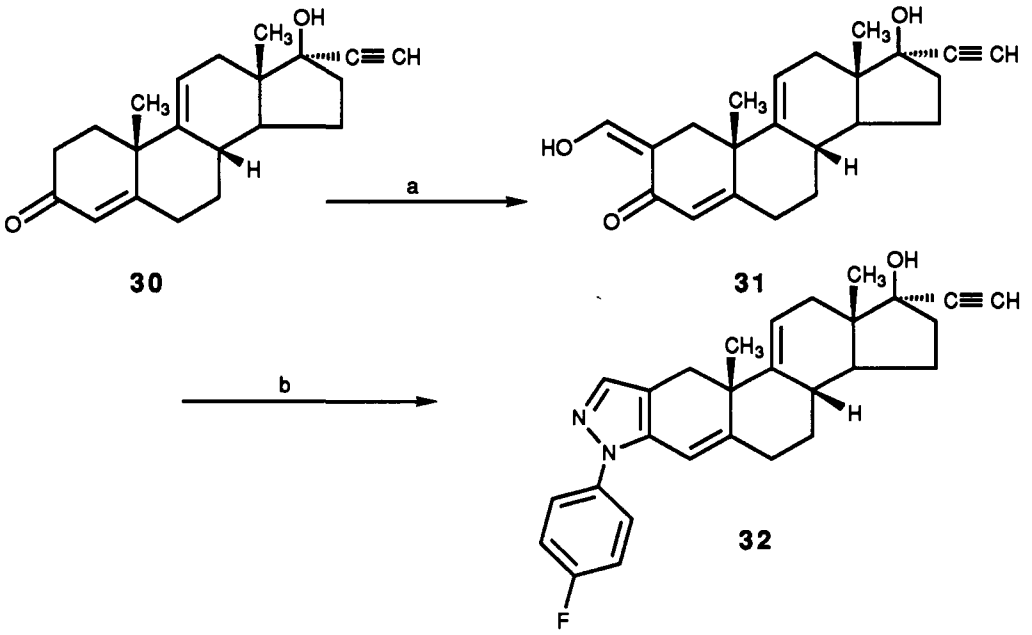
Most of the compounds presented in Table I exhibited significant affinity to the glucocorticoid receptor. Compounds **4**, **5**, **9**, **10**, and **12a** bound with approximately the same affinity as the synthetic radioligand, dexamethasone. The pyridine derivative **8** bound more strongly than the ligand. The derivative **6** in which the fluorophenyl ring is replaced by a pyridine had much reduced affinity. The dihydro derivative **7** had no detectable affinity for the receptor. Any departures from the N-Me group of **12a** among those variations examined resulted in a precipitous

Scheme III[a]



[a] Reagents: (a) ninhydrin, toluene; (b) LDA, 4,4′-dipyridyl disulfide, THF; (c) alloxan, Δ, xylenes; (d) mCPBA, $CHCl_3$; (e) NaH, DMF, MeI; (f) Δ, toluene.

Scheme IV[a]



[a] Reagents: (a) HCO_2Me, NaOMe, MeOH; (b) 4-F-$C_6H_5NHNH_2$·HCl, NaOAc, HOAc, H_2O.

decrease in binding to the receptor as did the change to the cis isomer **12b**. Had the series been limited to an examination of the pyrimidinetetrone (alloxan) and indantrione (ninhydrin) adducts a coherent receptor binding

Table I. Physical Properties of Compounds (I)

compd	R	R1	R2	R3	yield (%)	mp, °C	solvent	formula
4	4-F-C_6H_4			O	54	210–211	EtOH	$C_{28}H_{23}FN_2O_3$
5	C_6H_5			O	48	209–210	Et_2O	$C_{28}H_{24}N_2O_3$
6	4-pyridyl			O	43	213–215	Et_2O/EtOAc	$C_{25}H_{25}N_5O_4$ 1/3 H_2O
7	4-pyridyl (4,5-dihydro; 5α)			O	13	195–198	EtOAc/CH_2Cl_2	$C_{25}H_{27}N_5O_4$
8	4-F-C_6H_4	H	H	CH-pyridyl	30	154–156	Et_2O	$C_{27}H_{26}FN_3$
9	4-F-C_6H_4	H	Ac	CHAc	16	157–159	CH_2Cl_2/isooctane	$C_{28}H_{27}FN_2O_2$
10	4-F-C_6H_4	H	H	$C(CO_2Et)_2$	27	133–135	MeOH	$C_{28}H_{31}FN_2O_4$
11	4-F-C_6H_4			O	55	235–236	EtOH	$C_{24}H_{21}FN_4O_4$
12a	4-F-C_6H_4			O	42	188–189	Et_2O	$C_{28}H_{25}FN_4O_4$
12b	4-F-C_6H_4			O	1	166–168	i-PrOH	$C_{28}H_{25}FN_4O_4$
13	4-F-C_6H_4			O	48	171–172	CH_2Cl_2–heptane	$C_{28}H_{23}FN_4O_4$
14	4-F-C_6H_4			O	32	81–82	hexane–EtOAc	$C_{30}H_{33}FN_4O_4$
15	4-F-C_6H_4			O	40	95–96	hexane–EtOAc	$C_{30}H_{33}FN_4O_4$
16	4-F-C_6H_4			O	37	70–72	Et_2O	$C_{30}H_{26}FN_4O_4$
17	4-F-C_6H_4			O	33	109–111	$CHCl_3$	$C_{30}H_{25}FN_4O_4$
18	4-F-C_6H_4			O	44	145–146	Et_2O	$C_{30}H_{29}FN_4O_8$
19	C_6H_5			O	26	253–254	Me_2CO	$C_{24}H_{22}N_4O_4$

