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Antiinflammatory phospholipase-A_2 inhibitors. I

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Summary — A novel series of dehydroabietylamine derivatives has been synthesized and shown to be inhibitors of phospholipase-A_2 (PLA_2). These compounds exhibit *in vivo* antiinflammatory activity in the rat carrageenan paw edema assay and support the concept of PLA_2 inhibition as an approach to the discovery of novel antiinflammatories.

Résumé — **Inhibiteurs de la phospholipase-A_2 à activité anti-inflammatoire.** *On a réalisé la synthèse d'une nouvelle série de dérivés de déhydroabiétylamine et mis en évidence leurs propriétés inhibitrices de la phospholipase-A_2 (PLA_2). Ces composés manifestent une action anti-inflammatoire* in vivo *dans l'essai de l'œdème à la carragéénine de la patte du rat et corroborent le rôle de l'inhibition de la PLA_2 dans la méthodologie de découverte de nouveaux agents anti-inflammatoires.*

dehydroabietylamine / phospholipase-A_2 / inhibition / anti-inflammatory activity / SAR, amino-, alcohols, amides, oximes and ketones / diamines

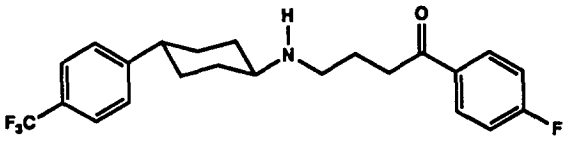
Introduction

Phospholipase-A_2 (PLA_2) is a hydrolase responsible for the release of arachidonic acid from the *sn*2 position of phospholipids. The released arachidonic acid is then converted to mediators of inflammation, such as the prostaglandins and leukotrienes, by the enzymes prostaglandin synthetase (cyclooxygenase) and 5-lipoxygenase, respectively. The inhibition of PLA_2 should lead to a decrease in the release of arachidonic acid and, consequently, the inflammatory mediators.

Attempts to discover PLA_2 inhibitors with anti-inflammatory activity have been made by many research groups with varying degrees of success [1, 2]. We would like to report the synthesis and biological profile of 4 derivatives of dehydroabietylamine that resulted from our efforts to discover anti-inflammatory PLA_2 inhibitors.

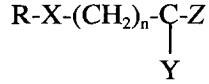
Chemistry

Based on our study of the X-ray crystal structure of porcine pancreatic phospholipase-A_2 (PAN-PLA_2) [3] and the report by Wallach *et al* [4, 5] on compounds like U-3538, we initially proposed that a PLA_2 inhibitor should possess: 1) a large and/or lipophilic group to bind to that portion of the enzyme occupied by the alkenyl portion of arachidonate; and 2) a group to coordinate with calcium in the active site. U-3538 met these conditions by having a carbonyl function capable of coordinating with calcium in the active site and the lipophilic 4-trifluoromethylphenyl-4-cyclohexyl group. However, we felt that an alcohol or an amine would better coordinate, or might even displace calcium from the active site of the enzyme [6–8].



U-3538

Based on the structural components of U-3585, we proposed a more expansive template for the PLA_2 inhibitor:

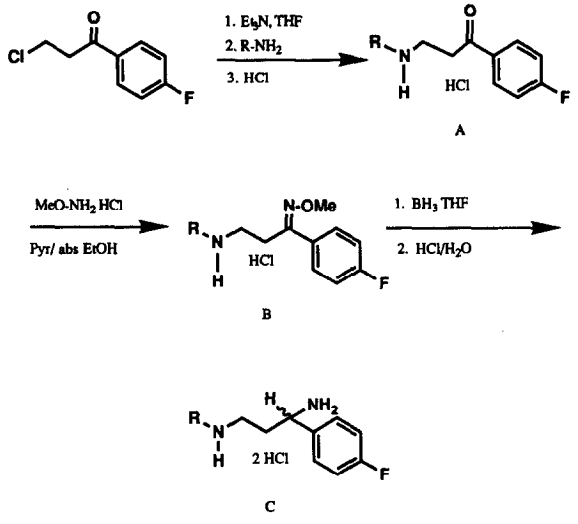


where R is large and/or lipophilic; X is NH, S, O, or CH_2; n is 1 to 3; Y is O=, OH, or NH_2; and Z is aromatic, substituted aromatic, or lower alkyl.

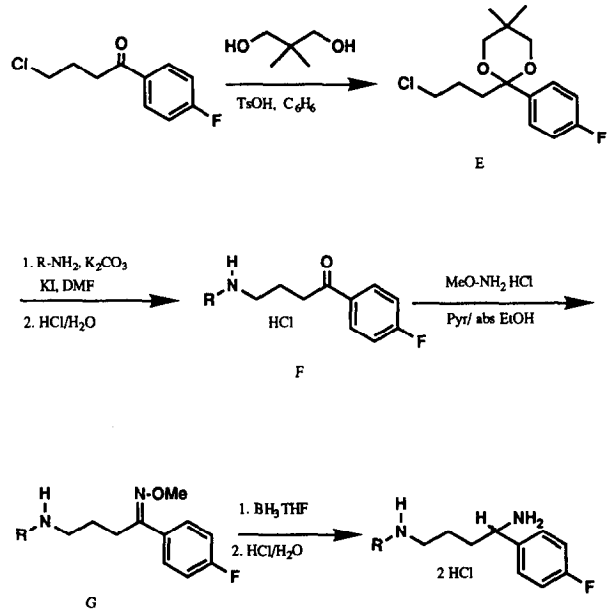
Synthetic efforts resulted in the synthesis of 600+ amino-amides, -ketones, alcohols, -oximes, and diamines which revealed the following general SAR for PLA_2 inhibition: for Y, NH_2 > OH > MeO-N= > O=; for n, 2 > 3 > 1; for X, NH > O > S > CH_2=N-CH_3 > N-CO; for Z, 4-F-Phe > Phe > 4-MeO-Phe > 4-MeS-Phe; and R is large and or lipophilic (Wilkerson, in preparation) [9]. The general routes to the synthesis of these compounds are shown in schemes 1 to 4.

