Eur J Med Chem (1992) 27, 595–610

Anti-inflammatory phospholipase-A_2 inhibitors. II. Design, synthesis and structure–activity relationship

W Wilkerson, I DeLucca, W Galbraith, J Kerr

DuPont Merck Pharmaceutical Company, E354/347 Experimental Station, Wilmington, DE 19880-0353, USA

(Received 26 April 1991; accepted 5 March 1992)

Summary — The design and synthesis of a novel series of phospholipase-A_2 (PLA_2) inhibitor with antiinflammatory activity was based on a systematic structure–activity relationship (SAR) analysis.

phospholipase-A_2 (PLA_2) / antiinflammatory / structure–activity relationship (SAR)

Introduction

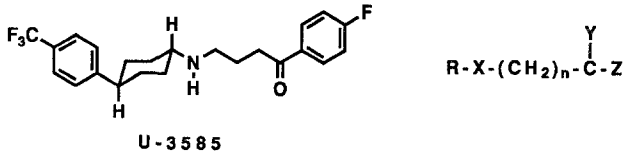
Recently we reported on the synthesis and antiinflammatory profile of a series of dehydroabietylamine derivatives with anti-PLA_2 activity [1]. This class of compounds was the result of a massive synthetic effort based on the X-ray crystal structure of porcine pancreatic PLA_2 [2], and the analysis of a series of structures reported by Wallach [3, 4] to be PLA_2 inhibitors with antiinflammatory activity. The results of these analyses led to the development of a template for the design of a novel series of antiinflammatory PLA_2 inhibitor.

The template was based on the observation that the active site of porcine pancreatic PLA_2 (PAN–PLA_2) contained calcium, a large lipophilic pocket, and many potential hydrogen bonding sites, and the structural unit of **U-3585** [3, 4]. These observations suggested that a PLA_2 inhibitor should, at a minimum, contain a large and/or lipophilic group to occupy that region of the enzyme normally occupied by the alkenyl portion of arachidonic acid, and a group capable of coordinating with the calcium ion at the active site. The general composition of this template is shown in scheme 1, where R is large and/or lipophilic; X is NH, S, O, or CH_2; n is a tether of 1 to 4 carbons to link X and Y; Y is = O, OH, or NH_2 to coordinate with calcium; and Z is a relatively small group that might influence the electron density on Y. The observation that Z should be relatively small was further dictated by the fact that the calcium ion of the enzyme is deep within the enzyme active site. The ultimate objective was to develop a better drug by 'dissecting' a reported antiinflammatory PLA_2 inhibitor **(U-3585)** into various molecular components, determining the contribution of those parts to biological activity, and incorporating that information and understanding into the design of novel compounds with a better *in vitro* and *in vivo* activity profile than the lead compound **(U-3585)**.

Tables I and II contain some of the 600 compounds and biological data used in this study.

Chemistry

The proposed inhibitors shown in table I, where $n = 2$, were generally synthesized by reacting a 2-haloketone or ester with a primary amine or thiol as shown in scheme 2. This approach was not successful when the halide was reacted with an alcohol. Following the course of the reactions by TLC indicated the disappearance of the starting materials, but upon work-up, the desired ketoether (X = O) was not obtained; only the starting alcohol was isolated. The conclusion was made that the desired product was formed during the reaction, but during work-up the product underwent a 'retro-Michael' reaction. In some instances,



Scheme 1.

Table I. Physical data on compounds **1–106**.

R-X-$(CH_2)_n$-C(Y)-Z

Compd	*R*[a]	*X*	*n*	*Y*	*Z*	*mp (°C)*	*Yield (%)*	*Formula*[b]
1	DHA	NH	3	H, OH	n-Hexyl	oil	86	$C_{30}H_{51}NO$
2	DHA	NH HCl	2	H, OH	H	foam	97	$C_{23}H_{37}NO$ HCl
3	DHA	NH HCl	3	H, OH	Me	foam	58	$C_{25}H_{41}NO$ HCl
4	DHA	NH HCl	3	H, NH_2 HCl	Me	foam	73	$C_{25}H_{42}N_2$ 2HCl
5	DHA	NH HCl	3	H, OH	4-F-Phe	208–209	91	$C_{30}H_{42}NOF$ HCl
6	DHA	NH HCl	3	H, OH	Phe	205–207	57	$C_{30}H_{43}NO$ HCl
7	DHA	NH HCl	2	H, NH_2 HCl	4-F-Phe	215 dec	96	$C_{29}H_{41}N_2F$ 2HCl
8	DHA	NH HCl	2	H, NH_2 HCl	Phe	262–264	41	$C_{29}H_{42}N_2$ 2HCl
9	DHA	NH HCl	2	H, NH_2 HCl	4-MeO-Phe	212 dec	99	$C_{30}H_{44}N_2O$ 2HCl
10	DHA	NH HCl	2	H, NH_2 HCl	4-MeS-Phe	210 dec	76	$C_{30}H_{44}N_2S$ 2HCl
11	NAP-1	NH HCl	2	O=	OEt	115–118	30	$C_{17}H_{21}NO_2HCl$
12	NAP-2	NH HCl	2	O=	4-F-Phe	206–208	71	$C_{21}H_{20}NOF$ HCl
13	NAp-2	NH HCl	2	O=	4-MeO-Phe	195–196	51	$C_{22}H_{23}NO_2$ HCl
14	NAP-2	NH HCl	2	O=	4-MeS-Phe	190–191	62	$C_{22}H_{23}NOS$ HCl
15	CHM	NH HCl	2	O=	4-F-Phe	200–202	68	$C_{16}H_{22}NOF$ HCl
16	CHM	NH HCl	2	O=	4-MeS-Phe	186–188	81	$C_{17}H_{25}NOS$ HCl
17	CHM	NH HCl	2	O=	OEt	213–215	50	$C_{12}H_{23}NO_2$ HCl
18	DHA	NH HCl	3	O=	OEt	145–148	35	$C_{26}H_{41}NO_2$ HCl
19	DHA	NH HCl	3	O=	Me	152–155	92	$C_{25}H_{39}NO$ HCl
20	DHA	NH HCl	3	O=	4-F-Phe	170–171	35	$C_{30}H_{40}NOF$ HCl
21	DHA	NH	2	MeO-N=	4-MeO-Phe	oil	99	$C_{31}H_{44}N_2O_2$
22	DHA	NH HCl	2	H, OH	4-MeO-Phe	136 dec	88	$C_{30}H_{43}NO_2$ HCl
23	DHA	NH TsOH	2	O=	4-MeO-Phe	186–187	46	$C_{30}H_{41}NO_2C_7H_8O_3S$
24	DHA	NH HCl	2	H, OH	4-MeS-Phe	158 dec	75	$C_{30}H_{43}NOS$ HCl
25	DHA	NH TsOH	2	O=	4-MeS-Phe	197–198	83	$C_{30}H_{41}NOSC_7H_8O_3S$
26	DHA	NH	2	MeO-N=	4-MeS-Phe	oil	96	$C_{31}H_{44}N_2OS$
27	DHA	NH TsOH	2	O=	4-F-Phe	157–159	48	$C_{29}H_{38}NOFC_7H_8O_3S$
28	DHA	NH HCl	2	H, OH	4-F-Phe	192–194	82	$C_{29}H_{40}NOF$ HCl
29	DHA	NH HCl	3	H, NH_2 HCl	4-F-Phe	155 dec	99	$C_{30}H_{43}N_2F$ 2HCl
30	n-Dodecyl	NH HCl	2	O=	4-F-Phe	169–172	74	$C_{21}H_{34}NOF$ HCl
31	n-Dodecyl	NH HCl	2	H, OH	4-F-Phe	235 dec	55	$C_{21}H_{36}NOF$ HCl
32	n-Dodecyl	NH HCl	2	MeO-N=	4-F-Phe	81–82	93	$C_{22}H_{37}N_2OF$ HCl
33	n-Dodecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	243–245	91	$C_{21}H_{37}N_2F$ 2HCl
34	NAP-2	NH HCl	1	H, NH_2 HCl	4-F-Phe	186–189	37	$C_{20}H_{21}N_2F$ 2HCl
35	NAP-2	NH HCl	2	H, NH_2 HCl	4-F-Phe	280–281	96	$C_{21}H_{23}N_2F$ 2HCl
36	NAP-2	NH HCl	3	H, NH_2 HCl	4-F-Phe	182–185	38	$C_{22}H_{25}N_2F$ 2HCl
37	n-Decyl	CH_2	2	H, NH_2 HCl	4-F-Phe	145–147	91	$C_{20}H_{34}NF$ HCl
38	n-Decyl	S	2	H, NH_2 HCl	4-F-Phe	110–112	88	$C_{19}H_{32}NFS$ HCl
39	n-Decyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	233–234	99	$C_{19}H_{33}N_2F$ 2HCl
40	NAP-1	NEt HCl	2	H, NH_2 HCl	4-F-Phe	120 dec	95	$C_{23}H_{27}N_2F$ 2HCl
41	NAP-1	NH HCl	2	H, NH_2 HCl	4-F-Phe	263–265	94	$C_{21}H_{23}N_2F$ 2HCl
42	4-Me-Phe	S	3	H, NH_2	4-F-Phe	oil	99	$C_{17}H_{20}NFS$
43	4-Me-Phe	S	3	H, NHAc	4-F-Phe	69–73	86	$C_{19}H_{22}NOFS$
44	DHA	NH AcOH	3	H, NH_2 AcOH	Phe	97–101	99	$C_{30}H_{44}N$ $2C_2H_4O_2$
45	DHA	NAc	3	H, NHAc	Phe	glass	99	$C_{34}H_{48}N_2O_2$
46	n-Butyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	255–257	58	$C_{13}H_{21}N_2F$ 2HCl
47	n-Hexyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	240–241	77	$C_{15}H_{25}N_2F$ 2HCl
48	n-Heptyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	172–174	85	$C_{16}H_{27}N_2F$ 2HCl
49	n-Octyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	222–224	52	$C_{17}H_{29}N_2F$ 2HCl
50	n-Undecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	204–206	80	$C_{20}H_{35}N_2F$ 2HCl
51	n-Tridecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	226–228	70	$C_{22}H_{39}N_2F$ 2HCl
52	n-Tetradecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	245 dec	68	$C_{23}H_{41}N_2F$ 2HCl
53	n-Hexadecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	246 dec	80	$C_{25}H_{45}N_2F$ 2HCl
54	n-Octadecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	248 dec	87	$C_{27}H_{49}N_2F$ 2HCl
55	t-Octyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	139 dec	59	$C_{18}H_{31}N_2F$ 2HCl
56	n-Butyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	218–220	77	$C_{14}H_{23}N_2F$ 2HCl
57	n-Heptyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	204–206	83	$C_{17}H_{29}N_2F$ 2HCl