model could probably have been developed. However, inclusion in the group of the high affinity and structurally divergent group of adducts **8–10** apparently undermines that endeavor.

A Δ^4-3-ketone or $\Delta^{1,4}$-3-ketone are structural features present in all the potent nonheterocyclic steroidal glucocorticoids. These functional groups are present in the compounds **24, 25a, 25b,** and **29** which lack the arylpyrazole moiety. None of them exhibited receptor binding. That a double bond at C-9.C-11 is compatible with binding to the receptor in this 3-keto series is demonstrated by the significant affinity for the receptor of compound **32**.

The indantrione adduct **4** exhibited a profound effect on all four parameters at low doses. In comparison with 6-α-methylprednisolone **20**, adduct **4** was about 5 times less potent as an antiinflammatory agent but considerably more potent as an adrenal suppressant. In a side by side assay, **4** was 17 (7.0–39.3)[17] times as potent as **20** as a suppressant of adrenal weight and 2 (0.7–4.1) times as potent as a thymolytic agent as **20**. Compound **4** is apparently also significantly more catabolic than **20**. Removal of the fluorine atom in **4** resulted in compound **5** which is markedly less potent than **4**. The corresponding alteration of a steroidal pyrazole also results in a sharp decrease in potency.[18]

The activity exhibited by compounds **9–12** was not notable with respect to potency but did serve to illustrate how widely the structure of the dienophile could be varied

Table II. Receptor Affinity, Glucocorticoid Profile, and Antiinflammatory Activity of I

compd	RBA[a] (%) for the glucocorticoid receptor	mg/kg (po)	glucocorticoid profile (rat) % change from control[b] thymus	adrenals	body wt gain	antiinflammatory activity (rat) α-tocopherol pouch ED_{50}, mg/kg (po)
4	72.5 ± 9.3	1	−61	−56	−129	10
		5	−79	−64	−224	
5	77.1 ± 8.5	5	−51	−38	−62	inactive
		25	−62	−50	−86	
6	0.72 ± 0.10[c]		NT[d]			NT
7	0[c]		NT			NT
8	180 ± 49.1	100	−64	−48	−88	inactive @ 100
9	118 ± 14.0	5	−59	−40	−80	23
10	82 ± 9.0	5	0	0	0	inactive
		100	−80	−47	−186	@ 100
11	18.7 ± 3.1	1	0	0	−65	4
		5	−72	−30	−140	
		25	−84	−53	−180	
12a	118 ± 5.3	0.2	−28	−25	−55	2
		1	−71	−57	−127	
		5	−83	−63	−236	
12b	4 ± 1.5		NT			NT
13	118 ± 1.3	25	−34	0	0	inactive
		100	−34	−45	−75	@ 25
14	118 ± 3.5	50	0	0	0	inactive @ 25
15	2.6 ± 1.3	50	0	0	0	inactive @ 25
16	2.9 ± 1.1	50	−34	−37	−58	inactive @ 48
17	1.8 ± 0.1	5	0	0	0	41% inhibition @ 29
18	3.2 ± 1.5	25	0	0	0	inactive @ 70
19	9.3 ± 3.5	50	−34	0	0	inactive
		100	0	0	0	@ 50
32		1	−41	−35	0	NT
(Scheme IV)		5	−76	−60	−145	
24 (Scheme III)	0.04 ± 0.01[c]		NT			
25a (Scheme III)	0.02 ± 0.001[c]		NT			
25b (Scheme III)	0.03 ± 0.03[c]		NT			
29 (Scheme III)	0.02 ± 0.006[c]		NT			
hydrocortisone	9.6 ± 1.8		NT			18
20	99 ± 14	1	−40	0	−45	2
(6α-methylprednisolone)		5	−70	−42	−79	

[a] RBA is defined as relative binding affinity: concentration of dexamethasone @ 50% binding inhibition + concentration of competitor @ 50% binding inhibition at the rat thymus glucocorticoid receptor. The RBA of dexamethasone was set at 100. See ref 15 for the experimental procedure. [b] See ref 15 for the experimental details. [c] These compounds were evaluated by incubation with glucocorticoid receptor derived from liver cytosol from adrenalectomized rats. [^{3}H]RU28362 was used as the ligand. Dexamethasone has an RBA = 57 ± 2% in this assay. A "zero" means that binding was too low to measure. [d] NT means not tested.

and yet afford an adduct which possessed, at least qualitatively, some of the properties of a glucocorticoid.

