(DMSO-d_6) δ 8.43 (b s, 1 H), 8.19 (m, 1 H), 8.01 (m, 1 H), 7.82 (m, 2 H), 4.72 (b m, 2 H), 4.30 (b m, 4 H); CIMS m/z (relative intensity) 266 ($[M + H]^+$, 100).

Pharmacology. In Vitro Investigations. The methods used in the determination of in vitro binding affinities have been previously reported. Briefly, dopamine D-2 affinity was assayed against [^{3}H]spiperone in homogenized rat corpus striatum tissues.[15] The affinity for 5-HT_{1A} and 5-HT_1 receptors was determined in homogenized rat hippocampal tissues versus [^{3}H]8-OH-DPAT and [^{3}H]5-HT, respectively.[19] The remaining in vitro studies utilized homogenized rat cerebral cortex tissues. The reference ligand used in the determination of 5-HT_2 affinity[20] was [^{3}H]-spiperone; [^{3}H]WB-4101 was used to assay α_1-adrenergic receptor sites.[21] The calculation of the 50% inhibition of specific binding (IC_{50}) was performed in duplicate in the standard manner using a log-probit analysis with $n = 5$; where n is the number of different test ligand concentrations used to calculate the IC_{50}.

In Vivo Investigations. Procedures for the conditioned avoidance response (CAR) test, the inhibition of apomorphine-induced stereotypy (APO) test, and the induction of catalepsy test have all been previously described.[15,17,18]

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Design of Potent Linear PAF Antagonists

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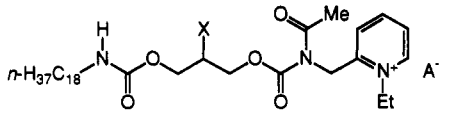
A new series of linear PAF antagonists have been prepared as simplified models of our previously described tetrahydrofuran and dioxolane derivatives.

Introduction

As part of the continuing effort of our laboratories in the area of PAF antagonists, we initiated a research program based on the design of simple, easy-to-make, achiral PAF analogues.[1] A preceding paper described and evaluated a number of PAF-related structures featuring a disubstituted tetrahydrofuran or dioxolane ring as the key modification of the PAF glycerol framework.[2] Those compounds, which were envisioned as conformationally restricted PAF analogues, showed excellent activities and surpassed in some cases that of the chemically related reference compound 2-[[*N*-acetyl-*N*-[[2-methoxy-3-[(octadecylcarbamoyl)oxy]propoxy]carbonyl]amino]-methyl]-1-ethylpyridinium chloride (CV-6209, **1**).[3] In general, we found that the activity of the compounds was maintained within the same order of magnitude independently of the nature of the five-membered ring pattern shown in Figure 1. Furthermore, the presence of a well-defined acyl carbamate pyridinium group (B) at one end and a lipophilic chain (A) at the other proved to be essential for attaining high levels of activity.

Prompted by these results and in search of more simplified compounds, we sought to examine new entities in which the ring would be replaced by a linear, achiral framework maintaining the nature of the substituents. Ideally, these compounds would have an expeditious synthesis and avoid the issue of manufacturing diastereomeric and enantiomeric mixtures. Finally, their evaluation would determine the putative influence upon bioactivity of the C-2 substituent present in several other known glycerol-derived PAF antagonists (e.g. **3** vs **1** and **2**).[3,4] In this paper we report the synthesis and biological evaluation of a series of linear PAF antagonists as substitutes of our previously reported five-membered ring systems.



1: X = OMe; A = Cl (Takeda; CV-6209)
2: X = 3-isoxazolyloxy; A = Cl (Sankyo)
3: X = H; A = I

Chemistry

The synthesis of the target compounds was accomplished by minor variations of known methodologies.[2,3b] The compounds were easily synthesized from inexpensive glycols according to the five-step sequence outlined for the sulfide series in Scheme I, which in general terms comprises (1) derivatization of one of the glycol hydroxyl groups by reaction with either an alkyl halide (ether series,

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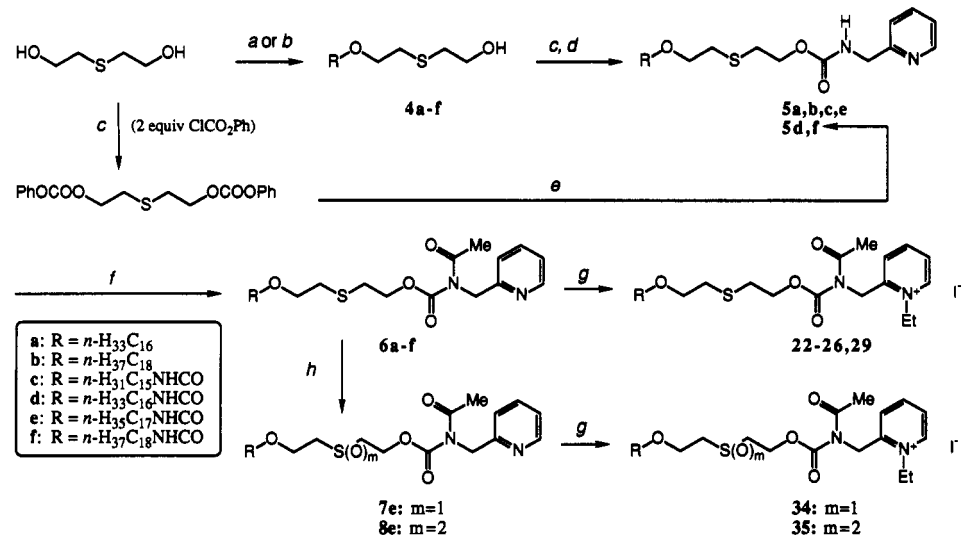
Scheme I[a]



[a] (a) NaH, n-$H_{2n+1}C_nBr$, DMF, room temperature, 18 h; (b) n-$H_{2n+1}C_nN{=}C{=}O$, pyr; (c) $ClCO_2Ph$, pyr, $CHCl_3$, 10 °C, 2 h; (d) 2-picolylamine, $CHCl_3$, reflux, 20 h; (e) n-$H_{2n+1}C_nNH_2$, $CHCl_3$, reflux, 20 h, then 2-picolylamine, $CHCl_3$, reflux, 20 h; (f) AcCl, TEA, $CHCl_3$, room temperature, 48 h; (g) EtI, CH_3CN, 65 °C, 24–48 h; (h) MCPBA (m = 1, 1 equiv; m = 2, 2 equiv), $CHCl_3$, −78 °C to room temperature.