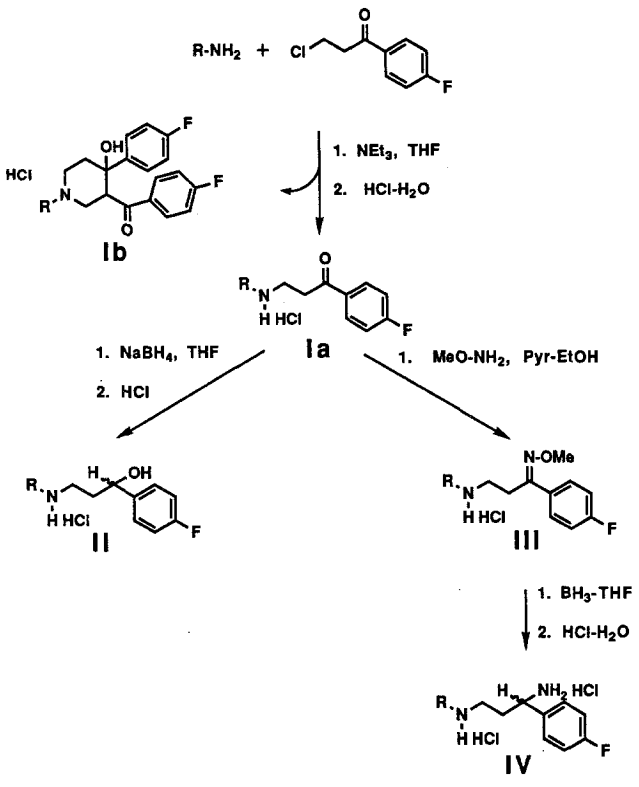
Table I. *Continued.*

Compd	*R*[a]	*X*	*n*	*Y*	*Z*	*mp (°C)*	*Yield (%)*	*Formula*[b]
58	n-Octyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	114–116	99	$C_{18}H_{31}N_2F$ 2HCl
59	n-Decyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	182–184	67	$C_{20}H_{35}N_2F$ 2HCl
60	n-Undecyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	206–208	91	$C_{21}H_{37}N_2F$ 2HCl
61	n-Dodecyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	183–184	84	$C_{22}H_{39}N_2F$ 2HCl
62	n-Tridecyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	180–182	52	$C_{23}H_{41}N_2F$ 2HCl
63	n-Tetradecyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	227–229	84	$C_{24}H_{43}N_2F$ 2HCl
64	n-Hexadecyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	185–188	99	$C_{26}H_{47}N_2F$ 2HCl
65	n-Octadecyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	154–156	58	$C_{28}H_{51}N_2F$ 2HCl
66	4-Me-Phe	S	3	O=	4-F-Phe	oil	99	$C_{17}H_{17}NOS$
67	n-Undecyl	NH HCl	2	O=	4-F-Phe	174 dec	71	$C_{20}H_{32}NOF$ HCl
68	n-Undecyl	NH HCl	2	MeO-N=	4-F-Phe	90–93	97	$C_{21}H_{35}N_2OF$ HCl
69	$HOC(CF_3)_2$-Phe	NH	2	H, NH_2	4-F-Phe	136–137	69	$C_{18}H_{17}N_2OF_7$
70	$HOC(CF_3)_2$-Phe	NMe	2	H, NH_2	4-F-Phe	142–144	91	$C_{19}H_{19}N_2OF_7$
71	(4-Pyr) (Phe)CH	NH	2	O=	4-F-Phe	115 dec	99	$C_{21}H_{19}N_2OF$ 2HCl
72	(4-Pyr) (Phe)CH	NH	2	H, NH_2	4-F-Phe	183 dec	98	$C_{21}H_{22}N_3F$ 3HCl H_2O
73	t-Octyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	242–244	99	$C_{17}H_{29}N_2F$ 2HCl
74	n-Hexadecyl	NH HCl	1	O=	4-F-Phe	129–131	54	$C_{24}H_{40}NOF$ HCl
75	n-Octadecyl	HN-C=O	2	O=	Phe	91–92	77	$C_{28}H_{47}NO_2$
76	n-Octadecyl	NH HCl	2	H, NH_2 HCl	Phe	248–249	99	$C_{28}H_{52}N_2$ 2HCl
77	n-Tetradecyl	NH HCl	2	O=	4-F-Phe	118–120	55	$C_{23}H_{38}NOF$ HCl
78	t-Butyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	260–263	86	$C_{13}H_{21}N_2F$ 2HCl
79	s-Butyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	250–253	76	$C_{13}H_{21}N_2F$ 2HCl
80	n-Hexyl	NH HCl	2	H, OH	4-F-Phe	166 dec	91	$C_{15}H_{26}NOF$ HCl
81	n-Octyl	S	3	H, NH_2 HCl	4-F-Phe	143	59	$C_{18}H_{30}NFS$ HCl
82	n-Octyl	HN-C=O	2	O=	Phe	75–76	89	$C_{18}H_{27}NO_2$
83	n-Octyl	CH_2	2	H, NH_2 HCl	4-F-Phe	129–132	94	$C_{18}H_{30}NF$ HCl
84	n-Decyl	S	2	H, OH	4-F-Phe	oil	99	$C_{19}H_{31}OFS$
85	n-Decyl	NH HCl	3	H, H	Phe	187 dec	96	$C_{20}H_{35}N$ HCl
86	n-Decyl	NH HCl	3	H, NH_2 HCl	Phe	186–190	98	$C_{20}H_{36}N_2$ 2HCl
87	n-Decyl	NH HCl	2	O=	4-F-Phe	183–184	67	$C_{19}H_{30}NOF$ HCl
88	n-Decyl	NH HCl	2	MeO-N=	4-F-Phe	94–98	97	$C_{20}H_{32}N_2OF$ HCl
89	n-Decyl	HN-C=O	2	O=	4-F-Phe	93–94	78	$C_{20}H_{30}NO_2F$
90	n-Decyl	NH HCl	3	H, NH_2 HCl	4-F-Phe	187–188	94	$C_{20}H_{35}N_2F$ 2HCl
91	n-Undecyl	NH HCl	2	H, H	Phe	199 dec	96	$C_{20}H_{35}N$ HCl
92	CHM	NH HCl	3	H, OH	4-F-Phe	138–140	56	$C_{17}H_{26}NOF$ HCl
93	3-^{t}Bu-Phe	O	3	H, OH	4-F-Phe	oil	91	$C_{20}H_{26}O_2F$
94	3-^{t}Bu-Phe	O	3	H, NH_2 HCl	4-F-Phe	187–190	83	$C_{20}H_{26}NOF$ HCl
95	4-Me-Phe	S	3	H, NH_2 HCl	4-F-Phe	177–180	86	$C_{17}H_{20}NFS$ HCl
96	DHA	NH TsOH	3	O=	4-F-Phe	180–182	70	$C_{30}H_{40}NOF$ $C_7H_8O_3S$
97	4-Biphenyl-methyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	246–248	99	$C_{22}H_{23}N_2F$ 2HCl
98	4-Me-Phe	NH HCl	2	H, NH_2 HCl	4-F-Phe	236–238	67	$C_{16}H_{19}N_2F$ 2HCl
99	4-Decyl-Phe	NH HCl	2	H, NH_2 HCl	4-F-Phe	208–210	89	$C_{25}H_{37}N_2F$ 2HCl
100	DHA	NH TsOH	2	O=	Phe	162–164	20	$C_{29}H_{39}NO_2$ $C_7H_8O_3S$
101	DHA	HN-C=O	2	H, OH	H	foam	99	$C_{24}H_{37}NO_2$
102	DHA	NH HCl	3	H, OH	H	171–173	60	$C_{24}H_{39}NO$ HCl
103	DHA	NH HCl	2	H, OH	Phe	131 dec	85	$C_{29}H_{41}NO$ HCl
104	DHA	NH HCl	1	H, OH	Phe	226–228	36	$C_{28}H_{39}NO$ HCl
105	NAP-2	NH HCl	2	H, NH_2 HCl	4-MeO-Phe	240 dec	98	$C_{22}H_{26}N_2O$ 2HCl
106	DHA	NAc	2	H, NHAc	4-F-Phe	98–102	99	$C_{33}H_{45}N_2O_2F$

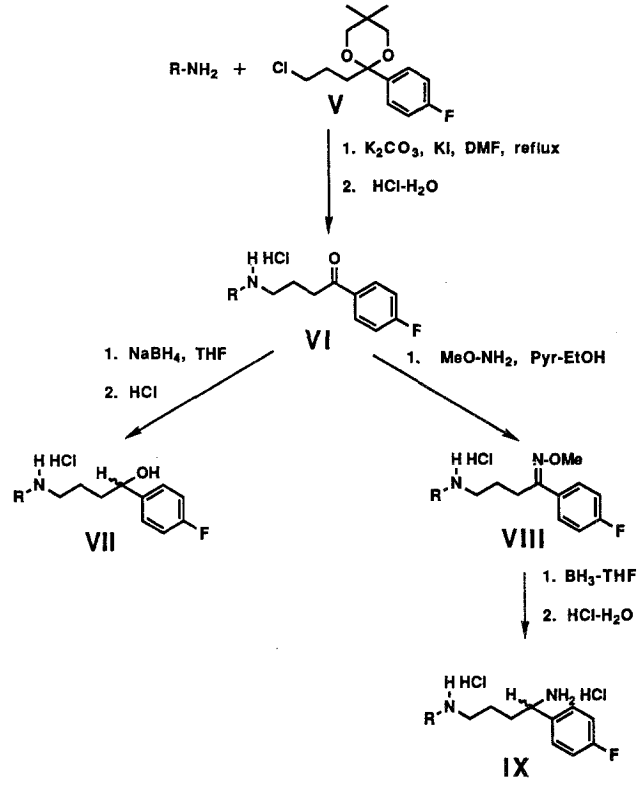
[a]DHA = dehydroabietyl; NAP-2 = naphthyl-2-ethyl; NAP-1 = naphthyl-1-ethyl; CHM = cyclohexanemethylene. [b]All compounds were analyzed for C, H, N and S where necessary and found to be within ± 0.4% of theoretical values.