The choice of dehydroabietylamine for R was made after investigating amines as small as *n*-butylamine and as large and/or lipophilic as cholestaneamine. Of these many structural types, we found the dehydroabietylamine derivatives to be more consistently active in the *in vivo* models of inflammation.

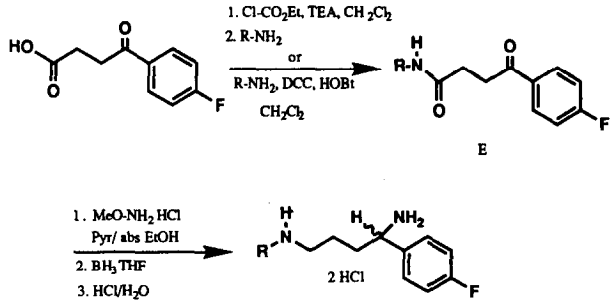
Specifically, when *n* was 2, the dehydroabietylamine was alkylated with 3-chloro-4-fluoropropiophenone to give the corresponding amino-ketone, which could be directly reduced to the amino-alcohol, or converted to the amino-oxime which was reduced to the diamine. This approach was found to be unsatisfactory when the alkylating agent was 4-chloro-4-fluorobutyrophenone. The relative inertness of the 4-chloro group required the addition of sodium iodide, strong basic conditions, and prolonged heating to give the desired amino-ketone. The synthesis of the 4-amino-ketone was accomplished in good yields by coupling 4-fluorobenzoyl propionic acid to dehydroabietylamine using dicyclohexyl carbodiimide, the mixed anhydride of ethyl chloroformate, or the acid chloride. The corresponding 4-amino-ketone was



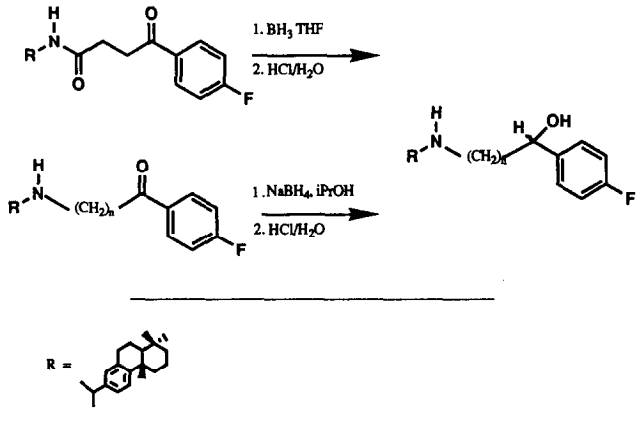
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

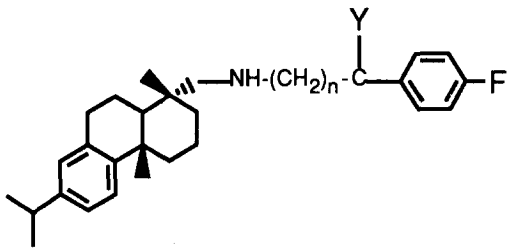
reduced with refluxing borane-tetrahydrofuran (BH_3-THF) to give the 4-amino-alcohol. The 4-amino-ketone was reacted with methoxyamine to give the corresponding amino-oxime which was reduced to the diamine by refluxing BH_3-THF. The physical data for the dehydroabietylamine derivatives are shown in table I.

The synthetic efforts, aimed at the improvement of the activity of U-3538 through structural modifications, were guided by the aim to optimize the enzyme inhibitory activity followed by the optimization of antiinflammatory activity as demonstrated by the carrageenan paw edema (CARR PAW) and tetradecanoyl phorbol acetate induced ear edema assay (TPA).

Biological investigation and results

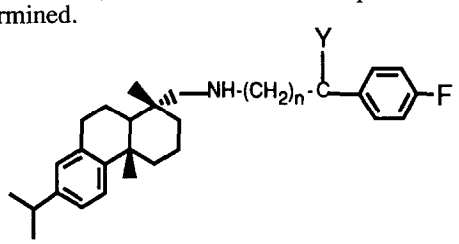
These compounds were tested *in vitro* for activity against the enzymes associated with the arachidonic acid cascade; an extracellular and intracellular source of PLA_2: porcine pancreatic phospholipase-A_2 (PAN-PLA_2) [10] and rat polymorphonuclear phospholipase-A_2 (PMN-PLA_2) [11] respectively; prostaglandin synthetase from the bovine seminal vesicle (BSV-PGS) [12]; and 5-lipoxygenase from the rat basophilic leukemia cell (RBL-5LO) [13–15]. Our *in vivo* assays were conducted in the rat carrageenan paw edema (CARR PAW) [16], mouse contact sensitivity assay (CONT SENS) [17], and the zymosan peritonitis assay (ZYM PERIT) [18]. We used the 12-*O*-tetra-

Table I. Physical data for derivatives **1a–d** and **2a–d**.



Compd	*n*	*Y*	*Yield (%)*	*mp (°C)*	*°[α]$^{25}_D$ (c, 0.6, MeOH)*	*Formula*
1a	2	O=	58	158-160	19.4	$C_{29}H_{38}NOF$ HCl
1b	2	HO	64	194-202	26.9	$C_{29}H_{40}NOF$ HCl
1c	2	MeO-N=	88	185-187	21.3	$C_{30}H_{41}N_2OF$ HCl
1d	2	NH_2 HCl	91	218-223	10.3	$C_{29}H_{41}N_2F$ 2HCl
2a	3	O=	70	166-167	20.3	$C_{30}H_{40}NOF$ TsOH
2b	3	HO	76	195-198	15.8	$C_{30}H_{42}NOF$ HCl
2c	3	MeO-N=	97	oil	19.9	$C_{31}H_{43}N_2OF$
2d	3	NH_2 HCl	82	165-168	4.6	$C_{30}H_{43}N_2F$ 2HCl 1.5H_2O