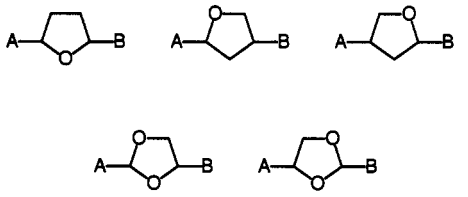
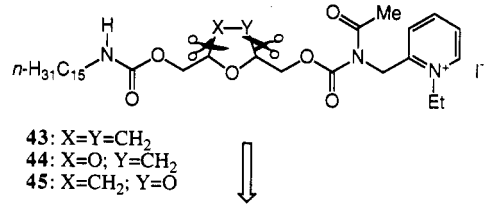


Figure 1.



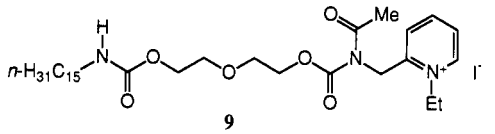


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Figure 2.

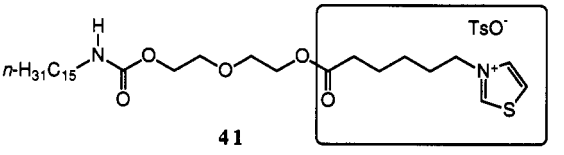

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Figure 3.

step *a*) or an alkyl isocyanate (urethane series, step *b*), (2) reaction of the remaining free hydroxyl with phenyl chloroformate in pyridine–chloroform (step *c*), (3) urethane formation by reaction of the resulting phenyl carbonate with 2-picolylamine (step *d*), (4) N-acetylation by internal delivery of the acetyl group from an acylpyridinium intermediate using acetyl chloride (step *f*), and (5) pyridine quaternization using an alkyl iodide (step *g*). Pharmaceutically acceptable salts were obtained by ion-exchange chromatography. When the fatty amine was commercially available, the synthesis leading to the urethane series was further simplified as the bis-carbamate derivative could be provided by the stepwise addition of the fatty amine and 2-picolylamine to the glycol bis-phenyl carbonate, in one pot (step *e*). In the sulfide-containing compounds, a byproduct identified as ethyl sulfide **21** (Table I) was detected in minor quantities (<10%) in the last step. The formation of this undesired product, presumably derived from an intermediate sulfonium ion, was reduced by shortening the reaction time and lowering temperature. Compounds featuring a sulfoxide (**34**) or sulfone (**35**) function were obtained by stoichiometric oxidation of the sulfides with MCPBA at low temperatures (step *h*), prior to quaternization of the pyridine nitrogen. The ease of execution of these preparations, the good yield obtained for every step, and the crystallinity of the intermediates allowed multigram productions without the need of silica purifications. As a representative example, 66 g of compound **27** was obtained in five steps from 2,2′-thiodiethanol in 42% overall yield.

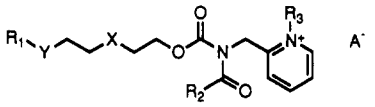
Results and Discussion

The activity of the compounds was monitored by their ability to inhibit the in vitro PAF-induced rabbit platelet aggregation and in vivo PAF-induced hypotension in rats. In general, the relative order of potency of the various analogues in the in vivo assay did not always faithfully mirror that seen in vitro. As a matter of fact, the hypotension test showed a lower sensitivity to structural changes, and many of the most active compounds in vitro had an ID_{50} in the 0.01–0.02 mg/kg iv range in vivo. For this reason, we used the in vitro values for structure optimization and structure–activity relationship (SAR) discussion. The results of both tests are given in Table I.

For the purposes of our initial investigation, we prepared compound **9**, resulting from the removal of the top moiety of products **43–45**, as shown in Figure 2. We were delighted to see that this key simplification maintained, or even improved, the PAF antagonist activity (Table I). The effectiveness of compound **9** gave support to the underlying hypothesis and prompted the synthesis and evaluation of new linear entities.

As expected by the results obtained in our earlier work, the acyl carbamate substituent gave a remarkably higher potency than the thiazolium counterpart depicted in Figure 3 (Table I, entry **41**). We, therefore, carried out all the optimization work directly on compounds bearing the former group. Nonetheless, to make sure that the