Table II. Biological data on compounds **1–106** and standards.

Cmpd	PAN–PLA_2 (IC_{50}, μM)	PMN–PLA_2 (IC_{50}, μM)	Car paw (% nh) (100 mg/kg)	Car paw (ED_{30}, mg/kg)
1	48.0	> 750.0		
2	1.4	45.0	10–100	
3	2.1	260.0	24–100	82.0
4	1.5	5.4	66–100	7.0
5	14.0	7.5	49–50	5.9
6	180.0	15.0	41–50	22.3
7	2.3	5.6	81–50	8.0
8	3.5	12.0	60–100	44.9
9	5.0	14.0	30–100	100.0
10	3.8	9.0	51–100	58.8
11	300.0			
12	11.0			
13	6.5	58.0		
14	25.0			
15	> 25.0			
16	5.6			
17	> 25.0			
18	2.5	470.0	32–50	46.9
19	1.8	110.0		
20	1.00	> 750.0	8–100	
21	> 25.0	12.0		
22	15.0	220.0		
23	55.0	310.0		
24	2.9	> 75.0	34–100	85.0
25	230.0			
26	> 25.0			
27	40.0	> 750.0	54–50	8.0
28	5.0	25.0	49–50	3.6
29	2.6	2.7	63–50	5.0
30	19.0	48.0	17–100	
31	8.0	4.9	43–50	18.2
32	20.0			
33	3.1	4.9	72–100	22.0
34	14.0			
35	2.0	150.0	51–100	54.3
36	16.0			
37	14.0		34–100	71.8
38		13.0	27–100	78.9
39	4.5	25.0	63–100	41.9
40	20.0	93.4		
41	7.5	47.0	49–100	57.0
42	2.3	44.0		
43	> 25.0			
44		11.0	77–100	29.0
45	> 750.0			
46	805.1	576.8	32–100	73.8
47	400.0	403.6	41–100	64.5
48	18.0	169.7	49–100	58.6
49	5.4	110.0	56–100	49.1
50	2.9	3.3	81–100	26.0
51	3.1	4.7	74–100	33.0
52	3.0	4.4	71–100	35.0
53	3.6	1.8	43–100	60.0
54	7.7	1.6	36–100	69.7
55	120.0	> 750.0		
56		430.5	41–100	65.7
57		191.4	43–100	62.5
58		180.0	46–100	50.2
59		30.0	76–100	27.7
60	2.6	10.7	69–100	35.0
61		7.4	61–100	35.0
62		3.4	69–100	34.9
63		3.5	58–100	45.2
64		1.8	68–100	35.1
65		2.4	56–100	49.0
66	0.54	> 750.0	30–100	100.0
67	0.44	25.0	44–100	59.0
68	6.6			
69	5.6	7.4		
70	3.2	< 750.0		
71	0.20	420.0		
72	1.9	310.0	32–50	50.0
73	41.7	750.0	62–100	42.9
74		9.9	31–100	100.0
75		> 750.0	16–100	
76		6.3	44–100	59.0
77		7.6	45–100	58.2
78	> 25.0	> 750.0	30–100	100.0
79	> 25.0	> 750.0	15–100	
80		36.8	54–50	16.8
81	1.8	13.0	10–100	
82		> 750.0	20–100	
83		40.0	64–100	39.1
84		> 750.0	9–100	
85		38.0	23–100	
86		6.2	72–100	32.6
87	1.1	65.5	21–100	
88			42–100	63.1
89		> 750.0	17–100	
90		6.3	79–100	28.7
91		37.0	36–100	72.0
92	> 25.0		45–100	59.2
93	> 25.0	> 750.0	28–20	
94	4.2	12.0	17–20	
95	3.0	54.0	20–100	
96	32.0	> 750.0	43–50	9.0
97	16.0	39.0	26–100	
98	13.0		50–100	40.8
99	7.5	1.8	10–100	
100	4.8	150.0	32–50	40.8
101	> 25.0	23.3	44–100	60.5
102	8.0	52.0	46–100	57.9
103	22.1	120.0		
104	100.0	380.0		
105	22.0			
106	30.8	90.5	37–100	81.0
U-3585	2.3	> 250.0	53–100	> 20.0
Mepacrin	22.0	100.0	48–50	3.5
Dexamethasone	> 25.0	> 750.0	48–10	< 1.0
Indomethacine	315.0	250.0	43–9	1.0

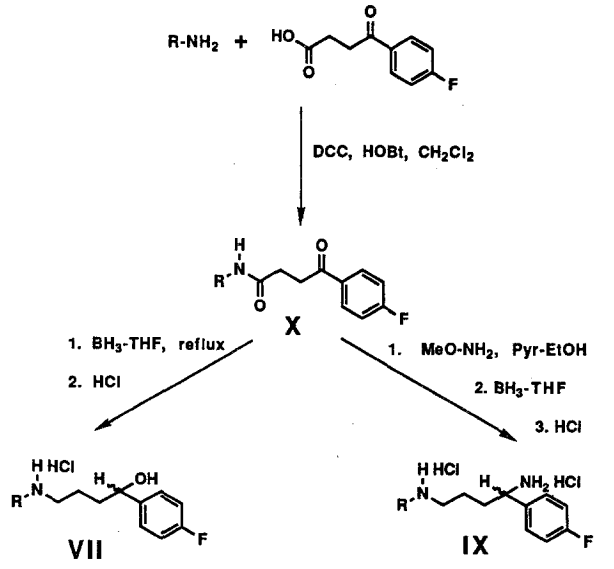


Scheme 2. Synthetic approach to the propyl series.



Scheme 3. Synthetic approach to the butyl series.

when a 2-halopropiophenone was reacted with a primary amine, another product was isolated. This compound was later identified and confirmed by directed synthesis as a piperidine (**Ib**) [5], which resulted from the dialkylation of the amine followed by a very facile internal Aldol condensation. We found that by preforming the Michael acceptor (enone) *in situ* and reacting the enone with the acid addition salt of the amine, we could reduce the amount of **Ib** formed. Attempts to synthesize inhibitors where $n = 3$ by the procedure discussed above were unsuccessful, specifically where R-XH represented a primary amine. The chlorogroup of the 3-chloroketones were extremely resistant to nucleophilic displacement by the amines. To obtain the desired 3-aminoketone (**VI**), we had to protect the ketone as the ketal (**V**) and conduct the reaction in refluxing DMF in the presence of excess base and sodium iodide. Generally the yields of **VI** were low following the procedure shown in scheme 3. This approach was applicable for the nucleophilic thiols and alcohols. During these studies, we discovered that some of the better antiinflammatory PLA_2 inhibitors were the 3-aminoalcohols (**VII**) and diamines (**IX**), and as a consequence, a better synthetic route was desired. Using the procedure shown in scheme 4, we were able to react an amine with a benzoyl carboxylic acid, using dicyclohexylcarbodiimide (DCC), to give the ketoamide (**X**) in good to excellent yields. Other



Scheme 4. Alternative approach to the butyl series.

methods of activating the carboxyl, such as forming the mixed anhydride of ethyl chloroformate or the acid chloride, were investigated and found to be less desirable.

The aminoketones (**Ia** and **VI**) were reduced to the aminoalcohols (**II** and **VII** respectively) using sodium borohydride or borane–tetrahydrofuran complex (BH_3–THF). The ketoamide (**X**) was also reduced to **VII** using BH_3–THF. These reduction methods were also acceptable for converting the ketosulfides and ketoethers to the corresponding alcoholsulfides and alcoholether, respectively.

The ketones (**Ia**, **VI** and **X**) were treated with methoxylamine hydrochloride in a mixture of dry pyridine and absolute ethanol (1:1) to give the corresponding oximes, which were reduced to the diamines (**IV** and **IX**) using BH_3–THF under refluxing conditions. Again, this approach was applicable to the ketosulfides and ketoethers.

Generally, all *in vitro* and *in vivo* studies were conducted on racemates or mixtures of diastereomers (cases where R was optically pure). In one study, enantiomerically enriched preparations of **50**, **(+)50** and **(–)50**, were obtained by the asymmetric reduction of the corresponding oxime using a modification of the method described by Sakito *et al* [6].

Results and discussion

The search for antiinflammatory drugs based on the inhibition of PLA_2 was initiated using the following protocol: compounds were synthesized and submitted for screening against PAN–PLA_2 [7] or PMN–PLA_2 [8]. Compounds having IC_{50}s of less than 25 or 100 μM, respectively, were then submitted for carrageenan paw edema inhibition (car paw) [9] evaluation. Compounds that elicited a 40% or greater inhibition in edema at 100 mg/kg orally were then subjected to dose response studies to determine car paw ED_{30} expressed in mg/kg. However, some compounds not conforming to the above protocol were fully characterized if we thought that the additional data would be useful in developing the SAR. Over time, this protocol underwent many changes such as choice of enzyme and screening doses. Most of the initial studies were based on PAN–PLA_2 that was abandoned in favor of the 'more relevant' PMN–PLA_2. Initial screening dose for car paw was 20 mg/kg, which was abandoned in favor of 100 mg/kg because of inconsistency in data replication. Unfortunately, many data points were not repeated using the updated protocol(s). The driving force, which motivated some of these changes, was to find a safe orally active antiinflammatory based on an anti-PLA_2 mechanism of action.

Enzymes

At the beginning of these studies, the best characterized and most available PLA_2 was from the porcine pancreas (Sigma Chemical Co). Thus, early SAR studies were conducted on this enzyme. The general opinion, however, was that the PAN–PLA_2, an extracellular digestive enzyme, was not an ideal enzyme for antiinflammatory drug design. To address this problem, we undertook the isolation and characterization of the PLA_2 from rat PMNs [8].