With respect to antiinflammatory activity the most potent compounds were the pyrimidinetetrone adduct **11** and its *N,N*-dimethyl derivative **12a**. Other closely related N,N-disubstituted derivatives **13–19** were inactive or at best weakly active. The doses of **11** and **12** which caused significant adrenal suppression in the two-week glucocorticoid profile test were comparable to those which resulted in significant activity in the 5-day antiinflammatory test. Furthermore, when antiinflammatory activity and adrenal suppressant activity were determined in the same test following Steelman's procedure,[19] significant adrenal suppression was also observed at doses which caused an antiinflammatory response. It has not been possible, therefore, to separate adrenal suppressant and antiinflammatory activity with these compounds.

In conclusion, we have demonstrated that it is not necessary to retain an intact steroid nucleus in order to retain significant affinity for the glucocorticoid receptor. Indeed, major departures from the steroid structures are permitted in both the C- and D-ring regions. In vivo, in the rat, administration of the compounds caused a significant antiinflammatory response and also resulted in adrenal suppression, thymolysis, and a catabolic effect.

Experimental Section

^{1}H NMR spectra were recorded on a Varian Model HA-100 spectrometer with Me_4Si as external standard. Chemical shifts are expressed in δ units. Coupling constants (*J*) are expressed in Hertz (Hz). MS determinations were carried out using a JEOLCO JMS-0ISC Model instrument. Preparative liquid chromatography was performed on a Waters Prep LC 500 instrument using two Prep PAK columns. Analyses are indicated by symbols of the elements and are within ±0.4% of the theoretical values. Mp's are uncorrected.

5-Ethenyl-3-(hydroxymethylene)-4,4a,7,8-tetrahydro-4a-methyl-2(3*H*)-naphthalenone (2). A solution of 5-ethenyl-4,4a,7,8-tetrahydro-4a-methyl-2(3*H*)-naphthalenone (1) (50.0 g, 0.265 mol) in 350 mL of THF was cooled to −5 °C in an ice–MeOH bath and stirred under N_2 atmosphere while 57.2 g (1.06 mol) of NaOMe was added. The resulting mixture was stirred for 30 min at −5 °C, and then a solution of methyl formate (114 mL, 1.85 mol) in 100 mL of THF was added slowly. The mixture was stirred overnight at room temperature and then poured onto a mixture of ice–H_2O (1500 mL) and 6 N HCl (265 mL). The product was extracted with Et_2O, and the combined extracts were washed with H_2O. The dried ($MgSO_4$) extract was concentrated in vacuo to afford an oil. This oil was triturated with hexane (4 × 250 mL), and the combined triturates were dried ($MgSO_4$) and concentrated in vacuo to afford 55.37 g of a red oil (2): ^1H NMR ($CDCl_3$) δ 13.58 (broad s, 1H), 7.54 (s, 1H, =CHCO), 6.30 (dd, 1H, J = 9.0 Hz, CH=CH_2), 6.04–5.91 (m, 2H, COCH= and C=CHCH$_2$), 5.40 (dd, 1H, J = 2.0 Hz, CH=CH_2), 5.09 (dd, 1H, J = 1.5 Hz, CH=CH_2), 2.6–2.2 (m, 8H), 1.23 (s, 3H, CH_3).

1-Ethenyl-6-(4-fluorophenyl)-3,4,9,9a-tetrahydro-9a-methyl-6*H*-naphtho[2,3-*c*]pyrazole (3a). 4-Fluorophenylhydrazine hydrochloride (45.85 g, 0.282 mol) and NaOAc (23.14 g, 0.282 mol) were added to a solution of crude 2 (55.37 g, 0.256 mol) in 225 mL of glacial HOAc. The mixture was stirred overnight at room temperature and then concentrated in vacuo to afford a semisolid. This material was suspended in Et_2O (1 L) and filtered to remove NaCl. The Et_2O filtrate was washed with H_2O (4 × 250 mL), saturated $NaHCO_3$ (until weakly basic), and saturated NaCl (100 mL). The dried ($MgSO_4$) and charcoaled extract was concentrated in vacuo to afford an oil. This oil was triturated with Et_2O–hexane (1:2) (3 × 750 mL) to afford 69.6 g of dark brown oil. An analytical sample was prepared by using high-performance liquid chromatography with 1:3 Et_2O–hexane as solvent. The resulting yellow oil was triturated with pentane to afford yellow solid (3a), mp 70–72 °C, with a ^1H NMR spectrum consistent with the assigned structure. Anal. ($C_{20}H_{19}FN_2$) C, H, N.