Table I. PAF Antagonist Activities of Linear Compounds



compd	X	R_1	Y	R_2	R_3	A	platelet aggregation: IC_{50},[a] μM	blood pressure: ID_{50},[b] mg/kg iv	formula[c]	anal.[d]
9	O	$H_{31}C_{15}$	$NHCO_2$	Me	Et	I	0.022 (0.014–0.035)	0.041 (0.037–0.045)	$C_{31}H_{54}IN_3O_6 \cdot H_2O$	C, H, N
10	O	$H_{31}C_{15}$	$NHCO_2$	EtO	Et	I	0.023 (0.016–0.033)	0.032 (0.021–0.051)	$C_{32}H_{56}IN_3O_7 \cdot {}^1/_2H_2O$	C, H, N
11	O	$H_{31}C_{15}$	$NHCO_2$	Me	Pr	I	0.23 (0.17–0.30)	0.68 (0.35–1.3)	$C_{32}H_{56}IN_3O_6 \cdot {}^3/_4H_2O$	C, H, N
12	O	$H_{35}C_{17}$	$NHCO_2$	Me	Et	I	0.017 (0.012–0.026)	0.067 (0.031–0.14)	$C_{33}H_{58}IN_3O_6 \cdot H_2O$	C, H, N
13	O	$H_{35}C_{17}$	$NHCO_2$	Me	Me	I	0.055 (0.034–0.089)	0.13 (0.092–0.19)	$C_{32}H_{56}IN_3O_6 \cdot 2H_2O$	C, H, N
14	O	$H_{35}C_{17}$	$NHCO_2$	EtO	Et	I	0.0088 (0.0053–0.014)	0.021 (0.015–0.030)	$C_{34}H_{60}IN_3O_7 \cdot {}^3/_4H_2O$	C, H, N
15	O	$H_{37}C_{18}$	$NHCO_2$	Me	Et	I	0.018 (0.016–0.021)	0.019 (0.017–0.021)	$C_{34}H_{60}IN_3O_6$	C, H, N
16	O	chx-CH_2	$NHCO_2$	Me	Et	I	2.0 (1.2–3.2)	0.50 (0.26–0.93)	$C_{23}H_{36}IN_3O_6$	C, H, N
17	O	$Ph(CH_2)_3$	$NHCO_2$	Me	Et	I	1.3 (1.1–1.5)	0.88 (0.64–1.2)	$C_{25}H_{34}IN_3O_6 \cdot {}^5/_2H_2O$	C, H; N[e]
18	O	Me_3C	CO_2	Me	Et	I	1.7 (1.1–2.4)	>5	$C_{20}H_{31}IN_2O_6 \cdot {}^3/_2H_2O$	C, H, N
19	O	$H_{37}C_{18}$	O	Me	Et	I	0.022 (0.010–0.051)	0.024 (0.014–0.042)	$C_{33}H_{59}IN_2O_5 \cdot H_2O$	C, H, N
20	OCH_2CH_2O	$H_{31}C_{15}$	$NHCO_2$	Me	Et	I	0.012 (0.0077–0.018)	0.028 (0.017–0.046)	$C_{33}H_{58}IN_3O_7 \cdot {}^1/_2H_2O$	C, H, N
21	S		H	Me	Et	I	0.19 (0.13–0.28)	0.37 (0.15–0.85)	$C_{15}H_{23}IN_2O_3S$	H, N; C[f]
22	S	$H_{33}C_{16}$	O	Me	Et	I	0.0051 (0.0037–0.0070)	0.014 (0.010–0.019)	$C_{31}H_{55}IN_2O_4S$	C, H; N[g]
23	S	$H_{37}C_{18}$	O	Me	Et	I	0.015 (0.0079–0.028)	0.016 (0.012–0.021)	$C_{33}H_{59}IN_2O_4S \cdot H_2O$	C, H; N[h]
24	S	$H_{31}C_{15}$	$NHCO_2$	Me	Et	I	0.0084 (0.0056–0.013)	0.040 (0.022–0.073)	$C_{31}H_{54}IN_3O_5S \cdot 2H_2O$	C, H, N
25	S	$H_{33}C_{16}$	$NHCO_2$	Me	Et	I	0.014 (0.0095–0.020)	0.010 (0.0060–0.017)	$C_{32}H_{56}IN_3O_5S \cdot {}^3/_2H_2O$	C, H, N
26	S	$H_{35}C_{17}$	$NHCO_2$	Me	Et	I	0.0056 (0.0048–0.0065)	0.013 (0.0080–0.014)	$C_{33}H_{58}IN_3O_5S \cdot H_2O$	C, H, N
27	S	$H_{35}C_{17}$	$NHCO_2$	Me	Et	Cl	0.012 (0.0089–0.016)	0.018 (0.016–0.020)	$C_{33}H_{58}ClN_3O_5S \cdot H_2O$	C, H, N
28	S	$H_{35}C_{17}$	$NHCO_2$	Me	Et	NO_3	0.0086 (0.0083–0.0090)	0.013 (0.0068–0.026)	$C_{33}H_{58}N_4O_8S$	C, H, N
29	S	$H_{37}C_{18}$	$NHCO_2$	Me	Et	I	0.0059 (0.0042–0.0082)	0.015 (0.012–0.019)	$C_{34}H_{60}IN_3O_5S$	C, H, N
30	S	$H_{35}C_{17}$	$NHCO_2$	2-$MeOC_6H_4$	Et	I	0.0091 (0.0056–0.015)	0.019 (0.014–0.024)	$C_{39}H_{62}IN_3O_6S \cdot H_2O$	C, H, N
31	S	$H_{35}C_{17}$	$NHCO_2$	$H_{31}C_{15}$	Et	I	8.7 (8.4–9.0)	>5	$C_{47}H_{86}IN_3O_5S$	C, H, N
32	S	$H_{35}C_{17}$	$NHCO_2$	*t*-Bu	Et	I	0.15 (0.13–0.18)	0.33 (0.23–0.47)	$C_{36}H_{64}IN_3O_5S \cdot H_2O$	C, H, N
33	S	$H_{35}C_{17}$	$NHCO_2$	EtO	Et	I	0.018 (0.015–0.021)	0.028 (0.020–0.038)	$C_{34}H_{60}IN_3O_6S \cdot {}^1/_2H_2O$	C, H, N
34	SO	$H_{35}C_{17}$	$NHCO_2$	Me	Et	I	0.027 (0.015–0.048)	0.025 (0.019–0.033)	$C_{33}H_{58}IN_3O_6S \cdot 3H_2O$	C, H, N
35	SO_2	$H_{35}C_{17}$	$NHCO_2$	Me	Et	I	0.011 (0.010–0.012)	0.019 (0.012–0.032)	$C_{33}H_{58}IN_3O_7S$	C, H, N
36	CH_2	$H_{31}C_{15}$	$NHCO_2$	Me	Et	I	0.024 (0.017–0.034)	0.057 (0.044–0.072)	$C_{32}H_{56}IN_3O_5 \cdot {}^1/_2H_2O$	C, H, N
37	CH_2	$H_{25}C_{12}$	CH_2	Me	Et	I	0.87 (0.53–1.4)	0.17 (0.10–0.30)	$C_{29}H_{51}IN_2O_3$	C, H, N
38	CH_2	$H_{29}C_{14}$	CH_2	Me	Et	I	0.16 (0.12–0.21)	0.33 (0.22–0.51)	$C_{31}H_{55}IN_2O_3$	C, H, N
39	CH_2	$H_{33}C_{16}$	CH_2	Me	Et	I	1.4 (1.1–1.8)	0.53 (0.37–0.76)	$C_{33}H_{59}IN_2O_3$	C, H, N
40							24 (4.5–120)	>5	$C_{13}H_{19}IN_2O_3$	C, H, N
41							12 (3.5–39)	>5	$C_{36}H_{60}N_2O_8S_2$	C, H, N
42							1.4 (1.3–1.5)	0.50 (0.31–0.80)	$C_{31}H_{56}IN_3O_5$	C, H, N
43							0.072 (0.039–0.13)	0.098 (0.032–0.30)	$C_{33}H_{56}IN_3O_6 \cdot {}^1/_2H_2O$	C, H, N
44							0.026 (0.016–0.043)	0.11 (0.069–0.17)	$C_{32}H_{54}IN_3O_7 \cdot {}^3/_4H_2O$	C, H, N
45							0.0075 (0.0052–0.011)	0.085 (0.062–0.12)	$C_{32}H_{54}IN_3O_7 \cdot {}^1/_2H_2O$	C, H, N
3							0.023 (0.015–0.036)	0.020 (0.011–0.036)	$C_{33}H_{58}IN_3O_5$	C, H, N
1 (I^-)							0.011 (0.0078–0.015)	0.011 (0.0063–0.018)	$C_{34}H_{60}IN_3O_6 \cdot H_2O$	C, H, N
1 (Cl^-)							0.012 (0.010–0.014)	0.0078 (0.0056–0.011)	$C_{34}H_{60}ClN_3O_6 \cdot H_2O$	C, H, N