A comparison of 92 proposed inhibitors of PAN–PLA_2 and PMN–PLA_2 indicated that there was little correlation between the 2 enzymes with respect to the inhibitors as shown in Eq 1 where n is the number of data points, r^2 is the coefficient of determination, and s is the standard deviation of the regression.

$$\text{PMN–PLA}_2 = 0.58\ (\text{PAN–PLA}_2) + 99.23 \qquad (1)$$
$$n = 92, \quad r^2 = 0.12, \quad s = 154.23$$

Inhibitor design

The synthesis of compounds based on a hypothesis of drug-active site interaction of a 'lead' compound is simply referred to as 'analoging'. By using computer modeling techniques and the X-ray crystal data for PAN–PLA_2 [2], each fragment optimization of the lead molecule was rationally designed. The use of the template strategy was an attempt to organize the synthetic approach and fragment selection [10]. The authors are well aware that the interaction between substrate–enzyme involves many physical chemical interactions, and that the alternation of one can greatly influence the others.

In our attempts to improve on the structure **U-3585**, we dissected the molecule and assigned structural functions based on our understanding of the X-ray data of PAN–PLA_2. This effort allowed us to make the following assumptions:

– 4-fluorophenyl (Z); a lipophilic group, electronically capable of influencing the electron density on the neighboring ketone, and having the ability to form hydrogen bond or participating in a π–π interaction with aromatic amino acids in the active site of the enzyme;
– ketone (Y); a group capable of forming hydrogen bonds and coordinating with calcium at the active site;
– n; a spacer between Y and X;
– secondary amine (X); a group capable of forming salt bridges and hydrogen bonds; and
– 4-trifluorophenyl-4'-cyclohexyl (R); a 'large' and/or lipophilic group possibly filling the space normally occupied by the alkenyl portion of arachidonic acid of the substrate.

Using this analysis, we proposed the target(s) described in scheme 1 where R is large and/or lipo-

philic; 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.

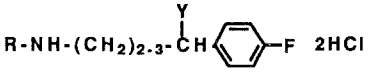
We initiated the study by investigating the nature and limitations of the Z-group. A series of compounds was synthesized where Z was aromatic, small alkyl, or H. The results from this study are shown in tables III and IV. The most potent inhibitors of PLA_2 were molecules where Z was a small group such as H, Me, or substituted aromatics. The Z group was limited to 4-substituted benzenoids (Z'-W) where W was H, F, MeO, and MeS mainly because of synthetic utility and availability of starting materials.

The data shown in table V demonstrate that the better inhibitors are produced when Y = NH_2 or OH. A similar pattern of activity can be seen in table III. Generally the primary amino-group produced better PLA_2 inhibition and antiinflammatory activity than the alcohol that was in turn, better than the ketone. Further evidence of the importance of Y = H, NH_2 can be seen in the comparison of **7** (Y = H, NH_2) and **106** (Y = H, NHAc) and **42** (Y = H, NH_2) and **43** (Y = H, NHAc).

The data in table VI indicate that there is a correlation between the 'spacer' *n* and PLA_2 inhibitory activity. As a result of these findings, we concentrated our efforts on species where *n* = 2 or 3. However, where comparisons could be made, we found little difference in the *in vivo* activity. A constant concern about adverse central nervous system effects further guided our choice of targets in that we avoided synthesizing many 2-amino-alcohols.

In an attempt to find the best moiety for X, we synthesized a very limited series of compounds where X was CH_2, S, or NH. It should be noted that attempts to prepare compounds where X was oxygen and *n* was 2 were not successful by the route shown in scheme 2; the resulting ketoether undergoes facile 'retro-Michael' reaction. The results from this study are shown in table VII. Although **37** and **38** are about twice as potent against the PMN–PLA_2 enzyme as **39**, they are about half as efficacious as **39** in the car paw. Generally, the data indicated that the best antiinflammatory PLA_2 inhibitor resulted when X was NH. Further indication that alkylation or acylation of X, where X = NH, resulted in loss of activities can be seen in the comparisons of **40** and **41**, **69** and **70**, and **7** and **106**; and those cases in tables I and II where *R*-NH-CO was transformed to R-NH-CH_2.

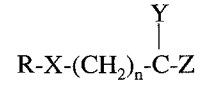
The data in tables I–VIII suggest that the best antiinflammatory PLA_2 inhibitors should have the general structure shown in formula II where the nature of the R-moiety has not been fully defined.



where Y is NH_2 or OH

II

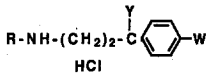
Table III. Activity as a function of Z.



Cmpd	*R*[a]	*X*	*n*	*Y*	*Z*	*PAN–PLA_2 (IC_{50}, μM)*	*PMN–PLA_2 (IC_{50}, μM)*	*Car paw ED_{30}, mg/kg*
1	DHA	NH HCl	3	H, OH	n-Hexyl	48.0	> 750.0	> 50.0
2	DHA	NH HCl	3	H, OH	H	1.4	< 7.5	> 50.0
3	DHA	NH HCl	3	H, OH	Me	2.1	260.0	82.0
4	DHA	NH HCl	3	H, NH_2 HCl	Me	1.5	5.4	7.0
5	DHA	NH HCl	3	H, OH	4-F-Phe	14.0	7.5	5.9
6	DHA	NH HCl	3	H, OH	Phe	180.0	15.0	23.0
7	DHA	NH HCl	2	H, NH_2 HCl	4-F-Phe	2.3	5.6	8.0
8	DHA	NH HCl	2	H, NH_2 HCl	Phe	3.5	10.0	44.9
9	DHA	NH HCl	2	H, NH_2 HCl	4-MeO-Phe	5.0	14.0	100.0
10	DHA	NH HCl	2	H, NH_2 HCl	4-MeS-Phe	3.8	9.0	58.8
11	2-NAP	NH HCl	2	=O	OEt	300.0		
12	2-NAP	NH HCl	2	=O	4-F-Phe	11.0		
13	2-NAP	NH HCl	2	=O	4-MeO-Phe	6.5		
14	2-NAP	NH HCl	2	=O	4-MeS-Phe	> 25.0		
18	DHA	NH HCl	3	=O	OEt	2.5		46.9
19	DHA	NH HCl	3	=O	Me	1.8		
20	DHA	NH HCl	3	=O	4-F-Phe	1.0		> 50.0

[a]DHA = dehydroabietyl; NAP-2 = naphthyl-2-ethyl.

Table IV. a. Activity as a function of substitution W on Z.



Cmpd	*R*[a]	*Y*	*W*	*PAN–PLA$_2$, IC$_{50}$, μM, Found*	*PAN–PLA$_2$, IC$_{50}$, μM, Calcd*
22	DHA	H, OH	MeO	15.0	14.4
24	DHA	H, OH	MeS	2.9	2.2
28	DHA	H, OH	F	5.0	5.3
103	DHA	H, OH	H	22.1	22.4
23	DHA	=O	MeO	55.0	55.4
25	DHA	=O	MeS	230.0	229.6
27	DHA	=O	F	40.0	41.8
100	DHA	=O	H	4.8	3.0
10	NAP-2	H, NH_2 HCl	MeS	3.8	4.3
35	NAP-2	H, NH_2 HCl	F	0.7	0.3
105	NAP-2	H, NH_2 HCl	MeO	22.0	21.9
12	NAP-2	=O	F	11.0	11.1
13	NAP-2	=O	MeO	6.5	6.4
14	NAP-2	=O	MeS	25.0	25.0

Table IV. b. Selected aromatic substituent constants for **W**.

W	π	σ*p*	*F*	*R*	*MR*
H	0.00	0.00	0.00	0.00	1.03
F	0.14	0.06	0.43	−0.34	0.92
MeO	−0.02	−0.27	0.26	−0.51	7.87
MeS	0.61	0.00	0.20	−0.18	13.82

[a]DHA = dehydroabietyl; NAP-2 = naphthyl-2-ethyl.

We had observed that slight differences in the R-group affected the biological profile of the inhibitors. Typical examples are shown in table IX. We finally turned our efforts to understanding the requirements of the R-group. Throughout these studies, the only requirement for R was that it be large and/or lipophilic. This allows us to progress initially from the *n*-butyl group to the dehydroabietyl group without fully understanding the requirements.

In an attempt to understand the requirements for R, we studied those compounds in table II where Z was 4-fluorophenyl, Y was NH_2 (HCl), *n* was 2 or 3, and X was NH (HCl); PMN PLA_2 IC_{50}s had been determined; and car paw ED_{30}s had been calculated. This selection process provided us with a series of antiinflammatory PLA_2 inhibitors where R varied only in log P, 'bulkiness' as represented by MR, the distance between X and Y, and molecular shape. We used computer calculated molar refractivity (C-MR) and the computer calculated log P (ClogP) of the molecules although they were co-linear for many members of the series, and the computer-generated log P's for some of the members of the series were unrealistic. However, the ClogP values were co-linear with a homologous series of alkylamines used by Lien and Wang [11]. Attempts to determine true log P's from octanol–water partitioning using UV spectroscopy on some members of the series were unsuccessful.

The intent was to obtain a simple qualitative understanding of the contribution R had on biological activity. Plots of log (PMN PLA_2 IC_{50}) *vs* ClogP and C-MR, as shown in Fig 1a, b indicated that PLA_2 inhibition was related to inhibitor lipophilicity and bulkiness though not to the degree, as had been anticipated. Curve-fitting to these 2 plots (Fig 1a and Eqs 2, 3) resulted in the anticipated parabolas. Analysis of these curves indicated that for this series of compounds, the best PMN PLA_2 inhibitors should have ClogP = 11–13 and C-MR = 14–16. Alter-

Table V. Activity as a function of Y.