Table II. Biological profile of the derivatives **1b**, **1d**, **2b**, and **2d**. Compounds were tested as mixtures of diastereomers. Separate experiments of ten. nd = not determined.



cmpd	*1b*	*2b*	*1d*	*2d*
n	*2*	*3*	*2*	*3*
Y	*CH*	*CH*	*NH_2HCl*	*NH_2HCl*
	Mean (+/– SE), n = 3			
PAN-PLA_2 IC_{50}, μM	4.0 (1.1)	9.0 (1.5)	1.4 (0.1)	1.8 (0.6)
PMN-PLA_2 IC_{50}, μM	25.0 (2.3)	11.4 (2.3)	5.6 (0.5)	1.9 (0.7)
BSV-PGS IC_{50}, μM	220.0 (14.4)	335.0 (23.3)	73.0 (2.6)	53.0 (3.8)
RBL-5LO IC_{50}, μM	5.2 (0.2)	14.7 (2.6)	4.7 (0.2)	18.9 (2.5)
CARR PAW PO, ED_{30} mg/kg	2.8 (0.7)	3.9 (1.8)	8.0 (1.5)	4.8 (1.2)
CONT. SENS. PO, Day 5&6, ED_{35} mg/kg	2.0 (0.3)	35.0 (3.6)	7.0 (0.8)	16.0 (1.9)
ZYM. PERIT. PO, % Contrl @ 50 mg/kg	57.0	55.0 (2.0)	93.0 (2.5)	83.0 (4.9)
PO, ED_{50}, mg/kg	nd	20.0 (1.3)	nd	nd
TPA EAR, Mouse Top., ED_{50} μg/ear	34.0 (5.5)	44.0 (5.3)	26.5 (4.7)	100.0 (4.1)
Top., %Inh @ 100 ug/ear	46.0 (5.0)	54.3 (2.0)	89.0 (3.8)	50.0 (3.0)
AA EAR, Mouse Top., % Inh @ 100 μg/ear	9.0	19.0	13.0	8.0
Thymus Involution PO, % @ 100 mg/kg	8.0 (5.0)	9.0 (6.8)	9.0 (3.0)	5.0 (3.0)
GI Lesion, Rat TD_{50}, mg/kg	>300.0	>300.0	>300.0	>300.0

decanoylphorbol-13-acetate induced inflammation (TPA EAR) [19] and the mouse arachidonic acid ear edema (AA-EAR) [20] assays to measure the topical antiinflammatory activity of these compounds. Because we were concerned about a PLA_2 inhibitor mimicking the steroids and causing thymic involution [21], we included an assay to study this effect. The biological profiles for derivatives **1b**, **1d**, **2b**, and **2d**, and the standards are shown in tables II and III, respectively.

To insure that the *in vivo* activities demonstrated in the CARR PAW and TPA EAR assays were not due to endogenous steroid formation caused by stress, we had serum levels of corticosterone measured on rats dosed with **2b** and used in the CARR PAW assay [8]. No significant difference was found between test and control animals, and all animals had levels within the normal range of 5–70 μg/d* (see table IV).

The data in table V show the relationship between the Y-moiety and the biological activity. These data

Table III. Biological profile of standards. nd = not determined. IND: indomethacin. DEX: dexamethasone. MEP: mepacrine.

Cmpd	*IND*[c]	*DEX*[d]	*MEP*[e]
	Mean (+/– SE)		
PAN-PLA_2 IC_{50} +/- s.e, μM	315.0 (26.0)	>250.0	22.0 (2.0)
PMN-PLA_2 IC_{50}, μM	250.0 (5.8)	>750.0	100.0 (3.3)
BSV-PGS IC_{50}, μM	1.8 (0.3)	nd	36.0 (3.0)
RBL-5LO IC_{50}, μM	>250.0	120.0 (8.6)	130.0 (11.2)
CARR PAW PO, ED_{30} mg/kg	2.6 (0.8)	0.02 (0.005)	5.8 (0.7)
CONT. SENS. PO, Day 5&6, ED_{35} mg/kg	4.5 (4.2)	0.7 (0.1)	23.9 (2.6)
ZYM. PERIT. PO, % Contrl @ 50 mg/kg	95.0 (5.0)	61.0 (3.0)	73.0 (6.0)
PO, ED_{50}, mg/kg	>100.0	3.0 (0.23)	25.7 (1.5)
TPA EAR, Mouse Top., ED_{50} μg/ear	nd	nd	>100.0
Thymus Involution PO, % @ dose, mg/kg	O @ 10.0	40 @ 3.9	14 @ 100.0
GI Lesion, Rat TD_{50}, mg/kg	10.1 (1.4)	>75.0	>300.0

*Met Path Inc, Kensington, MD, USA.

Table IV. Steroid induction study for **2b** and standards. Male Sprague–Dawley rats were used (6 animals per group).

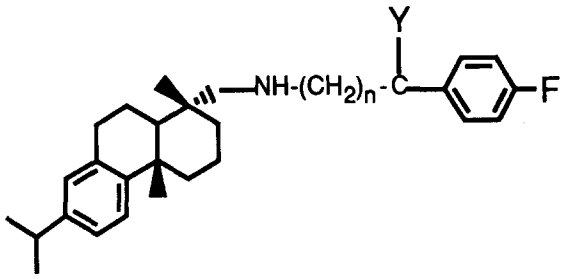
Test group	*Serum levels corticosterone (μg/d)*
Methocel controls	37.0 ± 6
Carrageenan controls	36.5 ± 7
Drug **2b**, 20.0 mg/kg, *po*	39.0 ± 4
IND @ 0.9 mg/kg, *po*	33.0 ± 5
DEX @ 1.0 mg/kg, *ps*	< 2.5
MEP @ 80.0 mg/kg, *po*	35.0 ± 4

illustrate that the amines or alcohols are better PLA_2 inhibitors than ketones or oxime ethers. A similar relationship exists for the *in vivo* antiinflammatory activity.