[a] Concentration required to inhibit PAF-induced maximum aggregation by 50%. Parentheses contain 95% confidence limits. [b] Dose required to reduce the lowering of the arterial blood pressure caused by PAF by 50%. Parentheses contain 95% confidence limits. [c] Empirical formula with amount of water of hydration. [d] Analytical results for the indicated elements are within ±0.4% of the calculated values, unless indicated otherwise. [e] N: calcd, 6.51; found, 6.02. [f] C: calcd, 41.10; found, 41.56. [g] N: calcd, 4.13; found, 3.59. [h] N: calcd, 3.86; found, 3.32.

structure–activity trends found within the acyl carbamate moiety in the previous series were maintained in these related compounds, we prepared several products featuring different R_2CO (acyl) and R_3 (alkyl) groups. Thus, replacing the acetyl group by ethoxycarbonyl (entries **10** vs **9**, **14** vs **12**, and **33** vs **26**) roughly maintained the activity, whereas lack of an acyl substituent at this site dramatically decreased the potency (**42**, i.e. deacetylated **9**). A long acyl

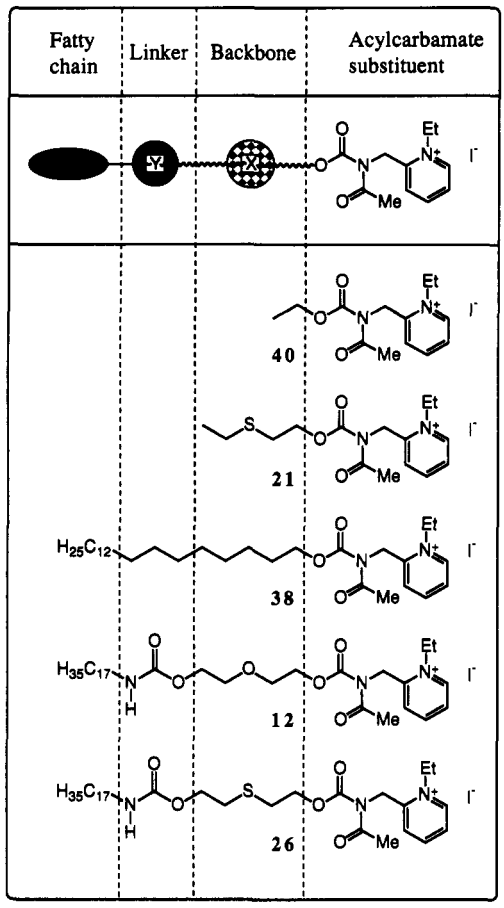


Figure 4. Structural variables on linear antagonists.

chain was badly tolerated (**31**) and a pivaloyl group decreased the activity by 1 order of magnitude (**32**). The previously reported 2-methoxybenzoyl group[5] did maintain potency (**30**), but the product obtained was extremely hygroscopic. Changing the ethyl group at the pyridinium nitrogen by methyl (**13**) or propyl (**11**) also resulted in an impairment of activity. We next focused on the linear side of the molecule (Figure 4). First, we explored the role of the central framework by employing spacers (X) which differed in length and nature. The question of whether an heteroatom was mandatory at all in the central backbone was addressed by preparing 1,5-pentanediol derivative **36**. To our surprise, this compound exhibited a very similar activity to that of its oxygen counterpart, product **9**. On the other hand, substitution of the oxygen by sulfur led to a 3-fold enhancement in potency in the urethane series (Y = $NHCO_2$) (**26** vs **12**, **24** vs **9**) and a somewhat less pronounced improvement in the ether series (Y = O) (**23** vs **19**). In fact, the sulfides (X = S) turned out to be the best suited compounds of the whole series. Higher oxidation states of the sulfur atom (sulfoxide **34** and sulfone **35**) resulted in a 2–4-fold impairment of activity. In regard to the size of the spacer, and given a similar overall length for the compound, shortening the distance between the linker and the acyl carbamate group from five to three methylene units had no effect upon activity (**36** vs **3**). In a similar way, lengthening that distance by one ethylene glycol unit (**20**) resulted in no effect. In general, thus, a variety of functional groups and lengths were well-tolerated on the central backbone.