Compound 3b was prepared in a similar manner and characterized through its ^1H NMR spectra.

8′-(4-Fluorophenyl)-2′,3′,5′,6′,11′,11a′-hexahydro-11a′-methylspiro[2*H*-indene[2,3′]-3*H*-pyrazolo[4″,5″:7′,6′]naphtho[2,1-*b*]pyran]-1,3-dione (4). A mixture of 3a (15.3 g, 0.05 mol) and 1,2,3-indantrione monohydrate (9.8 g, 0.055 mol) in 150 mL of xylene was refluxed for 2 h. The cooled reaction mixture was filtered through silica gel and concentrated in vacuo. The residue was crystallized from EtOH to afford 7.19 g of tan solid (4): ^1H NMR ($CDCl_3$) δ 7.38–7.85 (m, 9H, aromatic), 6.34 (s, 1H, =CH), 5.86 (m, 1H, =CH), 4.98 (m, 1H, CH—O), 1.86–3.28 (m, 8H), 1.30 (s, 3H, CH_3). Anal. ($C_{29}H_{23}FN_2O_3$) C, H, N.

4a′,5′,6′,8′,11′,11a′-Hexahydro-11a′-methyl-8-phenylspiro-[2*H*-indene-[2,3′]-3*H*-pyrazolo[4″,5″:7′,6′]naphtho[2,1-*b*]pyran]-1,3-dione (5). A mixture of 3b (14.42 g, 0.05 mol) and 1,2,3-indantrione monohydrate (9.88 g, 0.06 mol) in 150 mL of xylene was refluxed for 2 h, then allowed to stand at room temperature for 55 h, and filtered to afford a green solid. This material was dissolved in CH_2Cl_2, filtered through silica gel, and solvent removed in vacuo. The residue was triturated with Et_2O to afford a yellow solid (5), 11.0 g: ^1H NMR ($CDCl_3$) δ 7.2–8.2 (m, 9H, aromatic), 6.34 (s, 1H, =CH), 5.86 (m, 1H, =CH), 4.98 (m, 1H, CH—O), 1.70–3.40 (m, 8H), 1.30 (s, 3H, CH_3). Anal. ($C_{29}H_{24}FN_2O_3$) C, H, N.

Compound 6 was prepared from 4-hydrazinopyridine hydrochloride as described for 3a and 7.

(4a*α*,6a*α*,11a*β*)-4a,5,6,6a,7,8,11,11a-Octahydro-1′,3′,11a-trimethyl-8-(4-pyrrolinyl)spiro[[1]benzopyrano[5,6-*f*]indazole-3(2*H*),5′(2′*H*)-pyrimidine]-2′,4′,6′(1′*H*,3′*H*)-trione (7). To a cold (0 °C) suspension of NaOEt [prepared from 60% NaH (5.3 g, 0.131 mol) and absolute EtOH (0.74 mL)] in 125 mL of anhydrous Et_2O under a N_2 atmosphere was added solid 21[20] (25 g, 0.131 mol). After stirring for 15 min ethyl formate (16.12 mL, 0.199 mol) was added dropwise over a period of 1 h. The reaction mixture was stirred for 72 h at room temperature and quenched with 16 mL of H_2O. The product was extracted with Et_2O (3 × 200 mL) after acidification with 6 N HCl. The ether layer was washed with H_2O, followed by saturated NaCl solution, and dried over $MgSO_4$. Removal of solvent gave 22 as a brown oil, 21.5 g (75%).

A mixture of 22 (4.0 g, 0.018 mol), 4-hydrazinopyridine hydrochloride (2.6 g, 0.018 mol), and KOAc (1.8 g, 0.018 mol) in 100 mL of AcOH was stirred at room temperature for 16 h and then heated on a steam bath for 4 h. The solvent was removed under reduced pressure, and the product was extracted with EtOAc, washed with H_2O, and dried over $MgSO_4$. Removal of solvent gave a crude brown oil which was purified on a silica gel column with CH_2Cl_2 to give 23 as a light yellow oil, 5.0 g (96%).

A solution of 23 (5.0 g, 0.0172 mol) and dimethylalloxan[21] (4.85 g, 0.0258 mol) in 50 mL of dry toluene was heated on a steam bath under a N_2 atmosphere for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2, washed with water, dried over $MgSO_4$ and evaporated to dryness to give a brown oil. The product was filtered through a Florisil column, eluting with (1) CH_2Cl_2, (2) EtOAc, and then recrystallized from EtOAc to give 1 g of a tan solid (7): MS (m/e) 461 (M^+), 446 (M^+ – Me); IR (KBr) 1690, 1680, 1585 cm^{-1}; ^1H NMR δ 1.10 (s, 3H), 3.20 (s, 3H), 3.25 (s, 3H), 4.66 (m, 1H), 5.80 (m, 1H), 7.50 (s, 1H), 7.65 (d, J = 5.0 Hz, 2H), 8.70 (d, J = 4.8 Hz, 2H). Anal. ($C_{25}H_{27}N_5O_4$) C, H, N.