Discussion

Compounds **1b**, **1d**, **2b**, and **2d** have been found to be good inhibitors of porcine pancreatic PLA_2 and an enriched preparation of rat PMN PLA_2, and relatively inactive against the BSV–PGS. However, they inhibit RBL 5-LO, causing some problem in defining the mechanism of their antiinflammatory activity. The compounds are active in the mouse TPA ear assay and inactive in the mouse AA ear test, suggesting that the observed effect is not due to the inhibition of 5-lipoxygenase or cyclooxygenase.

Table V. Selected biological activity as a function of n and Y. R for U-3585 = 4-trifluoromethylphenyl-4-cyclohexyl.



cmpd	*n*	*Y*	*PAN-PLA₂* *IC_{50}, μM*	*PMN-PLA₂* *IC_{50}, μM*	*CARR PAW* *ED_{30} mg/kg*	*TPA EAR* *ED_{50}, μg/ear*
1a	2	O=	27.0	270.0	>20.0	>100.0
1b	2	OH	4.0	25.0	2.8	34.0
1c	2	MeO-N=	>25.0	310.0	>100.0	>100.0
1d	2	NH_2 HCl	1.4	5.6	8.0	26.5
2a	3	O=	32.0	>750.0	9.0	71.0
2b	3	OH	9.0	11.4	3.9	44.0
2c	3	MeO-N=	100.0	>750.0	>100.0	>100.0
2d	3	NH_2 HCl	1.8	1.9	4.8	100.0
U-3585	3	O=	2.3	>250.0	>20.0	>100.0

Attempts to measure the ability of these compounds to inhibit the release of arachidonic acid and its products from rat PMNs was complicated by the cytotoxicity of the compounds. A similar observation made for the production of platelet-activating factor (PAF) by U937 cells [22].

Only the alcohols **1b** and **2b** showed activity in the zymosan peritonitis assay, where oral treatment with drug, at 50 mg/kg, showed that the pleural fluid volume was only 57 and 55% of that found in the untreated zymosan controls (100%), respectively. The amines **1d** and **2d** were considered inactive because the pleural fluid volumes were 93 and 85% of control, respectively. The differences in activity of the alcohols and amines is not presently explainable.

The compounds are systemically active as anti-inflammatories, as demonstrated in the mouse contact sensitivity model and the rat CARR PAW edema assay. The results from the CARR PAW assay would suggest that the compounds are not antiinflammatory because of anti-histamine or serotonin activity [16]. The lack of thymic involution and induction of steroids indicates that the compounds are not mimicking steroids, and the antiinflammatory activity is not the result of stress induced formation of steroids. The gastro-intestinal safety would imply a non-cyclooxygenase antiinflammatory mechanism. These data together would suggest that **1b**, **1d**, **2b**, and **2d** are PLA_2 inhibitors with antiinflammatory activity.

Conclusion

These compounds represent a novel class of anti-inflammatory agents, and we attribute their antiinflammatory activity to their ability to inhibit PLA_2. Based on the biochemical and antiinflammatory profiles discussed above, we have further concluded that the compound **2b** was the best antiinflammatory PLA_2 inhibitor.

Experimental protocols

Chemical synthesis

Melting points were determined with a Thomas–Hoover melting point apparatus and are uncorrected. The NMR spectra were recorded with an IBM/Bruker WPS 200 spectrometer, IR spectra were recorded with a Perkin–Elmer 1600 FTIR spectrophotometer, optical rotations were determined on a Perkin Elmer PE 241 polarimeter, and mass spectra were performed with a Hewlett Packard HP5988A GC-MS system. Thin layer chromatography (TLC) was performed on silica gel plates.

*3-((1,4A-Dimethyl)-7-(1-methylethyl)-1,2,3,4,4A,9,10,10A-octahydro-1-phenanthrenyl)methylamino)-1-(4-fluorophenyl)-1-propanone hydrochloride **1a***

A solution of 3-chloro-4'-fluoropropiophenone (18.7 g, 100 mmol) in 100 ml Et_2O was treated with triethylamine (10.1 g, 100 mmol) and stirred for 3 h to form the corresponding enone. The mixture was filtered, and the filtrate was treated with dehydroabietylamine (28.5 g, 100 mmol) and stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*, and the residue was triturated with 200 ml 1N HCl. The aqueous phase was decanted, and the gummy solid was twice washed with water and triturated with 200 ml Et_2O. The resulting solid was collected by filtration, washed with Et_2O and dried to give the product in 58% yield (27.3 g); mp = 158–160°C; IR (nujol): C=O ν 1679 cm^{-1}; NMR ($CDCl_3$ TMS): δ 0.6–2.1 (m, 21H), 2.33 (d, 1H, Ar'CH), 2.88 (m, 2H, $Ar'CH_2$), 3.00 (t, 2H, CH_2CO), 3.43 (m, 2H, NCH_2), 3.80 (t, 2H, NCH_2), [6.88 (s, 1H), 6.97 (d, 1H), 7.17 (d, 1H) Ar'], [7.40 (d of d, 2H), 8.07 (m, 2H) 4-F-Phe], 9.10, 9.25 (d, 2H, H_2N+Cl^-). Anal calcd for $C_{29}H_{38}NOF$ HCl, MW 472.07: C, 73.78; H, 8.33; N, 2.97. Found: C, 73.70; H, 8.65; N, 3.22. Mass spec *m/e* 435; $[\alpha]_D^{25}$ + 19.4° (c, 1.00, MeOH).