R-X-$(CH_2)_n$-C(Y)-Z

Cmpd	*R*[a]	*X*	*n*	*Y*	*Z*	*PAN–PLA$_2$, IC$_{50}$, μM*	*PMN–PLA$_2$, IC$_{50}$, μM*	*Car paw, ED$_{30}$, mg/kg*
21	DHA	NH	2	MeO-N=	4-MeO-Phe	> 25.0	12.0	
9	DHA	NH HCl	2	H, NH_2 HCl	4-MeO-Phe	5.0	14.0	
22	DHA	NH HCl	2	H, OH	4-MeO-Phe	15.0	220.0	
23	DHA	NH TsOH	2	O=	4-MeO-Phe	55.0	310.0	
24	DHA	NH HCl	2	H, OH	4-MeS-Phe	2.9	> 75.0	85.0
10	DHA	NH HCl	2	H, NH_2 HCl	4-MeS-Phe	3.8	< 7.5	58.8
25	DHA	NH TsOH	2	O=	4-MeS-Phe	230.0		
26	DHA	NH	2	MeO-N=	4-MeS-Phe	> 25.0		
27	DHA	NH HCl	2	O=	4-F-Phe	40/0	> 750.0	8.0
28	DHA	NH HCl	2	H, OH	4-F-Phe	5.0	25.0	3.6
7	DHA	NH HCl	2	H, NH_2 HCl	4-F-Phe	1.8	5.6	8.0
20	DHA	NH HCl	3	O=	4-F-Phe	1.0	> 750.0	> 50.0
5	DHA	NH HCl	3	H, OH	4-F-Phe	14.0	7.5	5.9
29	DHA	NH HCl	3	H, NH_2 HCl	4-F-Phe	2.6	2.7	5.0
30	*n*-Dodecyl	NH HCl	2	O=	4-F-Phe	19.0	48.0	> 100.0
31	*n*-Dodecyl	NH HCl	2	H, OH	4-F-Phe	8.0	4.9	18.2
32	*n*-Dodecyl	NH HCl	2	MeO-N=	4-F-Phe	20.0		
33	*n*-Dodecyl	NH HCl	2	H, NH_2 HCl	4-F-Phe	3.1	4.9	22.0

[a]DHA = dehydroabietyl

Table VI. Activity as a function of *n*.

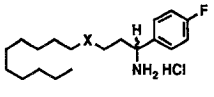
R-X-$(CH_2)_n$-C(Y)-Z

Cmpd	*R*[a]	*X*	*n*	*Y*	*Z*	*PAN–PLA$_2$, IC$_{50}$, μM*	*PMN–PLA$_2$, IC$_{50}$, μM*	*Car paw, ED$_{30}$, mg/kg*
28	DHA	NH HCl	2	H, OH	4-F-Phe	5.0	25.0	3.6
5	DHA	NH HCl	3	H, OH	4-F-Phe	14.0	7.5	5.9
7	DHA	NH HCl	2	H, NH_2 HCl	4-F-Phe	1.8	5.6	8.0
29	DHA	NH HCl	3	H, NH_2 HCl	4-F-Phe	2.6	2.7	5.0
34	NAP-2	NH HCl	1	H, NH_2 HCl	4-F-Phe	14.0		
35	NAP-2	NH HCl	2	H, NH_2 HCl	4-F-Phe	0.7	150.0	54.3
26	NAP-2	NH HCl	3	H, NH_2 HCl	4-F-Phe	16.0		

[a]DHA = dehydroabietyl; NAP-2 = naphthyl-2-ethyl

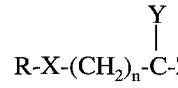
natively, the data in table X could be fitted to a linear model as shown in graph 1b and Eqs 4 and 5. A comparison of Eq 2 *vs* Eq 4 and Eq 3 *vs* Eq 5 showed that the relationship between enzyme inhibitory activity and ClogP and CMR was better expressed by the parabolic model than the linear model. Table XII lists the relevant statistical data on Eqs 2–5. There was little statistically significant difference between the 2 models. The linear model possibly describes one leg of the parabola. Further, a parabolic (quadratic equation) interpretation is logical, suggesting an ideal lipophilic and size requirement; whereas a linear model would be total nonsense, suggesting no limit to

Table VII. Activity as a function of X.



Cmpd	*X*	*PMN–PLA$_2$, IC$_{50}$, μM*	*Car paw ED$_{30}$, mg/kg*
37	CH_2	14.0	71.8
38	S	13.0	78.9
39	NH HCl	25.0	41.9

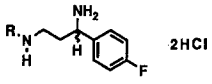
Table VIII. Activity as a function of X and Y.



Cmpd	*R*[a]	*X*	*n*	*Y*	*Z*	*PAN–PLA$_2$ (IC_{50}, μM)*	*PMN–PLA$_2$ (IC_{50}, μM)*	*Car paw ED_{30}, mg/kg*
40	NAP-1	NEt HCl	2	NH_2 HCl	4-F-Phe	20.0		
41	NAP-1	NH HCl	2	NH_2 HCl	4-F-Phe	7.5	47.0	57.0
42	4-MeS-Phe	S	3	NH_2 HCl	4-F-Phe	2.3	44.0	
43	4-MeS-Phe	S	3	NHAc	4-F-Phe	> 25.0	11.0	
44	DHA	NH AcOH	3	NH_2 AcOH	Phe		11.0	29.0
45	DHA	NAc	3	NHAc	Phe		> 750.0	

[a]DHA = dehydroabietyl; NAP-1 = naphthyl-1-ethyl.

Table IX. Activity as a function of the structure of R.



Cmpd	*R*	*PAN–PLA$_2$, IC_{50}, μM*	*PMN–PLA$_2$, IC_{50}, μM*	*Car paw, ED_{30}, mg/kg*
35		0.7	150.0	54.3
41		7.5	47	57.0

the size or lipophilicity of the inhibitors of the enzyme. Our interpretation of these data have not been accepted without controversy [12].

$$\log (PLA_2\ IC_{50}) = 4.3–0.7\ (\log P) + 3.0e^{-2}\ (\log P)^2 \quad (2)$$
$$n = 28 \quad r^2 = 0.90 \quad s = 0.31 \quad F = 106.48$$

$$\log (PLA_2\ IC_{50}) = 9.1–1.1\ (MR) + 3.3e^{-2}\ (MR)^2 \quad (3)$$
$$n = 28 \quad r^2 = 0.78 \quad s = 0.46 \quad F = 43.77$$

$$\log (PLA_2\ IC_{50}) = 3.5–0.4\ (\log P) \quad (4)$$
$$n = 28 \quad r^2 = 0.87 \quad s = 0.34 \quad F = 181.71$$

$$\log (PLA_2\ IC_{50}) = 5.6–0.5\ (MR) \quad (5)$$
$$n = 28 \quad r^2 = 0.76 \quad s = 0.47 \quad F = 80.46$$

Plots of car paw ED_{30}, μM/kg *vs* ClogP and C-MR are shown in figure 2a, b. Regression analysis, to determine which model (parabolic or linear) provided a better description of the data, produced Eqs 6–9. Again the parabolas provided the better fit, and indicated that the best antiinflammatories should have ClogP = 6–8 and C-MR = 11–13. These data would suggest that the lipophilicity

$$\text{Car paw } ED_{30} = 397.5–98.2\ (\log P) + 7.4\ (\log P)^2 \quad (6)$$
$$n = 28 \quad r^2 = 0.64 \quad s = 37.11 \quad F = 22.0$$

$$\text{Car paw } ED_{30} = 194.3–14.0\ (\log P) \quad (7)$$
$$n = 28 \quad r^2 = 0.29 \quad s = 51.0 \quad F = 10.6$$

$$\text{Car paw } ED_{30} = 941.0–143.7\ (MR) + 6.0\ (MR)^2 \quad (8)$$
$$n = 28 \quad r^2 = 0.59 \quad s = 39.6 \quad F = 17.9$$

$$\text{Car paw } ED_{30} = 314.1–19.2\ (MR) \quad (9)$$
$$n = 28 \quad r^2 = 0.41 \quad s = 46.4 \quad F = 18.1$$

and size requirements for optimal antiinflammatory are different from those required for PLA_2 inhibition (Clog P = 11–13 and C-MR = 14–16). A plot of car paw ED_{30} *vs* log (PMN–PLA_2) is shown in figure 3. The best representation for the data in table X, as plotted in figure 3, was a line with an r^2 = 0.41. Visual analysis of this plot showed 2 sets of outliers. When the outliers were removed from consideration, the linear correlation went from r^2 = 0.41 to 0.85 (Fig 4).

The outliers **53** and **54** belong to a homologous series of propyl diamines (**33**, **39**, **46–54**) differing only in the number of methylenes (n = 2) in R. A

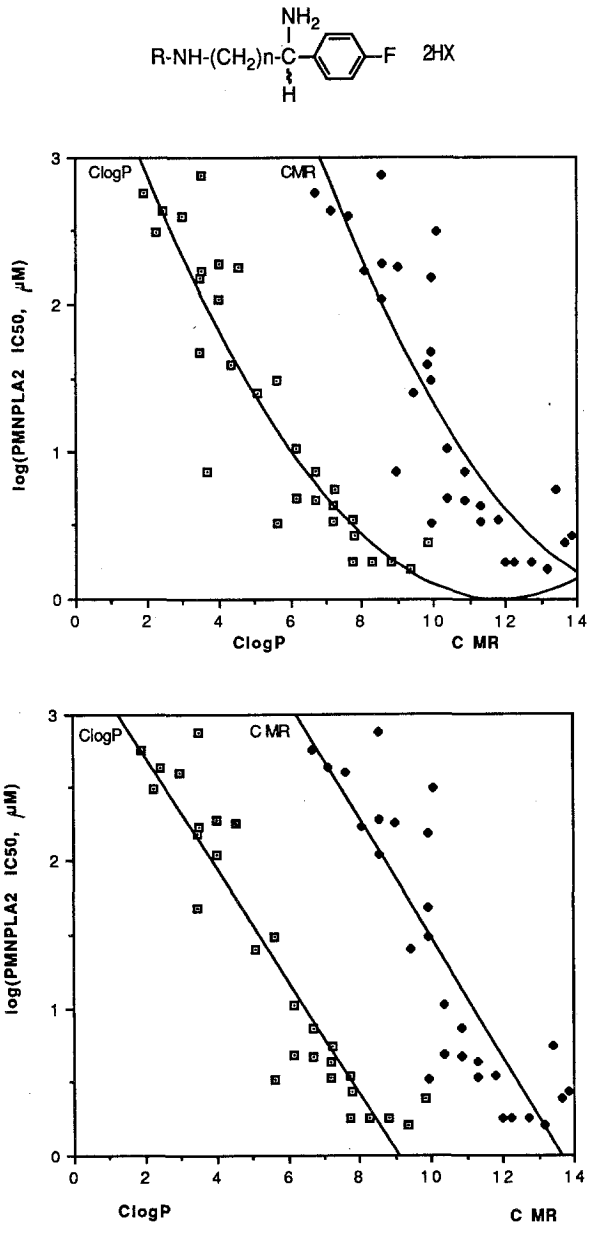


Fig 1. a. Plot of log(PMN PLA_2 IC_{50}, μM) *vs* Clog P and C-MR for compounds in table X: the polynomial regression. **b.** Plot of log(PMN PLA_2 IC_{50}, μM) *vs* Clog P and C-MR for compounds in table X: the linear regression.

visual analysis of figure 5 clearly suggest that except for **53** and **54**, the relationship between antiinflammatory activity and PLA_2 inhibition is linear. A similar result can be seen for the butyl diamine series (n = 3) in figure 6. In the absence of other data, we interpreted these deviations from linearity to be the result of poor gastrointestinal absorption or drug distribution. Compounds **72** and **99** are relatively bulky groups and as such may also be affected by poor drug metabolism. Compound **99** also exists as a possible mixture of four diastereomers. Clearly log P and MR do not explain the differences in biological activity observed for the sets of compounds in table IX. These observations suggest that secondary and/or tertiary structures play an important role in the biology of these compounds.