With regard to the nature of the functional group Y linking the lipophilic group, both carbamoyl and ethers were of similar activities when optimized (compare entry **22** with **26**). That the presence of at least one electronegative atom around these latitudes was important (either in Y or in X) was proved by compounds **37–39**. Indeed, these simplified products, featuring an all-carbon chain departing directly from the acyl carbamate group, were roughly 1 order of magnitude less active than the rest. Interestingly, compound **21**, which lacks both the fatty chain and the linker Y, but keeps the sulfur atom, showed a potency similar to that of compound **38**, suggesting that the fatty chain and the electron donor might share a comparable influence upon overall activity. To complete our work, we prepared compound **40**, featuring the acyl carbamate substituent only. The poor activity found for this product (over 1000 times lower than **26**) highlighted definitively the importance of the fatty chain and the electron donor as extra binding sites necessary to attain good levels of potency.

The length and nature of the alkyl chain R_1 was also assessed. Substituents R_1 carrying a ring-containing chain (entries **16** and **17**) were *ca.* 2 orders of magnitude less potent than those having a linear fatty chain. A pivaloyl ester showed poor activity too (entry **18**). Among the fatty urethanes, C_{15} (**9**), C_{17} (**12**), and C_{18} (**15**) chains were tested in the oxygenated backbone (X = O), and C_{15} (**24**), C_{16} (**25**), C_{17} (**26**), and C_{18} (**29**) in the sulfide analogues (X = S). The differences found were minor within each group.

The activity found for compound **3** is particularly relevant and further expands the SAR's in this area. Indeed, this compound, resulting from the removal of the methoxy group of **1**, showed an activity only slightly lower than that of its parent compound (ca. 2-fold), indicating that the PAF receptor is not highly demanding of a substituent and/or chirality at this position. This result is in accordance with the identical activities reported for both enantiomers of **1**.[3b]

We have shown that the substitution of the tetrahydrofuran or dioxolane ring of our previously reported PAF antagonists by a series of linear chains leads to retention of PAF antagonist activity in the resulting analogues. From this work one may tentatively conclude that *all that is needed to reach certain levels of anti-PAF potency is a long linear chain attached to the so-called acyl carbamate substituent* and that higher activities can be achieved by fine-tuning the position and nature of heteroatoms in the chain. In conclusion, our efforts in the search for the minimum structural requirements in this area have proved to be fruitful. Sulfides **23**, **26**, and **29** were chosen for further development because they met the criteria we had set forth at the beginning of the project, namely, same level of activity as other related products, absence of stereocenters, and a straightforward synthesis. Among them, compound **27** (UR-10324; the chloride form of **26**) proved to be preferred after being tested for topical antiinflammatory activity in several experimental models[6] and was selected for further development.

Experimental Section

A. Chemistry. Melting points were determined with a Mettler FP 80 central processor melting point apparatus and are uncorrected. Melting points of organic salts varied depending on the amount of water of the sample and should be regarded as

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approximated. Infrared spectra were recorded on a Perkin-Elmer 983 spectrophotometer. ^1H (80-MHz) and ^{13}C (20.1-MHz) NMR spectra were recorded on a Brücker AC 80 spectrometer and are reported in ppm on the δ scale, from the indicated reference. Combustion analyses were performed with a Carlo Erba 1106 analyzer. Liquid chromatography was performed with a forced flow (flash chromatography) of the indicated solvent system on SDS silica gel Chromagel 60 A C.C. (230–400 mesh). Analytical thin-layer chromatography (TLC) was performed with Macherey-Nagel 0.25 mm silica gel SIL G-25 plates. When necessary, solvents and reagents were dried prior to use. Tetrahydrofuran, diethyl ether, and toluene were distilled from sodium metal/benzophenone ketyl. Dichloromethane and triethylamine were distilled from calcium hydride. Unless otherwise specified, all nonaqueous reactions were conducted under a rigorously dried argon atmosphere, with oven-dried glassware.

C18-PAF-acether was synthesized from (*S*)-batyl alcohol[7] following a published procedure.[8] Compound **1** was prepared according to the literature[3b] and was selected as the reference compound.

2-[[2-[(Heptadecylcarbamoyl)oxy]ethyl]thio]ethanol (4e). A solution containing 2,2′-thiodiethanol (132 g, 1.08 mol) in dry pyridine (350 mL) was treated with a solution of heptadecyl isocyanate (nonpurified, 225 g) in pyridine (150 mL) and the resulting mixture was heated to 78 °C for 16 h. The mixture was concentrated and the resulting residue was dissolved in chloroform (4 L). The solution was washed successively with a 3 N HCl aqueous solution (2 × 0.5 L), 10% aqueous sodium bicarbonate (0.5 L), and brine (0.5 L). The organic layer was decanted and dried over anhydrous sodium sulfate, the drying agent was filtered off, and the filtrate was heated in the presence of decolorizing carbon. The mixture was filtered; the filtrate was concentrated to 1.5 L and was treated with hot acetone (1 L). Standing overnight at −20 °C afforded the product as white plates. A second crop was obtained after concentration and cooling (total weight 249 g, 77% yield, mp 87–90 °C). An analytical sample was obtained by flash chromatography (1:2 ethyl acetate–hexane): mp 85–86 °C; IR (KBr) ν 3304, 2948, 2912, 1677, 1541, 1464, 1377, 1316, 1293, 1275, 1146, 1046 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 5.0 (br s, 1 H, NH), 4.21 (t, J = 6.7 Hz, 2 H, $COOCH_2$), 3.74 (t, J = 6 Hz, 2 H, CH_2OH), 3.14 (q, J = 6 Hz, 2 H, RCH_2N), 2.69 (t, J = 6 Hz, 4 H, CH_2SCH_2), 2.6 (br s, 1 H, OH), 1.6–0.7 (m, ~33 H). Anal. ($C_{22}H_{45}NO_3S$) C, H, N.