*α-(2-((1,4A-Dimethyl-7-(1-methylethyl)-1,2,3,4,4A,9,10,10A-octahydro-1-phenanthrenyl)methylamino)ethyl)-4-fluorobenzenemethanol hydrochloride **1b***

A suspension of **1a** (10.0 g, 16 mmol) in 75 ml THF-iPrOH (2:1) was cooled in an ice bath and treated with $NaBH_4$ (1.6 g, 41 mmol). The mixture was stirred at room temperature until no starting ketone remained as evidenced by TLC ($CHCl_3$–MeOH, 9:1). The excess borate was decomposed with water, and the mixture was concentrated *in vacuo*. The residue was partitioned between 200 ml CH_2Cl_2 and 100 ml 1 N NaOH. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to an oil. The oil was triturated with excess 1 N HCl–Et_2O. The resulting solid was collected by filtration, washed with Et_2O, and dried to yield 5.0 g (66%); mp = 194–202°C; IR (nujol): OH ν 3344 cm^{-1}; NMR (DMSO–d_6 TMS): δ 0.6–1.9 (m, 21H), 2.07 (m, 2H, $NCCH_2$), 2.27 (d, 1H, Ar'CH), 2.6–3.17 (m, 6H, CH_2NCH_2 + $Ar'CH_2$), 4.70 (m, 1H, OCH), 5.73 (s, 1H, OH), [6.86 (s, 1H), 6.95 (d, 1H), 7.15 (d, 1H) Ar'], [7.17 (d of d, 2H), 7.39 (m, 2H) 4-F-Phe], 8.62 (s, 2H, $H_2N^+Cl^-$). Anal calcd for $C_{29}H_{40}NOF$ HCl, MW, 474.09: C, 73.46; H, 8.72; N, 2.96. Found: C, 73.51; H, 8.82; N, 2.91. Mass spec *m/e* 437; $[\alpha]_D^{25}$ + 26.90° (c, 1.03, MeOH).

*1-(4-Fluorophenyl)-3-((1,2,3,4,4A,9,10,10A-octahydro-1,4A-dimethyl-7-(1-methylethyl)-1-phenanthrenyl)methylamino)-1-propanone O-methyloxime hydrochloride (1R(1α,4Aβ)) **1c***

A mixture of **1a** (30.4 g, 50 mmol) and methoxyamine hydrochloride (8.4 g, 100 mmol) in 150 ml Pyr-EtOH (1:1) was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was cooled in an ice bath and triturated with 200 ml cold water. The resulting was collected by filtration, washed with cold water and Et_2O and dried to yield 22.0 g (88%); mp = 185–187°C; IR (nujol): no carbonyl; NMR (DMSO–d_6 TMS): δ 0.6–1.9 (m, 21H), 2.28 (m, 1H, Ar'CH), 2.7–3.1 (m, 6H, $Ar'CH_2$ + CH_2CH_2CN), 3.24 (m, 2H, NCH_2), [3.77, 3.97 (2s, 3H, OCH_3, 15:85)], [6.86 (s, 1H), 6.96 (d, 1H), 7.14 (d, 1H) Ar'], [7.28 (d of d, 2H), 7.87 (m, 2H) 4-F-Phe], 8.70 (broad s, 2H, H_2N+Cl^-). Anal calcd for $C_{30}H_{41}N_2OF$ HCl, MW, 501.13: C, 71.90; H, 8.45; N, 5.59. Found: C, 72.15; H, 8.13; N, 5.35. Mass spec *m/e* 464; $[\alpha]_D^{25}$ + 21.3° (c, 0.98, MeOH).

*N1-((1,4A-Dimethyl-7-(1-methylethyl)-1,2,3,4,4A,9,10,10A-octahydro-1-phenanthrenyl)methyl)-3-(4-fluorophenyl)-1,3-propanediamine dihydrochloride **1d***

A suspension of **1c** (14.8 g, 30 mmol) in 50 ml dry THF was treated with 1 M BH_3–THF (90 ml, 90 mmol) and refluxed for

16 h. The excess borane was decomposed with water, and the mixture was concentrated *in vacuo*. The mixture was digested with 6 N HCl for 1 h at 80°C and concentrated *in vacuo*. The residue was made alkaline with 1 N NaOH and extracted with 200 ml CH_2Cl_2. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated to an oil. The oil was triturated with excess 1 N HCl–Et_2O, and the resulting solid was collected by filtration, washed with Et_2O, and dried to yield 13.7 (91%); mp = 218–223°C, NMR (DMSO–d_6 TMS): δ 0.5–1.9 (m, 23H), 2.25 (m, 1H, Ar'CH), 2.3–3.1 (m, 6H, CH_2NCH_2 and Ar'CH_2), 4.43 (t, 1H, NCH), [6.83 (s, 1H), 6.93 (d, 1H), 7.13 (d, 1H), Ar'], [7.30 (d of d, 2H), 7.70 (m, 2H), 4-F-Phe], 8.83 (s, 2H, $H_2N^+Cl^-$), 9.00 (s, 3H, $H_3N^+Cl^-$). Anal calcd for $C_{29}H_{41}N_2F$ 2 HCl, MW 509.61: C, 68.35; H, 8.50; N, 5.50. Found: C, 68.77; H, 8.57; N, 5.37. Mass spec (FAB) *m/e* 437 (M+1); $[\alpha]_D^{25}$ + 10.3° (c, 1.05, MeOH).

1-(4-Fluorophenyl)-4-((1,2,3,4,4A,9,10,10A-octahydro-1,4A-dimethyl-7-(1-methylethyl)-1-phenanthrenyl)methylamino)-1-butanone 4-methylbenzenesulfonate (1R-(1α,4Aβ)) **2a**