A more rigorous statistical analysis (QSAR) of the compounds in table II, including other mediators of inflammation such as cyclooxygenase, 5-lipoxygenase, PAF, and IL-1 biosynthesis; and a limited amount of drug metabolism data is planned (Wilkerson W, unpublished observations).

Stereospecificity

As can be seen from the data in table XII, there was no significant difference in the biological data produced by the enriched samples over the racemate. This lack of stereospecificity may indicate allosteric binding [12].

Conclusion

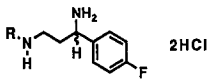
The data in this report suggests that compounds described in figure 2 where Z is 4-fluorophenyl; Y is H, NH_2 or H, OH; n is 2 or 3; X is NH; and R is large and/or lipophilic are orally active antiinflammatories that are more efficacious than indomethacin, and a large degree of the observed antiinflammatory activity can be attributed to the inhibition of PLA_2. We have attempted to correlate *in vitro* activity with *in vivo* activity without fully understanding the metabolism of the compounds. Studies are planned to determine the metabolic fate of some members of the above series, particularly those compounds that have antiinflammatory activity different from that predicted. The data obtained in this study will be used to refine our template so that we might be able to design and synthesize more potent and efficacious antiinflammatory PLA_2 inhibitors.

Experimental protocols

Chemical methods

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.

Table X. a. Activity as a function of the size of R for the propyl series. **b.** Calculated *vs* found Car paw ED_{30} using Eq 17[c].



a. Cmpd	R	PAN–PLA_2 IC_{50}, μM	PMN–PLA_2, IC_{50}, μM	Car paw, % Inh @ 100 mg/kg	Car paw ED_{30}, mg/kg	C-MR[a]	Clog P[a,b]
46	*n*-Butyl	805.1	576.8	32.0	73.8	6.688	1.921
47	*n*-Hexyl	400.0	403.6	41.0	64.5	7.616	2.979
48	*n*-Heptyl	18.0	169.7	49.0	58.6	8.079	3.508
49	*n*-Octyl	5.4	110.0	56.0	49.1	8.543	4.037
39	*n*-Decyl	4.5	25.0	63.0	41.9	9.471	5.095
50	*n*-Undecyl	2.9	3.3	81.0	16.0	9.935	5.624
33	*n*-Dodecyl	3.1	4.9	72.0	22.0	10.399	6.153
51	*n*-Tridecyl	3.1	4.7	74.0	33.0	10.862	6.682
52	*n*-Tetradecyl	3.0	4.4	71.0	35.0	11.326	7.211
53	*n*-Hexadecyl	3.6	1.8	43.0	60.0	12.254	8.269
54	*n*-Octadecyl	7.7	1.6	36.0	69.7	13.181	9.327

R-NH-$(CH_2)_2$-$CH(NH_2)$-(4-F-Phe) 2HCl

b. Cmpd	PMN–PLA_2, IC_{50}, μM	Car paw, % Inh @ dose, mg/kg	C-MR	Car paw, ED_{30}, mg/kg Found	Car paw, ED_{30}, mg/kg Calcd[c]
7	5.6	81–50	13.400	8.0	49.1
35	150.0	51–100	9.960	54.3	70.0
41	47.0	49–100	9.960	57.0	54.0
46	576.8	32–100	6.688	73.8	66.1
69	7.4	not done	8.982	not done	21.8
72	310.0	32–50	10.108	50.0	81.0
73	750.0	62–100	8.543	42.9	82.0
78	> 750.0	30–100	6.688	100.0	> 69.7
97	39.0	26–100	9.855	not done	51.0
99	1.8	10–100	11.982	not done	23.3
106	90.5	37–100	15.326	81.0	102.3

[a]Computer calculated for the 'free base', Paloma College; [b]the computer program 'flags' values > 7.0 as 'unreal in nature'. [c]Car paw, ED_{30} = 13.85 ln(PMN PLA_2, IC_{50}) + 7.02 (C MR) – 68.93.

The synthetic methods and schemes for some of the compounds discussed above have been disclosed earlier [1]. Depending on the nature of the reactants and the desired product, additional members of the series may be synthesized using the procedures shown below.

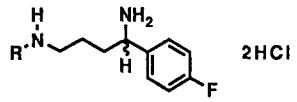
1-(4-Fluorophenyl)-3-(dodecylamino)-1-propanone hydrochloride ***30***

A solution of 3-chloro-4'-fluoropropiophenone (18.66 g, 0.1 mol) in 100 ml THF was treated with triethylamine (11.1 g, 0.11 mol) and stirred at room temperature for 1 h. The mixture was treated with n-dodecyl-amine hydrochloride (22.18 g, 0.1 mol) and refluxed for 6 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between 200 ml CH_2Cl_2 and 100 ml 5% $NaHCO_3$. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was triturated with 110 ml 1 N HCl–Et_2O, and the resulting solid was collected by filtration, washed with Et_2O, and dried to give the desired product in 66% (24.5 g) yield; mp 169–172°C; IR (nujol): 1673 cm^{-1} C = O; NMR ($CDCl_3$ TMS): δ 0.87 (t, 3H, CH_3), 1.2 [m, 18H, $(CH_2)_9$], 1.87 (m, 2H, CH_2-C-N), 3.0 (t, 2H, CH_2CO), 3.37 (m, 2H, CH_2-N), 3.67 (t, 2H, CH_2-N), [7.03 (d of d, 2H), 7.93 (m, 2H) 4-F-Phe]; mass spec *m/e* 335/180; Anal for $C_{21}H_{34}NOF$ HCl, MW 371.96 (C, H, N).

1-(4-Fluorophenyl)-3-(dodecylamino)-1-propanol hydrochloride ***31***

A suspension of **30** (4.5 g, 0.012 mol) in 50 ml THF and 15 ml *i*-PrOH was cooled in an ice bath and treated with $NaBH_4$

Table XI. Activity as a function of R for the butyl series.



Cmpd	*R*	*PMN–PLA$_2$, IC$_{50}$, μM*	*Car paw, % Inh @ 100 mg/kg*	*Car paw, ED$_{30}$, mg/kg*	*C-MR*	*Clog P*	*Caw paw, Calcd ED$_{30}$*[a]
55	*t*-Octyl	> 750.0	< 30	not done	9.007	3.956	
56	*n*-Butyl	430.5	41	65.7	7.152	2.450	55.1
57	*n*-Heptyl	191.4	44	62.5	8.543	4.037	55.8
58	*n*-Octyl	180.0	46	50.2	9.007	4.566	58.1
59	*n*-Decyl	30.0	76	27.7	9.935	5.624	46.0
60	*n*-Undecyl	10.7	69	35.0	10.399	6.153	38.6
61	*n*-Dodecyl	7.4	61	35.0	10.862	6.682	37.9
62	*n*-Tridecyl	3.4	69	34.9	11.326	7.211	33.0
63	*n*-Tetradecyl	3.5	58	45.2	11.790	7.740	36.3
64	*n*-Hexyldecyl	1.8	68	35.1	12.718	8.798	35.5
65	*n*-Octadecyl	2.4	56	49.0	13.645	9.856	44.3

[a]Calcd Car paw ED_{30} = 10.02 ln(PMN–PLA_2 IC_{50}) + 6.34 (C MR) – 51.03 from eq 24.

Table XII. Statistics on equations 2–5.

Equation	*Eq 2* $3.0\ e^{-2}\ (log\ P)^2 - 0.7\ (log\ P) + 4.3$	*Eq 4* $-0.4\ (log\ P) + 3.5$	*Eq 3* $3.3\ e^{-2}\ (MR)^2 - 1.1\ (MR) + 9.1$	*Eq 5* $-0.4\ (MR) + 5.6$
n	28	28	28	28
Coefficient of determination (r^2)	0.90	0.87	0.78	0.76
Adjusted coefficient (r^2)	0.89	0.87	0.76	0.75
Coefficient of correlation (*r*)	0.95	0.94	0.88	0.87
Standard error of estimate	0.31	0.34	0.46	0.47
F-ratio	106.48	181.71	43.77	80.46
Probability > *F*	0.000	0.000	0.000	0.000

Data obtained using Cricket Graph 1.3 and StatWorks 1.2 using a Macintosh IIx

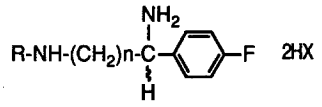
(0.9 gm, 0.024 mol) and stirred at room temperature until no starting material remained as evidenced by TLC ($CHCl_3$–MeOH, 9:1). The mixture was concentrated *in vacuo*, and the residue was triturated with 100 ml 1 N HCl. The resulting gelatinous product was collected with difficulty by filtration, washed with water, dried, washed with Et_2O, and redried to give the desired product in 55% (2.5 g) yield; mp 235°C dec; IR (nujol): 3400 cm^{-1} OH; NMR (DMSO–d_6 TMS): δ 0.86 (t, 3H, CH_3), 1.28 [m, 18H, $(CH_2)_9$], 1.53 (m, 2H, CH_2-C-N), 1.93 (m, 2H, CH_2C-O), 2.73 (m, 2H, CH_2-N), 2.87 (m, 2H, CH_2-N), 4.67 (m, 1H, H-C-O), [7.16 (d of d, 2H), 7.37 (m, 2H) 4-F-Phe]; mass spec *m/e* 337/164; Anal for $C_{21}H_{36}NOF$ HCl, MW 373.99 (C, H, N).