8-(4-Fluorophenyl)-2,3,4,4a,5,6,11,11a-octahydro-11a-methyl-4-(2-pyridinyl)-8*H*-phenanthro[2,3-*c*]pyrazole (8). A solution of 3a (18.91 g, 0.062 mol), 2-vinylpyridine (7.33 mL, 0.078 mol), and 1,2,3-benzenetriol (500 mg) in 200 mL of xylene was refluxed for 60 h. The cooled reaction mixture was extracted with 2 N HCl (4 × 100 mL). The combined HCl extracts were filtered, made basic with 5 N NaOH, and extracted thoroughly with CH_2Cl_2 (4 × 200 mL). The dried (Na_2SO_4) and charcoaled extract was concentrated in vacuo to afford a brown oil. The oil was triturated with Et_2O to give a beige solid, 7.8 g, as a mixture of isomers (8), mp 154–156 °C, as determined by ^1H NMR ($CDCl_3$) δ 7.00–8.50 (m, 9H, aromatic), 6.15 (d, 1H, =CH), 5.86 (m, 1H, =CH), 1.50–3.60 (m, 11H), 1.28, 1.24 (2s, 3H, CH_3). Anal. ($C_{27}H_{26}FN_3$) C, H, N.

1,1′-[8-(4-Fluorophenyl)-2,3,4,4a,5,6,11,11b-octahydro-11a-methyl-8*H*-phenanthro[2,3-*c*]pyrazole-3,4-diyl]bis[ethanone] (9). A solution of 3a (20 g, 0.065 mol) in benzene (200 mL) and 3-hexene-2,5-dione[22] (8.07 g, 0.072 mol) was stirred at reflux for 40 h under a N_2 atmosphere. The cooled reaction mixture was filtered through silica gel and concentrated in vacuo. The resultant oil was triturated with Et_2O to afford 7.5 g of a gold colored solid as a mixture of isomers, mp 138–142 °C, as determined by ^1H NMR. A 5-g sample of this mixture of isomers was separated using high-performance liquid chromatography with EtOAc–hexane (1:4). The major isomer was recrystallized from CH_2Cl_2–isooctane (1:1) to afford 3.0 g of a white solid (9), a single isomer as determined by ^1H NMR ($CDCl_3$) δ 7.05–7.53 (m, 5H, aromatic), 6.20 (s, 1H, =CH), 5.80 (m, 1H, =CH), 2.00–2.96 (m, 11H), 2.32 (2s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 1.24 (s, 3H, CH_3); MS (m/e) 418 (M^+), 403 ($M^+ - CH_3$), 375 ($M^+ - COCH_3$). Anal. ($C_{28}H_{27}FN_2O_2$) C, H, N.

Diethyl 8-(4-Fluorophenyl)-2,3,4,4a,5,6,11,11a-octahydro-11a-methyl-8*H*-phenanthro[1,2-*d*]pyrazole-4,4-dicarboxylate (10). A solution of 3a (25.8 g, 0.084 mol), diethyl methylenemalonate (18.1 g, 0.105 mol), and 1,2,3-benzenetriol (500 mg) in 200 mL of xylene was refluxed for 20 h. The cooled reaction mixture was filtered through silica gel and concentrated in vacuo to afford 41.9 g of a brown oil. The oil was purified by using high-performance liquid chromatography with EtOAc–CH_2Cl_2 (3:97) followed by recrystallization from MeOH to afford a white solid, 11.0 g (10): ^1H NMR ($CDCl_3$) δ 7.00–7.60 (m, 5H, aromatic), 6.20 (s, 1H, =CH), 5.70 (m, 1H, =CH), 4.20 (m, 4H, CH_2CH_3), 1.40–3.60 (m, 11H), 1.22 (m, 9H, 3CH_3). Anal. ($C_{26}H_{31}FN_2O_4$) C, H, N.

8-(4-Fluorophenyl)-2,3,5,6,11,11a-hexahydro-11a-methylspiro[3*H*-pyrazolo[4″,5″:7′,6′]naphtho[2,1-*b*]pyran-3,5′(2′*H*)-pyrimidine]-2′,4′,6′(1′*H*,3′*H*)-trione (11). A mixture of 3a (15.3 g, 0.05 mol) and 2,4,5,6(1*H*,3*H*)-pyrimidinetetrone (8.0 g, 0.05 mol) in 150 mL of toluene was refluxed in an apparatus fitted with a Dean-Stark trap for 24 h to remove H_2O. The mixture was cooled to room temperature and filtered to afford 21.55 g of a green solid. The green solid was recrystallized from EtOH to give 12.33 g of a grey solid (11): ^1H NMR ($CDCl_3$) δ

11.17, 11.03 (s, 2H, NH), 7.08–7.54 (m, 5H, aromatic), 6.26 (s, 1H, =CH), 5.78 (m, 1H, =CH), 4.9 (m, 1H, CH—O), 3.10–1.88 (m, 8H), 1.24 (s, 3H, CH_3). Anal. ($C_{24}H_{21}FN_4O_4$) C, H, N.