2-[[*N*-[[2-[[2-[(Heptadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]pyridine (5e). Compound **4e** (197 g, 0.49 mol) was dissolved in a mixture of chloroform (3 L) and pyridine (80 mL, 0.97 mol) and the solution was cooled to 10 °C. Phenyl chlorocarbonate (67.5 mL, 0.54 mol) was then added dropwise and the solution was stirred 30 min at 10 °C and 2 h at room temperature, under an argon atmosphere. The reaction mixture was quenched by the addition of a 3 N HCl aqueous solution (0.5 L). The aqueous phase was discarded, and the organic phase was washed successively with 1 N HCl aqueous solution (2 × 0.5 L), a 10% sodium bicarbonate solution (0.5 L), and brine (0.5 L). Anhydrous sodium sulfate was added, the mixture was filtered, and the filtrate was concentrated (water aspirator) to 2 L. The solution was then treated with 2-picolylamine (65.7 g, 0.61 mol) and was stirred at reflux for 20 h. After cooling to room temperature, a 2 N sodium hydroxide solution (4 × 0.2 L) was added and the organic phase was separated and washed with brine (0.5 L). Anhydrous sodium sulfate was added, the mixture filtered, and the filtrate treated with decolorizing carbon under heating. The mixture was filtered, the filtrate was concentrated, and the residue was recrystallized from acetone (overnight, −20 °C) to afford a white solid. A second crop was obtained from the mother liquors (total weight 227 g, 87% yield, mp 76–78 °C). An analytical sample was obtained by flash chromatography (1:1 ethyl acetate–hexane): mp 73–74 °C; IR (KBr) ν 3303, 3052, 2912, 2843, 1680, 1588, 1538, 1462, 1428, 1289, 1274, 1206, 1146, 1073, 1046, 1034, 982 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.53 (br d, J = 5 Hz, 1 H, pyr), 7.65 (br t, J = 7.5 Hz, 1 H, pyr), 7.3–7.0 (m, 2 H, pyr), 6.1 (br s, 1 H, NH), 5.0 (br s, 1 H, NH), 4.50 (d, J = 5.5 Hz, 2 H, pyr-CH_2), 4.3–4.1 (m, 4 H, $COOCH_2$, $COOCH_2$), 3.10 (q, J = 6.0 Hz, 2 H, RCH_2N), 2.79 (t, J = 6.5 Hz, 4 H, CH_2SCH_2), 1.7–0.7 (m, ~33 H). Anal. ($C_{29}H_{51}N_3O_4S$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(heptadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]pyridine (6e). To a cooled (10 °C) solution of compound **5e** (216 g, 0.4 mol) in chloroform (3 L) was slowly added acetyl chloride (33 mL, 0.46 mol). The resulting solution was stirred for 1 h at 10 °C and 20 h at room temprature. The solution was then cooled (10 °C) and treated with triethylamine (64.5 mL, 0.46 mol) and additional acetyl chloride (33 mL, 0.46 mol). After stirring for 20 h at room temperature, triethylamine was added (70 mL) and the mixture was washed with a 10% sodium bicarbonate aqueous solution (0.5 L), water (4 × 0.5 L), and brine (0.5 L). The solution was treated with decolorizing carbon under heating, filtered, dried over anhydrous sodium sulfate, and filtered, and the filtrate was concentrated *in vacuo* to a red-brown solid. Recrystallization from a 1:1 mixture of ethyl acetate–hexane afforded the title product as a white solid (208 g, 89%, mp 66–68 °C). An analytical sample was obtained by flash chromatography (1:1 ethyl acetate–hexane): mp 62–63 °C; IR (KBr) ν 3325, 2915, 2846, 1740, 1685, 1590, 1529, 1464, 1423, 1384, 1372, 1351, 1260, 1246, 1204, 1141, 1079 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.50 (br d, J = 4 Hz, 1 H, pyr), 7.60 (dt, J_t = 1.8 Hz, J_d = 7.4 Hz, 1 H, pyr), 7.3–7.0 (m, 2 H, pyr), 5.08 (s, 2 H, pyr-CH_2), 4.9 (br s, 1 H, NH), 4.30 (t, J = 7 Hz, 2 H, $COOCH_2$), 4.16 (t, J = 4.7 Hz, 2H, $COOCH_2$), 3.14 (q, J = 6 Hz, 2 H, RCH_2N), 2.68 (t, J = 6.7 Hz, 4 H, CH_2SCH_2), 2.62 (s, 3 H, CH_3), 1.7–0.7 (m, ~33 H). Anal. ($C_{31}H_{53}N_3O_5S$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(heptadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]-1-ethylpyridinium Iodide (26). A solution of compound **6e** (173.2 g, 0.30 mol) and ethyl iodide (215 mL, 2.7 mol) in acetonitrile (215 mL) was heated at 65 °C for 2 days (monitored by TLC). The volatiles were then removed *in vacuo*, and the residue was treated with acetone (1.5 L) and filtered, and the filtrate was concentrated under reduced pressure. The yellow residue was recrystallized from acetone–ether to afford the title product as a yellow powder (205.3 g, 93%, mp 55–63 °C). TLC analysis revealed minor amounts of compounds **21** and deacetylated **26**. An analytical sample was obtained by flash chromatography (1:8 MeOH–$CHCl_3$): mp 48–53 °C; IR (KBr) ν 3322, 2912, 2845, 1744, 1686, 1623, 1578, 1508, 1461, 1371, 1338, 1224, 1159 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.54 (br d, J = 6 Hz, 1 H, pyr), 8.47 (br t, J = 7.5 Hz, 1 H, pyr), 8.04 (br t, J = 7 Hz, 1 H, pyr), 7.85 (br d, J = 8 Hz, 1 H, pyr), 5.50 (s, 2 H, pyr-CH_2), 5.05 (q, J = 7 Hz, 3 H, NEt, NH), 4.47 (t, J = 6.2 Hz, 2 H, $COOCH_2$), 4.16 (t, J = 6.5 Hz, 2 H, $COOCH_2$), 3.2–2.6 (m, 6 H, RCH_2N, CH_2SCH_2), 2.67 (s, 3 H, CH_3), 1.72 (t, J = 7 Hz, 3 H, NEt), 1.7–0.7 (m, ~33 H). Anal. ($C_{33}H_{58}IN_3O_5S{\cdot}H_2O$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(heptadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]-1-ethylpyridinium Chloride (27). A mixture of iodide **26** (100 g, 136 mmol) in 3:1 methanol–water (4 L) was passed through a column of Amberlite IRA-410 ion-exchange resin (600 g) previously washed with a 0.5 N HCl aqueous solution, water, a 5% solution of sodium chloride, and water. The fractions containing the product were mixed, saturated with sodium chloride, extracted with dichloromethane (2×), dried over anhydrous sodium sulfate, and filtered, and the filtrate was concentrated to a yellowish powder. Two recrystallizations from acetone afforded the chloride salt (66 g, 75% yield) as a white powder, free from the chloride salts of compounds **21** and deacetylated **26**, as determined by TLC analysis. An analytical sample was obtained by flash chromatography (1:8 MeOH–$CHCl_3$): mp 47–48 °C; IR (KBr) ν 3403, 2913, 2845, 1744, 1692, 1624, 1579, 1524, 1462, 1372, 1362, 1338, 1291, 1253, 1222, 1159, 1089, 987 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.97 (br d, J = 6 Hz, 1 H, pyr), 8.55 (br t, J = 7.5 Hz, 1 H, pyr), 8.08 (br t, J = 7 Hz, 1 H, pyr), 7.80 (br d, J = 8 Hz, 1 H, pyr), 5.52 (s, 2 H, pyr-CH_2), 5.17 (q, J = 7 Hz, 3 H, NEt, NH), 4.45 (t, J = 6.2 Hz, 2 H, $COOCH_2$), 4.14 (t, J = 6.5 Hz, 2