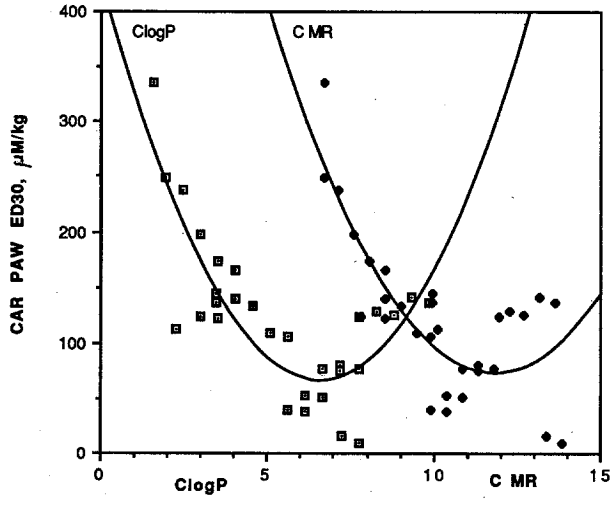
1-(4-Fluorophenyl)-3-(dodecylamino)-1-propanone-O-methyloxime ***32***

A mixture of **30** (15.0 g, 0.0403 mol) and methoxyamine hydrochloride (6.7 g, 0.081 mol) in 100 ml Pyr–EtOH (1:1) was stirred at room temperature for 24 h, refluxed for 3 h, and concentrated *in vacuo*. The residue was triturated with cold water, and the resulting solid was collected by filtration, washed with water, and dried to give the desired product in 93% (15.0 g) yield; mp 81–82°C; NMR ($CDCl_3$ TMS): δ 0.9 (t, 3H, CH_3), 1.23 (m, 18H), 1.87 (m, 2H, CH_2-C-N), 2.93 (t, 2H, CH_2-CN), 3.13 (m, 2H, CH_2-N), 3.70 (t, 2H, CH_2-N), 3.97 (s, 3H, OCH_3), [7.03 (d of d, 2H), 7.87 (m, 2H) 4-F-Phe]; mass spec *m/e* 364/346; Anal for $C_{22}H_{37}N_2OF$ HCl, MW 401.01 (C, H, N).

1-(4-Fluorophenyl)-3-(dodecylamino)-1-propaneamine dihydrochloride ***33***

To a cold suspension of **32** (10.0 g, 0.025 mol) in 50 ml dry THF was added 1 M BH_3–THF complex (100 ml), and the mixture was stirred at room temperature for 16 h and refluxed for 3 h. The mixture was cooled in an ice bath, and the excess borane was decomposed with MeOH. The mixture was concentrated *in vacuo*, treated with 100 ml 6 N HCl, heated at 80°C for 1 h, and concentrated *in vacuo*. The mixture was made alkaline with 1 N NaOH, and extracted with Et_2O. The ether layer was washed with 1 N NaOH, water, and brine; dried over $MgSO_4$; and filtered; and the filtrate was treated with 50 ml 1 N HCl–Et_2O while vigorously stirring in an ice bath. The result-





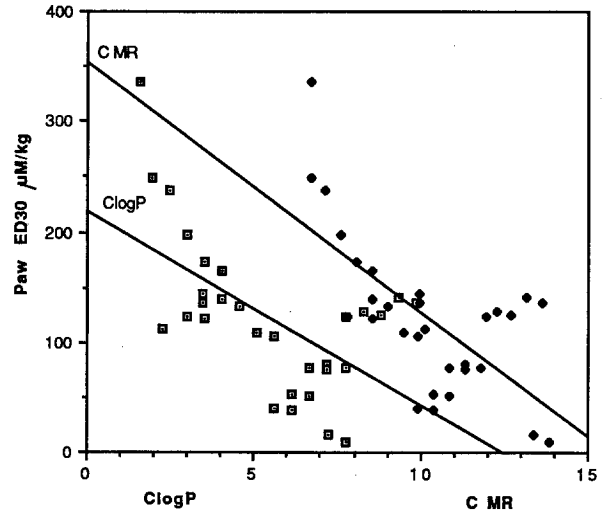
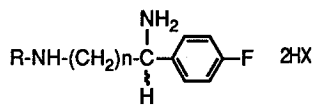


Fig 2. a. Car paw IC_{50}, µM/kg *vs* Clog P and C-MR for compounds in table X: the parabolic model. **b.** Car paw IC_{50}, µM/kg *vs* Clog P and C-MR for compounds in table X: the linear model.

ing white solid was collected by filtration, washed with Et_2O, and dried to give the desired product in 91% (9.3 g) yield; mp 243–245°C; NMR (DMSO–d_6 TMS): δ 0.83 (t, 3H, CH_3), 1.23 (m, 20H), 1.56 (m, 2H, CH_2-C-N), 2.1–3.0 (m, 6H, CH_2-N-CH_2-CH_2-C-N), 4.50 (m, 1H, H-C-N), [7.30 (d of d, 2H), 7.67 (m, 2H), p-F-Phe]; mass spec *m/e* 336/150; Anal for $C_{21}H_{37}N_2F$ 2 HCl, MW 409.46 (C, H, N).



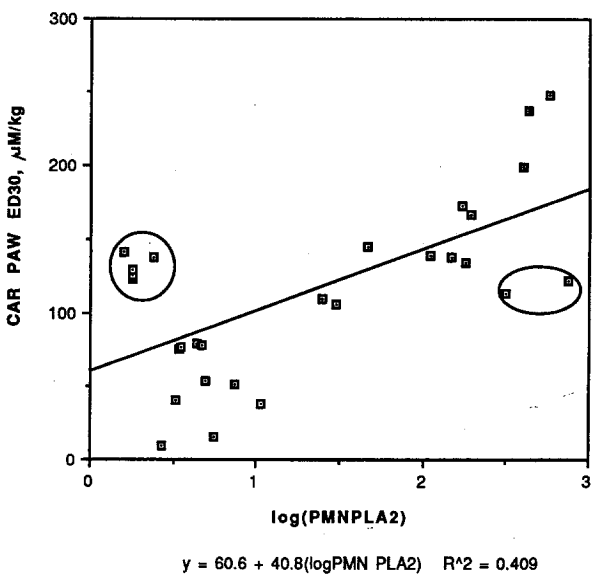


Fig 3. Plot of Car paw ED_{30}, µM/kg *vs* log(PMN PLA_2 IC_{50}, µM) for compounds in table X.

1-(4-Fluorophenyl)-4-(4'-methylphenyl)-1-butanone ***66***

A mixture of 4-thiocresol (24.8 g, 0.2 mol), 4-chloro-4'-fluorobutyrophenone (40.1 g, 0.2 mol) and triethylamine (22.3 g, 0.22 mol) in 150 ml THF was stirred under nitrogen for 16 h at room temperature, and concentrated *in vacuo*. The residue was partitioned between 300 ml Et_2O and 200 ml water, and the organic layer was washed with 5% $NaHCO_3$, water, and brine; dried over $MgSO_4$; filtered; and concentrated to an oil to give the product in 99% (57.1 g) yield; IR (neat): 1700 cm^{-1} C = O; NMR ($CDCl_3$ TMS): δ 1.8–1.95 (m, 2H, CH_2-C-CO), 2.26 (s, 3H, CH_3), 2.98 (t, 2H, CH_2-CO), 3.16 (t, 2H, CH_2-S), [7.1–7.4 (m, 6H), 7.9–8.1 (m, 2H) Ar]; mass spec *m/e* 288; Anal for $C_{17}H_{17}OFS$, MW 288.38 (C, H, S).

1-(4-Fluorophenyl)-4-[(2-methyl-2-propyl)phenoxy]-1-butanone ***93***

A suspension of NaH (2.6 g, 0.11 mol) and 4-chloro-4'-fluorobutyrophenone-2,2-dimethylpropylene ketal (28.7 g, 0.1 mol) in 100 ml dry DMF was cooled in an ice bath and treated with a solution of 3-*tert*-butylphenol (15.0 g, 0.1 mol) in 75 ml dry DMF. The mixture was stirred in the ice bath for 1 h, and refluxed for 16 h. The mixture was cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between 200 ml Et_2O and 100 ml 5% $NaHCO_3$, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to an oil (40.0 g) with an IR (neat) showing no hydroxyl or carbonyl. The ketalether (33.0 g, 0.082 mol) was dissolved in 100 ml MeOH, treated with 25 ml conc HCl, and stirred at room temperature until TLC ($CHCl_3$–MeOH, 9:1) indicated no starting material. The mixture was diluted to 500 ml with water, and the resulting

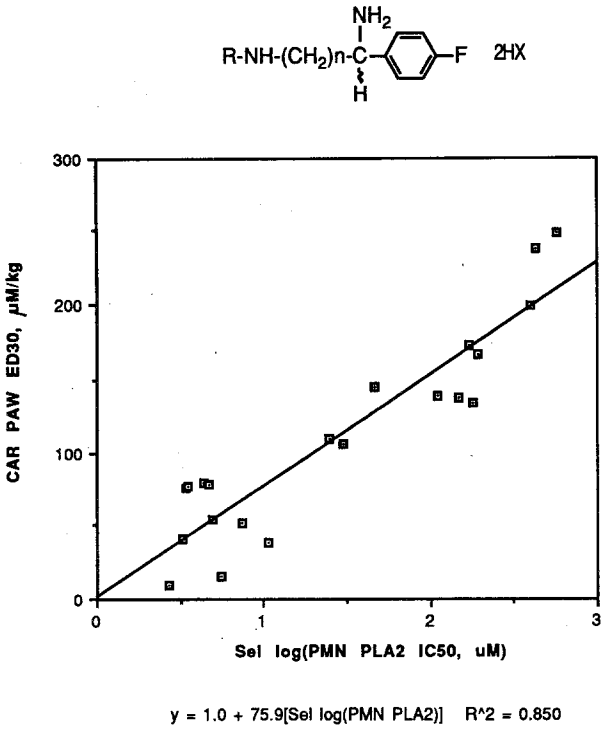


Fig 4. Plot of Car paw ED_{30}, µM/kg *vs* log (PMN PLA_2 IC_{50}, µM) for selected compounds in table X.