Compound **19** was prepared in a similar manner using **3b**.

8-(4-Fluorophenyl)-4a,5,6a,8,11,11a-hexahydro-11a,1′,3′-trimethylspiro[[1]-benzopyrano[5,6-*f*]indazole-3(2*H*),5′(2′*H*)-pyrimidine]-2′,4′,6′(1′*H*,3′*H*)-trione (12a). To a suspension of NaH (1.58 g, 0.066 mol) (previously washed with pentane and dried under a stream of N_2) in 200 mL of DMF at 0 °C under N_2 atmosphere was added slowly **11** (13.4 g, 0.03 mol) in DMF (130 mL). The reaction mixture was stirred at room temperature for 2 h under a N_2 atmosphere. Then MeI (4.11 mL, 0.066 mol) was added slowly, and the reaction was stirred at room temperature overnight. The reaction was poured onto H_2O (800 mL) to afford a solid which was isolated by filtration, washed with H_2O, and air dried to afford 9.36 g of a solid. This solid was dissolved in Et_2O–$CHCl_3$ and filtered through silica gel, and the solvent was removed in vacuo to afford a foam. The foam was crystallized from Et_2O to give an off-white solid (**12a**), as a mixture of isomers: ^1H NMR ($CDCl_3$) δ 7.70–7.10 (m, 5H, aromatic), 6.32 (s, 1H, =CH), 5.82 (m, 1H, =CH), 4.75 (m, 1H, CH—O), 3.80 (s, 3H, CH_3–N), 3.22 (s, 3H, CH_3–N), 3.10–1.40 (m, 8H), 1.25, 1.15 (s, 3H, $2CH_3$, 7:1 peak area ratio); MS (*m/e*) 476 (M^+), 461 ($M^+ - CH_3$), 306 ($M^+ - C_6H_6N_2O_4$). Anal. ($C_{26}H_{25}FN_4O_4$) C, H, N.

Compounds **13–18** were prepared in a similar manner using the appropriate alkylating agent.

(4aβ-11aβ)-8-(4-Fluorophenyl)-4a,5,6,8,11,11a-hexahydro-1′,3′,11a-trimethylspiro[1]-benzopyrano[5,6-*f*]indazole-3(2*H*),5′(2′*H*)-pyrimidine]-2′,4′,6′(1′*H*,3′*H*)-trione (12b). To a solution of **3a** (17.98 g, 0.058 mol) and 1,3-dimethyl-2,4,5,6-(1*H*,3*H*)pyrimidinetetrone[21] (14.0 g, 0.082 mol) in CH_2Cl_2 (360 mL) at −70 °C under a N_2 atmosphere was added dropwise anhydrous $SnCl_4$ (23.06 g, 0.09 mol). The reaction mixture was stirred for 4 h at −70 °C and then was raised to 0 °C. After stirring for 4 h at 0 °C, the reaction mixture was quenched by slow addition of water. The CH_2Cl_2 layer was separated, washed with H_2O and saturated NaCl solution, and dried ($MgSO_4$), and the solvent was removed under reduced pressure to give a syrup (23.5 g). The mixture was separated by a preparative HPLC (hexane/EtOAc, 3:1) into four major fractions. The first fraction (5.88 g) was mostly diene **3a**. The second fraction (6.3 g, 23%: mp 185–187 °C) was pure trans isomer (**12a**). The third fraction (8.0 g) consisted mostly of **12a** along with cis isomer as a minor product. The fourth fraction (1.2 g) consisted largely of the cis isomer (**12b**). Final purification was accomplished by use of preparative TLC: IR (KBr) 1690 (NCO) and 1515 cm^{-1} (C=C); MS (CI) *m/e* 477 (MH^+); ^1H NMR ($CDCl_3$) δ 7.40–7.10 (m, 4H, aromatic), 6.15 (s, 1H, =CH), 5.60 (s, 1H, =CH), 4.10 (m, 1H, O—CH), 3.35 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 3.00–1.20 (m, 8H), 1.15 (s, 3H, CH_3). Anal. ($C_{26}H_{25}FN_4O_4$) C, H, N.

2′,4a′,5′,6′,8′,9′,10′,10a′-Octahydro-10a′-methylspiro[2*H*-indene[2,3′]-3*H*-naphtho[2,1-*b*]]pyran-1,3,8′-trione (24). A suspension of **1** (7.52 g, 0.04 mol) and 1,2,3-indantrione hydrate (7.12 g, 0.04 mol) in 80 mL of toluene was refluxed with stirring for 2 h. Partial removal of solvent afforded a yellow solid which was collected by filtration and recrystallization from EtOH to give the product as light yellow solid (**24**), 10.18 g (73%): mp 186–188 °C; ^1H NMR ($CDCl_3$) δ 7.98 (bs, 4H), 6.00 (bs, 1H), 5.70 (bs, 1H), 4.55 (m, 1H), 1.60–3.00 (m, 9H), 1.46 (s, 3H). Anal. ($C_{22}H_{20}O_4$) C, H.