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H, $COOCH_2$), 3.2–2.6 (m, 6 H, RCH_2N, CH_2SCH_2), 2.67 (s, 3 H, CH_3), 1.72 (t, J = 7 Hz, 3 H, NEt), 1.7–0.7 (m, ~33 H); ^{13}C NMR (20.1 MHz, $CDCl_3$) δ (TMS) 172.71 (C), 156.10 (C), 153.10 (C), 152.81 (C), 147.21 (CH), 145.50 (CH), 127.04 (CH), 125.47 (CH), 66.23 (CH_2), 63.52 (CH_2), 53.93 (CH_2), 44.30 (CH_2), 40.99 (CH_2), 31.68 (CH_2), 30.96 (CH_2), 30.64 (CH_2), 29.80 (CH_2), 29.42 (CH_2), 29.09 (CH_2), 26.62 (CH_2), 26.14 (CH_3), 22.42 (CH_2), 15.96 (CH_3), 13.84 (CH_3). Anal. ($C_{33}H_{58}ClN_3O_5S·H_2O$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(heptadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]-1-ethylpyridinium Nitrate (28). In a similar manner, a solution of iodide **26** in 30% methanol–water was passed through a column of Amberlite IRA-410 ion-exchange resin previously washed with a saturated solution of sodium nitrate and water. The fractions containing the product were mixed, extracted with dichloromethane (2×), dried over anhydrous sodium sulfate, and filtered, and the filtrate was concentrated to a white powder: mp 47–48 °C; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.31 (br d, J = 6 Hz, 1 H, pyr), 8.40 (br t, J = 7.5 Hz, 1 H, pyr), 7.95 (br t, J = 7 Hz, 1 H, pyr), 7.73 (br d, J = 8 Hz, 1 H, pyr), 5.45 (s, 2 H, pyr-CH_2), 4.94 (q, J = 7 Hz, 3 H, NEt, NH), 4.44 (t, J = 6.3 Hz, 2 H, $COOCH_2$), 4.15 (t, J = 6.4 Hz, 2 H, $COOCH_2$), 3.2–2.6 (m, 6 H, RCH_2N, CH_2SCH_2), 2.65 (s, 3 H, CH_3), 1.70 (t, J = 7 Hz, 3 H, NEt), 1.7–0.7 (m, ~33 H). Anal. ($C_{33}H_{58}N_4O_8S$) C, H, N.

2-[[*N*-[[2-[[2-[(Octadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]pyridine (5f). To a cooled (0 °C) solution of 2,2′-thiodiethanol (21.2 g, 173 mmol) in chloroform (400 mL) and pyridine (56 mL) was added phenyl chloroformate (46 mL, 365 mmol) dropwise. After 30 min of stirring at 0 °C, the reaction mixture was quenched by the addition of a 6 N HCl aqueous solution (100 mL). The dicarbonate was isolated as a white solid following the procedure described in the preparation of **5e**. This compound was dissolved in chloroform (400 mL) and the solution was treated first with *n*-octadecylamine (47 g, 173 mmol, 20 h at reflux) and then with 2-picolylamine (18.8 g, 173 mmol, 20 h at reflux). The mixed urethane was isolated as for compound **5e** to afford 61.4 g (64%) of a white solid: mp 71–72 °C; IR (KBr) ν 3303, 3052, 2912, 2843, 1680, 1588, 1538, 1462, 1428, 1289, 1274, 1206, 1146, 1073, 1046, 1034, 982 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.53 (br d, J = 5 Hz, 1 H, pyr), 7.66 (dt, J_d = 1.6 Hz, J_t = 7.5 Hz, 1 H, pyr), 7.3–7.0 (m, 2 H, pyr), 6.0 (br s, 1 H, NH), 5.0 (br s, 1 H, NH), 4.48 (d, J = 5.4 Hz, 2 H, pyr-CH_2), 4.4–4.1 (m, 4 H, $COOCH_2$, $COOCH_2$), 3.12 (q, J = 6.0 Hz, 2 H, RCH_2N), 2.81 (t, J = 6.4 Hz, 4 H, CH_2SCH_2), 1.7–0.7 (m, ~35 H). Anal. ($C_{30}H_{53}N_3O_4S$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(octadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]pyridine (6f). Acetylation was performed according to the procedure described for the preparation of **6e** to afford the title compound as a white solid in quantitative yield: mp 61–63 °C; IR (KBr) ν 3355, 2915, 2843, 1742, 1683, 1590, 1516, 1455, 1426, 1386, 1373, 1347, 1254, 1195, 1156, 1082 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.50 (br d, J = 4 Hz, 1 H, pyr), 7.63 (dt, J_t = 1.8 Hz, J_d = 7.4 Hz, 1 H, pyr), 7.3–7.0 (m, 2 H, pyr), 5.08 (s, 2 H, pyr-CH_2), 4.9 (br s, 1 H, NH), 4.31 (t, J = 7 Hz, 2 H, $COOCH_2$), 4.16 (t, J = 4.7 Hz, 2 H, $COOCH_2$), 3.06 (q, J = 6 Hz, 2 H, RCH_2N), 2.69 (t, J = 6.7 Hz, 4 H, CH_2SCH_2), 2.62 (s, 3 H, CH_3), 1.7–0.7 (m, ~35 H). Anal. ($C_{32}H_{55}N_3O_5S$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(octadecylcarbamoyl)oxy]ethyl]thio]ethoxy]carbonyl]amino]methyl]-1-ethylpyridinium Iodide (29). Alkylation was performed according to the procedure described for **26** to afford the title compound as a yellow powder: mp 53–54 °C; IR (KBr) ν 3339, 2912, 2844, 1744, 1692, 1623, 1578, 1520, 1463, 1369, 1341, 1210, 1158 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.59 (br d, J = 6 Hz, 1 H, pyr), 8.56 (br t, J = 7.5 Hz, 1 H, pyr), 8.08 (br t, J = 7 Hz, 1 H, pyr), 7.85 (br d, J = 8 Hz, 1 H, pyr), 5.50 (s, 2 H, pyr-CH_2), 5.07 (q, J = 7 Hz, 3 H, NEt, NH), 4.47 (t, J = 6.2 Hz, 2 H, $COOCH_2$), 4.15 (t, J = 6.5 Hz, 2 H, $COOCH_2$), 3.2–2.6 (m, 6 H, RCH_2N, CH_2SCH_2), 2.67 (s, 3 H, CH_3), 1.74 (t, J = 7 Hz, 3 H, NEt), 1.7–0.7 (m, ~35 H). Anal. ($C_{34}H_{60}IN_3O_5S$) C, H, N.