A mixture of 4-chloro-4'-fluorobutyrophenone-2,2-dimethylpropyleneketal (28.7 g, 100 mmol), dehydroabietylamine (28.5 g, 100 mmol), K_2CO_3 (48 g, 350 mmol), and KI (1 g) in DMF (200 ml) was heated at reflux for 24 h and concentrated *in vacuo*. The residue was partitioned between Et_2O (300 ml) and water (200 ml). The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to an oil. The oil was dissolved in MeOH (300 ml), treated with conc HCl (50 ml), and stirred until no ketal was evidenced by TLC (CH_3Cl–MeOH, 9:1). The organic solvent was removed *in vacuo*, and the aqueous phase was adjusted to pH 8 with 2 N NaOH. The mixture was partitioned between CH_2Cl_2 (300 ml) and water (200 ml). The organic phase was washed with water and brine, dried over $MgSO_4$ filtered, and concentrated *in vacuo*. The residue was dissolved in Et_2O (400 ml) and treated with *p*-TsOH. The resulting solid was collected by filtration, washed with Et_2O, and dried to yield 43.3 g (70%) of the desired aminoketone; mp = 166–167°C; IR (nujol): C=O ν 1687 cm^{-1}; NMR (DMSO–d_6 TMS): δ 0.6–2.0 (m, 23H), 2.25 (m, 5H, Ar'CH + $PheCH_3$ + CH_2CCO), 2.5–3.4 (m, 6H, $CH_2NCH_2CCH_2$), [6.87 (s, 1H), 6.97 (d, 2H), 7.16 (d, 1H) Ar'], [7.10 (d, 2H), 7.48 (d, 2H) 4-SO_2-Phe], [7.38 (d of d, 2H), 8.06 (m, 2H) 4-F-Phe], 7.95 (s, 2H, H_2N^+). Anal calcd for $C_{30}H_{40}NOF$ $C_7H_8O_3S$, MW 621.85: C, 71.46; H, 7.78; N, 2.25; S, 5.61. Found: C, 71.63; H, 7.93; N, 2.18; S, 5.23. Mass spec (FAB) *m/e* 450 (M+1); $[\alpha]_D^{25}$ + 20.3° (c, 1.05, MeOH).

4-Fluoro-α-(3-((1,4A-dimethyl-7-(1-methylethyl)-1,2,3,4,4A-9,10,10A-octahydro-1-phenanthrenyl)methylamino)propyl)-benzenemethanol hydrochloride **2b**

A suspension of **2a** (10.0 g, 16.0 mmol) in THF-iPrOH (75 ml, 2:1) was cooled in an ice bath and treated with $NaBH_4$ (1.2 g, 32 mmol) and stirred at room temperature until no ketone was evidenced by TLC ($CHCl_3$–MeOH, 9:1). The mixture was concentrated *in vacuo*, and the residue was partitioned between Et_2O (150 ml) and 1 N NaOH (100 ml). The organic phase was washed with water and brine, dried over $MgSO_4$, and filtered. The Et_2O filtrate was treated with 1 N HCl–Et_2O (35 ml), stirred, and placed in the cold overnight. The resulting solid was collected by filtration, washed with Et_2O, and dried to give the title compound (6.0 g, 76%); mp = 195–198°C; IR (nujol): OH ν 3348 cm^{-1}; NMR (DMSO–d_6 TMS): δ 0.6–1.95 (m, 25H), 2.25 (d, 1H, Ar'CH), 2.8 (m, 6H, Ar'CH_2 + CH_2NCH_2), 4.55 (t, 1H, OCH), 5.43 (s, 1H, OH), [6.87 (s, 1H), 6.95 (d, 1H), 7.13 (d, 1H) Ar'], [7.15 (d of d, 2H), 7.37 (m, 2H) 4-F-Phe], 8.43 (s, 2H, $H_2N^+Cl^-$). Anal calcd for $C_{30}H_{42}NOF$ HCl, MW 488.11: C, 73.81; H, 8.88; N, 2.87. Found: C, 73.62; H, 8.96; N, 2.68. Mass spec *m/e* 451.368. $[\alpha]_D^{25}$ + 15.8° (c, 1.01, MeOH).

1-(4-Fluorophenyl)-4-((1,2,3,4,4A,9,10,10A-octahydro-1,4A-dimethyl-7-(1-methylethyl)-1-phenanthrenyl)methylamino)-1-butanone O-methyloxime (1R-(1α, 4Aβ)) **2c**

A mixture of **2a** (20.0 g, 32.2 mmol) and methoxyamine hydrochloride (5.4 g, 64.3 mmol) in 100 ml Pyr–EtOH (1:1) was refluxed for 6 h and concentrated *in vacuo*. The residue was partitioned between Et_2O (200 ml) and water (200 ml). The organic phase was washed with 1 N NaOH (100 ml), water, and brine; dried over $MgSO_4$; and concentrated to an oil 15.0 g (97%), IR (nujol): N-H ν 3350 cm^{-1}, no carbonyl; NMR ($CDCl_3$ TMS): δ 0.7–1.9 (m, 22H), 2.2–2.9 (m, 10H, CH_2-$NCH_2CH_2CH_2$ + Ar'CH_2], [3.80, 3.03 (2s, 3H, OCH_3)], [6.87 (s, 1H), 7.06 (d, 1H), 7.17 (d, 1H) Ar'], [7.00 (d of d, 2H), 7.62 (m, 2H) 4-F-Phe]; mass spectrum *m/e* 478: $[\alpha]_D^{25}$ + 19.9° (c, 0.97, MeOH).

1-(4-Fluorophenyl)-N4-(1,4A-dimethyl-7-(1-methylethyl)-1,2,3,4,4A,9,10,10A-octahydro-1-phenanthren-2-ylmethyl)-1,4-butanediamine dihydrochloride **2d**

A suspension of **2c** (10.0 g, 20.9 mmol) in dry THF (30 ml) was treated with 1 M BH_3–THF (100 ml, 100 mmol), stirred at room temperature for 2 h, and refluxed for 16 h. The excess borane was decomposed with MeOH, and the mixture was concentrated *in vacuo*. The residue was digested with 6 N HCl (100 ml) at 80°C for 1 h and concentrated *in vacuo*. The residue was made alkaline with 1 N NaOH and extracted with CH_2Cl_2 (200 ml). The CH_2Cl_2 solution was washed with 1 N NaOH, water, and brine; dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was triturated with 1 N HCl–Et_2O (100 ml), and the resulting solid was collected by filtration, washed with Et_2O, and dried to give the product (11.0 g, 100%); mp = 155°C dec; NMR (DMSO–d_6 TMS): δ 0.5–2.3 (m, 26H), 2.8 (m, 6H, CH_2NCH_2C-CH_2CN), 4.27 (m, 1H, NCH), 6.8–7.18 (m, 3H, Ar'), [7.28 (d of d, 2H), 7.67 (m, 2H) 4-F-Phe], 8.45, 8.56 (d, 2H, $H_2N^+Cl^-$), 8.85 (s, 3H, $H_3N^+Cl^-$); mass spec *m/e* 450; anal calcd for $C_{30}H_{43}N_2F$ 2 HCl, MW 523.61: C, 68.82; H, 8.66; N, 5.35. Found: C, 69.03; H, 8.62; N, 5.05. $[\alpha]_D^{25}$ + 4.7° (c, 1.02, MeOH).