2,4a,5,6,10a-Hexahydro-10a-methylspiro[[3*H*-naphtho-[2,1-*b*]pyran-3,5′(2′A)-pyrimidine]-2′,4′,6′,8(3′*H*,1′*H*,9*H*)-tetraone (25a). A suspension of **1** (7.52 g, 0.04 mol) and 2,4,5,6-(1*H*,3*H*)-pyrimidinetetrone (6.40 g, 0.04 mol) in 80 mL of toluene was refluxed for 20 h. The reaction mixture was cooled, and the resulting solid was collected by filtration and recrystallization from EtOH to give a white solid (**25a**), 7.13 g (54%): mp 227–228 °C; MS (*m/e*) 330 (M^+), 315 (M^+ – Me), 312 ($M^+ - H_2O$); ^1H NMR (DMSO-d_6) δ 5.88 (bs, 1H), 4.50 (m, 1H), 1.45 (s, 3H). Anal. ($C_{17}H_{18}N_2O_5$) C, H, N.

2,5,6,9,10,10a-Hexahydro-1′,3′,10a-trimethylspiro[[3*H*-naphtho[2,1-*b*]pyran-3,5′(2′*H*)-pyrimidine]-2′,4′,6′,8(1′*H*,3′*H*,4a*H*)-tetraone (25b). To a solution of **25a** (16.0 g, 0.048 mol) in 200 mL of DMF under a N_2 atmosphere was added 60% NaH (2.0 g, 0.05 mol). The reaction mixture was stirred at room temperature for 30 min, MeI (45.6 g, 0.32 mol) was added, and stirring continued for 24 h. After removal of solvent under reduced pressure the residue was partitioned between CH_2Cl_2 and H_2O, and the organic layer was separated, washed with 10% $NaHSO_3$, and H_2O, and dried over anhydrous $MgSO_4$. Removal of solvent under reduced pressure gave a gum which was purified on a Florisil column from CH_2Cl_2/Et_2O (9:1) to give a solid which was recrystallized from CH_2Cl_2/Et_2O (1:1) to give a tan solid (**25b**), 8.5 g (49%): mp 138–140 °C; IR (KBr) 1755, 1658, 1625 cm^{-1} (CO); MS (*m/e*) 358 (M^+), 343 ($M^+ - CH_3$); ^1H NMR ($CDCl_3$) δ 5.86 (bs, 1 H), 5.76 (bs, 1H), 4.58 (m, 1H), 3.36 (s, 3H), 3.28 (s, 3H), 1.60–3.10 (m, 10H), 1.47 (s, 3H). Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

5′,6′,8′,9′,10′,10a′-Hexahydro-10a′-methylspiro[2*H*-indene-2,3′[3*H*]-naphtho[2,1-*b*]]pyran-1,3,8′(6a′*H*)-trione (29). Following a literature procedure,[23] an LDA solution [prepared from *n*-BuLi (43.4 mL, 0.106 mol, 2.5 M in hexanes) and isopropylamine (15.8 mL, 0.113 mol) in 100 mL of anhydrous THF] at −78 °C, under a N_2 atmosphere, was added dropwise **1** (20 g, 0.106 mol) in 50 mL of anhydrous THF over 20 min. This mixture was stirred at −78 °C for another 30 min and a solution of 2,2′-dipyridyldisulfide (23.4 g, 0.106 mol) in 100 mL of THF added at −78 °C. The resulting solution was stirred at −78 °C for 2 h and then warmed to 0–5 °C. After stirring for 36 h, the reaction mixture was poured onto 100 mL of 10% NH_4Cl in ice/Et_2O. The Et_2O extracts (3 × 600 mL) were combined and washed with 6 N HCl (3 × 200 mL). The acidic solution was cooled in an ice-bath and neutralized with 35% NaOH to pH 10 and then begin acidified with H_3PO_4 to pH 6. The product was extracted with ether (3 × 600 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The product was purified on HPLC (solvent/85% hexane/15% EtOAc) to give a pale yellow solid which was recrystallized from cyclohexane to give **26** as pale yellow solid, 9.9 g (35%); mp 73–75 °C: MS (*m/e*) 297 (M^+). Anal. ($C_{16}H_{19}$-NOS) C, H, N.

To a cold (0–5 °C) stirred solution of **26** (1.5 g, 0.005 mol) in 25 mL of $CHCl_3$ was added a solution of 85% mCPBA (1.02 g, 0.005 mol) in 15 mL of $CHCl_3$ over a period of 20 min. After stirring the reaction mixture for 30 min at room temperature, it was quenched with 10 mL of 10% $NaHSO_3$. The $CHCl_3$ layer was separated and washed with 10% $NaHCO_3$, dried ($MgSO_4$), and evaporated to give **27**, 1.4 g (100%) which was used directly in the next reaction.