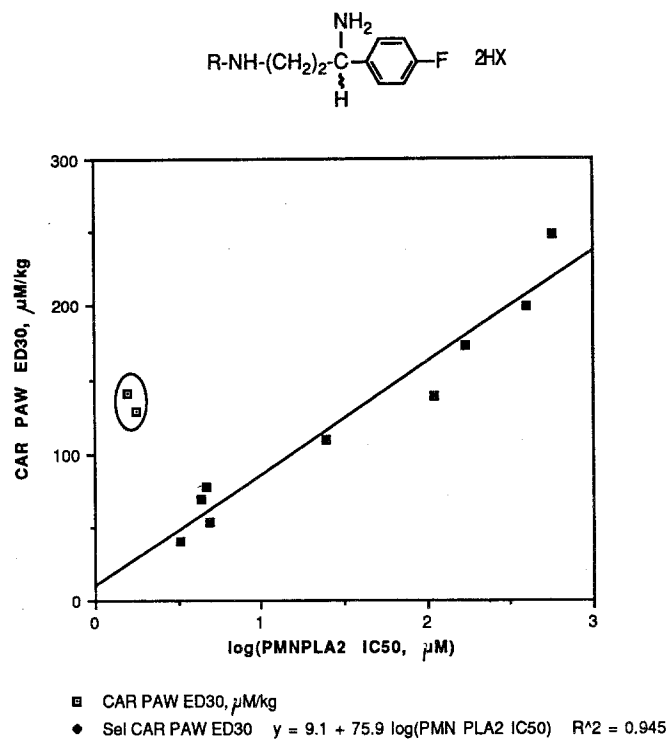


Fig 5. Plot of Car paw ED_{30}, µM/kg *vs* log (PMN PLA_2 IC_{50}, µM) for compounds in table X where $n = 2$.

ketone was collected by filtration, washed with water, and dried to give the product in 97% (25.0 g) yield; mp 69–70°C; IR (nujol): 1678 cm^{-1} C = O; NMR ($CDCl_3$ TMS): δ 1.33 (s, 9H, ^{t}Bu), 2.25 (d of t, 2H, CH_2), 3.20 (t, 2H, CH_2CO), 4.08 (t, 2H, CH_2-O), [6.73 (m, 1H), 6.8–7.3 (m, 5H), 8.00 (m, 2H), Ar]; mass spec *m/e* 314; Anal for $C_{20}H_{23}O_2F$, MW 314.38 (C, H).

1-(4-Fluorophenyl)-4-[(2-methyl-2-propyl)phenoxy]-1-butanol ***94***

A solution of 95 (6.0 g, 0.019 mol) in 50 ml dry THF was cooled in an ice bath and treated with 1 M BH_3–THF (25 ml) and stirred at room temperature for 4 h. The excess BH_3 was decomposed with water, and the mixture was concentrated *in vacuo*. The residue triturated with 50 ml conc HCl at 80°C for 1 h, cooled to room temperature, and extracted with 100 ml ether. The ether solution was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to an oil of constant weight. The product was obtained in 91% (5.5 g) yield; IR (neat): 3392 cm^{-1} OH; NMR ($CDCl_3$ TMS); δ 1.30 (s, 9H, ^{t}Bu); 1.6–2.0 (m, 4H, CH_2CH_2-C-O), 3.98 (t, 2H, CH_2-O), 4.73 (t, 1H, CH-O), 6.5–7.4 (m, 8H, Ar); mass spec *m/e* 316; Anal for $C_{20}H_{25}O_2F$, MW 316.40 (C, H).

Biological methods

Porcine pancreatic phospholipase-A_2 assay (PAN–PLA_2)

The assay was a modification of that reported by Hirata *et al* [7]. Inhibitors were dissolved in 0.2 M Tris–HCl (trihydroxymethyl aminoethane hydrochloride) at pH 8.5 or dissolved in DMSO and then diluted with Tris–HCl buffer (maximum DMSO concentration, 7%). The reaction was run in a total volume of 0.1 ml with the enzyme at a final concentration of 19 units/ml (0.025 µg protein/ml) which gave approximately 5000–8000 dpm (disintegrations per minute) of activity in a buffer containing 25 mM Tris (trihydroxymethyl aminoethane), 25 mM glycylglycine, 25 mM $CaCl_2$, and 0.75 mM EDTA (tetra sodium salt), pH 8.5. The test compound was added to the enzyme mixture, incubated for 2 min. To this mixture was added the substrate [(arachidonyl-1-^{14}C)-L-α-1-palmitoyl-2-arachidonyl phosphatidylcholine] at a final concentration of 7 µM (40 000 dpm) to begin the reaction that proceeded for 5 min at 37°C. The reaction was terminated by freezing in dry ice–ethanol slurry, and the arachidonic acid products were separated from the unreacted substrate using silica gel columns.

All reactions were run in duplicate, and each reported value represents the results of at least 3 sets of duplicates. IC_{50}, in µM, was calculated by linear regression analysis using 3 concentrations of drug, spanning the 50% inhibition point.

Rat polymorphonuclear leukocyte phospholipase-A_2 (PMN–PLA_2) [8]

A partially purified PLA_2 from casein-elicited rat neutrophils was used as the enzyme. The low speed subtenant 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

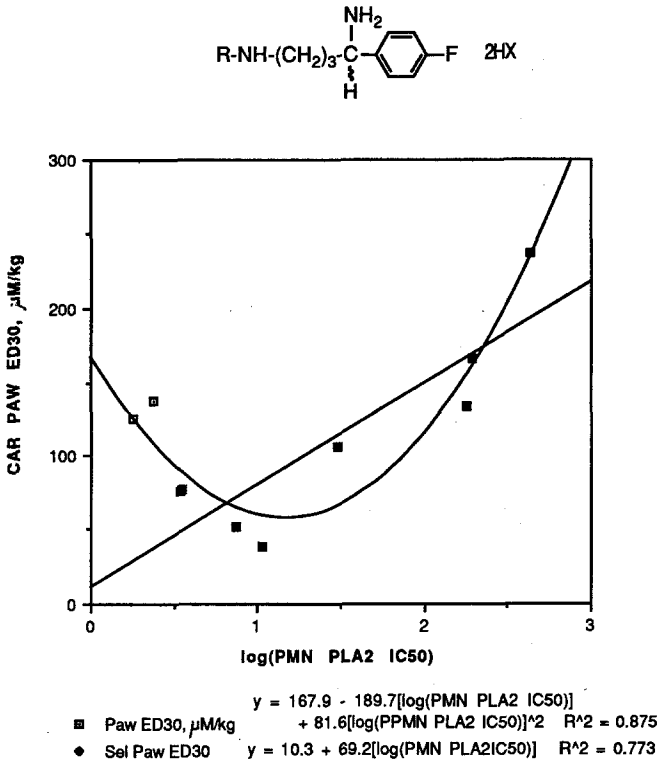


Fig 6. Plot of Car paw ED_{30}, μM/kg *vs* log (PMN PLA_2 IC_{50}, μM) for compounds in table X where $n = 3$.

incubation continued for 30 min. The reaction mixture was worked up as outlined above. IC_{50} value, in μM, was calculated by linear regression analysis using 3 concentrations of drug spanning the 50% inhibition point.

Carrageenan-induced edema in rat hind paw (car paw) [9]
The method is a modification of the method reported by Winters *et al* [9]. 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 recorded immediately after injection. Three hours later the volume of the paw was again measured. Activity is recorded as the calculated percentage inhibition of edema cause by the dose of 100 mg/kg of drug. ED_{30}, in mg/kg, was calculated by linear regression analysis using three concentrations of drug, spanning the 30% inhibition point.

Table XIV. Standards.

Standard	*PAN–PLA_2, IC_{50}, μM*	*PMN–PLA_2, IC_{50}, μM*	*Car paw % Inh @ dose, mg/kg (ED_{30}, mg/kg)*
Dexamethasone	> 25.0	> 750.0	48 10 (< 1.0)
Indomethacin	315.0	250.0	43% 9 (1.0)
Mepacrin	22.0	100.0	48% 50 (3.5)
U-3585	2.3	> 250.0	53% 100 (> 20.0)

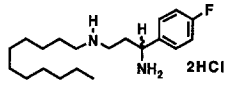
Acknowledgments

The authors would like to thank T Ferago, J Giannaras, R Goodman, L Mantegna, M McGowan, L Moody, D Pedicord, B Rule, T Stevens and D Treml for their assistance in conducting the biological assays.

References

1 Wilkerson W, DeLucca I, Galbraith W, Gans K, Harris R, Kerr J (1991) *Eur J Med Chem* 26, 667–676
2 Dijkstra B, Renetseder R, Kalk K, Hol W, Drenth J (1983) *J Mol Biol* 168, 163
3 Wallach DP (1980) US Pat 4, 239, 780
4 Wallach DP, Brown VJ (1981) *Biochem Pharmacol* 11, 1315
5 Draper MD, Keller K (19??) US Pat 3, 272, 838
6 Sakito Y, Yoneyoshi Y, Suzukamo G (1988) *Tetrahedron Lett* 29(2), 223
7 Hirata F (1980) *Proc Natl Acad Sci (USA)* 77, 2533
8 Galbraith W, Ignar D (1985) *Pharmacologist* 27, 177
9 Winters CA, Risley EA, Nuss GW (1962) *Proc Soc Biol Med* 111, 544
10 Franke R (1984) *In: Theoretical Drug Design Methods* Elsevier, New York
11 Lien EJ, Wang PH (1980) *J Pharm Sci* 69, 648
12 Monod J, Changeux P, Jacob F (1965) *J Mol Biol* 6, 306

Table XIII. Data on the enriched isomers of 50.



Cmpd	*PAN-PLA_2, IC_{50}, μM (± se)*[a]	*PMN–PLA_2, IC_{50}, μM (± se)*[a]	*Car paw, % Inh @ 100 mg/kg (± se)*[a]	*Isomer ratio*[b]	*$[a]^{25}D°$ (c, 1, MeOH)*[c]
50	2.9 (0.4)	3.3 (0.6)	81.0 (3)	50:50	0.0
(+)-50	1.5 (0.3)	4.0 (0.9)	73.0 (1)	82:18	+ 5.6
(–)-50	8.5 (0.5)	3.7 (0.6)	70.0 (3)	21:79	– 7.4

[a]Mean ± standard error, $n = 3$; [b]determined by analytical chiral HPLC, error ± 5%; [c]± absolute error of 0.5°.