1-(4-Fluorophenyl)-N4-(1,4A-dimethyl-7-(1-methylethyl)-1,2,3,4,4A,9,10,10A-octahydro-1-phenanthren-2-yl)methyl-1,4-butanediamine dihydrochloride **2d**. *Alternate method*

A. A solution of 90% dehydroabietylamine (31.7 g, 100 mmol), *N*-hydroxybenzotriazole (13.5 g, 100 mmol), and 3-(4-fluorobenzoyl)-propionic acid (19.6 g, 100 mmol) in 200 ml CH_2Cl_2 was cooled in an ice bath and treated with *N,N*-dicyclohexylcarbodiimide (20.6 g, 100 mmol). The mixture was stirred in an ice bath for 1 h and 6 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in 200 ml EtOAc, allowed to stand at room temperature for 1 h, and filtered to remove a small amount of dicyclohexylurea. The filtrate was washed with 2 x 100 ml 5% $NaHCO_3$, water, 2 x 100 ml 1 N HCl, water, and brine; dried over $MgSO_4$, filtered, and concentrated to 100 ml. The reddish solution was diluted to the 'cloud-point' with petroleum ether and placed in the cold overnight. The resulting solid was collected by filtration, washed with petroleum ether, and dried to give the desired ketoamide in 89.7% (41.6 g) yield: mp = 101–102°C; IR (nujol): NH ν 3280, C=O ν 1684 and 1665 cm^{-1}; NMR (DMSO–d_6 TMS): δ 0.6–2.1 (m, 21H), 2.27 (d, 1H, Ar'CH), 2.55 (m, 2H, CH_2CO), 2.78 (m, 3H, $NCOCH_2$ + Ar'CH), 3.20 (m, 3H, CH_2CO), [6.83 (s, 1H), 6.95

(d, 1H), 7.15 (d, 1H) Ar'], [7.32 (d of d, 2H), 8.00 (m, 2H) Ar], and 7.72 (t, 1H, NH); Anal calcd for $C_{30}H_{38}NO_2F$, MW 463.61: C, 77.72; H, 8.26; N, 3.02. Found: C, 77.78; H, 8.47; N, 3.09. Mass spec *m/e* 464 (M+1).

B. A solution of the above ketoamide (18.5 g, 40.0 mmol) in 100 ml Pyr–EtOH (1:1) was combined with methoxyamine hydrochloride (3.7 g, 44.0 mmol) and stirred at room temperature for 16 h, refluxed for 3 h, and concentrated *in vacuo*. The residue was partitioned between 200 ml Et_2O and 200 ml cold water. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give the oxime ether in 96% (18.9 g) yield as a gum; IR ($CHCl_3$ film): NH ν 3331, C=O ν 1658 cm^{-1}; NMR ($CDCl_3$ TMS): δ 0.6–2.1 (m, 21H), 2.27 (d, 1H, Ar'CH), 2.40 (m, $Ar'CH_2$), 2.83 (m, 2H, $CH_2C{=}N-$), 3.1 (m, 4H, CH_2NCH_2), 3.93, 3.98 (2s, 3H, OCH_3), 5.57 (m, 1H, NH), [6.8–7.2 (m, 5H) and 7.6 (m, 2H) Ar and Ar'].

C. The above oxime ether (18.9 g, 38 mmol) was treated with 1 M BH_3–THF (155 ml) and refluxed for 16 h. The excess borane was decomposed with cold water, and the mixture was concentrated *in vacuo*. The residue was digested with 100 ml 6 N HCl for 1 h at 80°C and concentrated *in vacuo*. The residue was made alkaline with 1 N NaOH and extracted with 200 ml Et_2O. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and treated with 100 ml 1 N HCl–Et_2O. The resulting white solid was collected by filtration, washed with Et_2O and dried to give the desired diamine in 82% (17.3 g) yield as a hygroscopic hydrate; mp = 165–168°C; NMR (DMSO–d_6 TMS): δ 0.5–2.17 (m, 25H), 2.27 (d, 1H, Ar'CH), 2.8 (m, 6H, CH_2NCH_2 and $Ar'CH_2$), 4.30 (m, 1H, NCH), [6.87 (s, 1H), 6.95 (d, 1H), and 7.15 (d, 1H) Ar'], [7.28 (d of d, 2H) and 7.67 (m, 2H), Ar]; Anal calcd for $C_{30}H_{43}N_2F$ 2 HCl 1.5 H_2O, MW 550.63: C, 65.46; H, 8.79; N, 5.09. Found: C, 65.82; H, 8.80; N, 5.06. Mass spec (FAB) *m/e* 451 (M+1); $[\alpha]_D^{25} + 4.6°$ (c, 1.04, MeOH).

Biological methods

Porcine pancreatic PLA_2 (PAN-PLA_2) [10]. The release of ^{14}C-arachidonic acid from 1-palmitoyl-2-^{14}C-arachidonoyl phosphatidylcholine by porcine pancreatic PLA_2 (Sigma) is the basic of the assay. The drug is added to the enzyme-buffer reaction mixture (pH 8.5) at time 0. Substrate (2 μM) is added after 2 min and the reaction proceeds for 5 min at 37°C in a shaking water bath. The reaction was stopped by freezing in a slurry of dry ice–EtOH, and the arachidonic acid was separated from the unreacted phosphatidylcholine by silica gel chromatography. The disintegrations per min (DPM) (counts per min, CPM, was converted to DMP to compensate for solvent quenching) of both product and unreacted substrate were determined. IC_{50}, in μM, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 50% inhibition point.