A solution of **27** (1 g, 0.0032 mol) in 50 mL of toluene was refluxed for 2 h under a N_2 atmosphere. The solvent was removed under reduced pressure to give an oil which was chromatographed on a silica gel column using ether/hexane (1:1). The product was redissolved in ether, washed with 2 N HCl (2 × 50 mL) and water, and dried over $MgSO_4$. Removal of solvent gave the tetraenone **28** as a pale yellow oil, 0.56 g (94%): ^1H NMR ($CDCl_3$) δ 7.10–7.20 (d, 1H), 6.10–6.60 (m, 2H), 5.85 (m, 1H), 5.05–5.50 (m, 2H), 2.20–2.90 (m, 4H), 1.45 (s, 3H).

To a solution of **28** (11.0 g, 0.06 mol) in 100 mL of toluene was added ninhydrin hydrate (10.7 g, 0.06 mol). The reaction mixture was refluxed for 10 min using a Dean Stark trap and cooled to room temperature. The solution was filtered through Solka Floc and concentrated to give a green foam. The foam was dissolved in 120 mL of EtOAc and cooled to 0 °C to give **29** as an off-white solid, 10.1 g (49%): mp 187–188 °C; MS (*m/e*) 346 (M^+); IR (KBr) 1750, 1720, 1660 cm^{-1}; ^1H NMR ($CDCl_3$), δ 7.90 (m, 4H), 7.10 (d, J = 5.0 Hz, 1H), 6.30 (m, 1H), 6.20 (bs, 1H), 6.00 (m, 1H), 4.50 (m, 1H), 2.00–3.00 (m, 6H), 1.50 (s, 3H). Anal. ($C_{22}H_{18}O_4$) C, H.

(17α)-2′-(4-Fluorophenyl)-2′*H*-pregna-2,4,9(11)-trien-20-yno[3,2-*c*]pyrazol-17-ol (32). To a solution of **30**[24] (20.75 g, 0.067 mol) in 473 mL of THF under N_2 atmosphere was added NaOMe (10.8 g, 0.20 mol) in one portion. The reaction mixture was stirred for 10 min. Methyl formate (20 mL, 0.32 mol) was added dropwise, and the resulting mixture was stirred at room temperature for 24 h. After addition of 100 mL of H_2O, solvent was removed under reduced pressure. The residue was triturated with 800 mL of H_2O, and the resulting solid was filtered, washed thoroughly with H_2O, and dried to give **31** (20.4 g, 90%); mp 175–180 °C: MS (*m/e*) 338 (M^+), 373 ($M^+ - CH_3$); ^1H NMR ($CDCl_3$) δ 7.50 (bs, 1H), 5.82 (bs, 1H), 5.63 (m, 1H), 2.70 (s, 1H), 1.24 (s, 3H), 0.84 (s, 3H).

To a suspension of 4-fluorophenylhydrazine hydrochloride (9.76 g, 0.06 mol) in 160 mL of HOAc was added a solution of $NaOAc \cdot 3H_2O$ (8.30 g, 0.06 mol) in 20 mL of water. After stirring for 15 min at room temperature 31 was added (20 g, 0.059 mol), and the solution was heated on a steam bath for 2 h. The reaction mixture was poured into excess of ice–H_2O, and the product was extracted with CH_2Cl_2 (3 × 200 mL). The CH_2Cl_2 layer was washed with H_2O, saturated $NaHCO_3$, and dried ($MgSO_4$). Removal of solvent gave crude 32 which was purified on a silica gel column from CH_2Cl_2/Et_2O (1:1) and recrystallized from MeOH to give 32 as an off-white solid (10.44 g, 41%): mp 125 °C (indef.); MS (*m/e*) 428 (M^+), 413 ($M^+ - CH_3$); IR (KBr) 3300, 1630, 1518 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.00–7.60 (m, 5H), 6.16 (bs, 1H), 5.65 (m, 1H), 2.60 (s, 1H), 1.20 (s, 3H), 0.90 (s, 3H). Anal. ($C_{25}H_{29}FN_2O$) C, H, N.

Antiinflammatory Activity.[25] Male rats which weighed 120 g were selected for testing. A rapid sc injection of 25 mL of air is made between the scapulae of each rat. This results in the establishment of an air filled pouch into which 0.5 mL of DL-α-tocopherol is injected. Test compounds are administered in daily oral doses for 7 days beginning on the day of pouch formation. Drugs to be tested were suspended in 1% gum tragacanth. Twenty-four hours after the last medication, the pouches are dissected free, and the fluid volume is measured.

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(25) All research involving animals described in this publication was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division (SWPRD) Policy on Animal Use and all national and federal legislation. All SWPRD animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).