Rat polymorphonuclear PLA_2 (PMN–PLA_2) [11]. A partially purified PLA_2 from casein-elicited rat neutrophils was used as the enzyme. The low speed supernatant from a neutrophil homogenate was fractionated on a discontinuous sucrose gradient. A brown band penetrating into 25% sucrose was the PLA_2 source. Enzyme (15–30 μg protein), calcium ion containing buffer (pH 7.5) and drug were incubated at 37°C for 2 min. Substrate, a sonicated dispersion of 1-palmitoyl-2-^{14}C-arachidonyl phosphatidylcholine (≈ 2 μM), was added, and incubation continued for 30 min. The reaction mixture was worked up as outlined above. IC_{50}, in μM, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 50% inhibition point.

Bovine seminal vesicle prostaglandin synthetase (BSV–PGS) [12]. The prostaglandin synthetase assay is based on the conversion of ^{14}C-arachidonic acid to ^{14}C-prostaglandin utilizing bovine seminal vesicles as the source of the enzyme. Inhibitors are added to the enzyme-buffer (Tris–Cl pH 8.5) reaction mixture at time 0. Substrate (20 μM) is added after 2 min to initiate the reaction which continues for 10 min at 37°C in a shaking water bath. The reaction tubes are placed in a dry ice–EtOH slurry to stop the reaction. The labelled prostaglandins are separated from labelled arachidonic acid by chromatography on Baker silica gel columns and quantitated. IC_{50}, in μM, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 50% inhibition point.

Rat basophilic leukemic 5-lipoxygenase (RBL 5-LO). The procedure of Jakschik *et al* was used [3–5]. The enzyme was prepared as a 1000 *g* supernatant from homogenized rat basophilic leukemic cells. Because of the variability in the enzyme content from culture to culture, an amount of supernatant was chosen to give a net production of 3300–3800 DPM of 5-HETE under the assay conditions (total cell protein 9–20 μg/assay). All reactions were conducted in duplicate. In a total volume of 100 μl, the appropriate amount of enzyme was incubated with test compound (prepared in 5% DMSO, 95% 0.2 M Tris, pH 8.5) in a phosphate buffer (45 mmol sodium phosphate, 0.83 mmol EDTA. 0.083% gelatin, 0.1 mmol glutathione, 0.83 mmol calcium chloride, 0.012 mmol indomethacin) at pH 7.0 and 37°C for 5 min. The reaction was initiated by the addition of 20 μl of a solution of arachidonic acid in phosphate buffer. The final concentration of the substrate in the assay was 0.042 mmol, including 0.167 μCi of [^{14}C]arachidonic acid (specific activity 50 mCi/mmol). The reaction was terminated after 2 min by freezing in dry ice–EtOH. 5-Lipoxygenase products were separated from unreacted arachidonic acid on silica gel columns with hexanes/ethyl acetate/acetic acid (82:17:1). 5-HETE was eluted with hexanes/tetrahydrofuran/ethyl acetate/acetic acid (65:30:10:1). The remaining products were eluted with methanol/water/acetic acid (70:30:1). The activity was measured as the total radioactivity in the 5-lipoxygenase products, and the inhibition was calculated as (1-D/C) x 100%, where D is activity in the presence of the test compound and C is the control activity. IC_{50}, in μM, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 50% inhibition point.

Carrageenan-induced edema in the rat hind paw (CARR PAW) [16]. Male Charles River CD rats were given a single oral dose (1.0 ml/100 g body weight) of compound suspended in 0.25% aqueous methocel. To insure uniform hydration, tap water was given immediately after dosing to a total of 5.0 ml/rat. One h later, 0.1 ml of carrageenan (1% solution in 0.9% NaCl) was injected into the plantar area of the left hind paw. Volume of the injected paw was again measured. Activity is recorded as the calculated percent inhibition of edema cause by the 100 mg/kg dose of drug. ED_{30}, in mg/kg, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 30% inhibition point.

Mouse contact sensitivity assay (CONT SEN). The assay was conducted as described by Claman *et al* [17].

Zymosan peritonitis. The assay was conducted as described by Lundy *et al* [18].

Tetradecanoyl phorbol acetate induced ear edema (TPA EAR). The assay is a modification from Furstenberger and Marks [19]. Tetradecanoyl phorbol acetate (1.6 mmol) in acetone was applied to the inner pinna of the right ear of mice. Test compounds were made up in acetone and applied in 10 μl of acetone to both ears just prior to the application of TPA. Four h after application of the TPA, the animals were eutha-

nized. The ears were removed and 6-mm punches were taken and weighed. The amount of swelling was determined by subtracting the compound treated ear punch mass (left ear) from the TPA treated ear punch mass (right ear). Results are reported as % inhibition caused by 100 μg/ear test compound, and represent the mean of at least 3 separate experiments conducted on 10 mice ± the standard error. ED_{50}, in μg/ear, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 50% inhibition point. The standard error for a large group of similar compounds was found to be less than 10%.

Arachidonic acid ear edema assay (AA EAR). A modification of the procedure of Young *et al* was used [20]. Groups of 10 CF_1 mice (18–20 g) were used. Arachidonic acid solution (100 μg/ml in acetone) was prepared fresh daily. Solutions of test compounds were prepared in acetone and applied to both ears just prior to challenge with 1 mg arachidonic acid (10 μl of solution) applied to the inner surface of one ear. The unchallenged ear served as a negative control. The animals were sacrificed by cervical dislocation 60 min after challenge. Disks (6-mm diameter) were removed from each ear with a skin biopsy punch, and the weights determined. The swelling was measured as the difference in weight between the punches from the challenged and unchallenged ears; this value varied < 10% between repeat experiments. Percent inhibition was calculated by using the [C-T/C] x 100%, where C is positive control swelling and T is the drug-tested swelling. Statistical difference was determined using the Student's *t*-test. Results are reported as % inhibition caused by 100 μg/ear test compound, and represents the mean of at least 3 separate experiments ± the standard error. The standard error for a large group of similar compounds was found to be < 10%.

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