2-[[*N*-Acetyl-*N*-[[3-[(octadecylcarbamoyl)oxy]propoxy]carbonyl]amino]methyl]-1-ethylpyridinium Iodide (3). In a similar manner to that described above, compound **3** was obtained from 1,3-propanediol: mp 50–80 °C; IR (KBr) ν 3441, 2912, 2844, 1745, 1691, 1625, 1577, 1524, 1463, 1392, 1342, 1212, 1161 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.61 (br d, J = 6 Hz, 1 H, pyr), 8.49 (br t, J = 7.5 Hz, 1 H, pyr), 8.03 (br t, J = 7 Hz, 1 H, pyr), 7.81 (br d, J = 8 Hz, 1 H, pyr), 5.47 (s, 2 H, pyr-CH_2), 5.08 (q, J = 7 Hz, 3 H, NEt, NH), 4.39 (t, J = 6.2 Hz, 2 H, $COOCH_2$), 4.05 (t, J = 6.5 Hz, 2 H, $COOCH_2$), 3.12 (q, J = 6.3 Hz, 2 H, RCH_2N), 2.66 (s, 3 H, CH_3), 2.02 (quint, J = 6 Hz, 2 H, $OCH_2CH_2CH_2O$), 1.74 (t, J = 7 Hz, 3 H, NEt), 1.7–0.7 (m, ~35 H). Anal. ($C_{33}H_{58}IN_3O_5$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(heptadecylcarbamoyl)oxy]ethyl]sulfinyl]ethoxy]carbonyl]amino]methyl]pyridine (7e). To a cooled (−78 °C) solution of compound **6e** (2.50 g, 4.31 mmol) in chloroform (100 mL) was added dropwise a dried solution containing MCPBA (55%, 1.35 g, 4.31 mmol) in chloroform (25 mL). The reaction mixture was stirred for 1 h at −78 °C and 30 min at room temperature and then quenched by the addition of a 5% sodium thiosulfate aqueous solution. The aqueous phase was separated and the organic phase was washed with 1 N sodium hydroxide aqueous solution (25 mL) and brine (25 mL). The remaining solution was dried over anhydrous sodium sulfate, the drying agent was filtered, and the filtrate was concentrated *in vacuo* to a white solid (4.2 g). Flash chromatography (1:15 MeOH–$CHCl_3$) afforded the title product as a white solid (2.48 g, 97% yield): mp 81–84 °C; IR (KBr) ν 3335, 2912, 2844, 1734, 1685, 1588, 1539, 1522, 1428, 1378, 1350, 1330, 1244, 1198, 1142, 1071 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 8.47 (br d, J = 4 Hz, 1 H, pyr), 7.66 (dt, J_t = 1.8 Hz, J_d = 7.4 Hz, 1 H, pyr), 7.3–7.0 (m, 2 H, pyr), 5.08 (s, 2 H, pyr-CH_2), 5.1 (br s, 1 H, NH), 4.60 (t, J = 5.5 Hz, 2 H, $COOCH_2$), 4.6–4.3 (m, 2 H, $COOCH_2$), 3.15 (q, J = 6 Hz, 2 H, RCH_2N), 3.1–2.8 (m, 4 H, CH_2SOCH_2), 2.63 (s, 3 H, CH_3), 1.7–0.7 (m, ~33 H). Anal. ($C_{31}H_{53}N_3O_6S$) C, H, N.

2-[[*N*-Acetyl-*N*-[[2-[[2-[(heptadecylcarbamoyl)oxy]ethyl]sulfinyl]ethoxy]carbonyl]amino]methyl]-1-ethylpyridinium Iodide (34). Following the general ethylation procedure described for compound **26**, the title compound was obtained as a yellow powder: mp 51–55 °C; IR (KBr) ν 3438, 2912, 2844, 1745, 1694, 1624, 1578, 1529, 1462, 1370, 1343, 1223, 1161 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ (TMS) 9.21 (br d, J = 6 Hz, 1 H, pyr), 8.49 (br t, J = 7.5 Hz, 1 H, pyr), 8.2–7.8 (m, 2 H, pyr), 5.56 (s, 2 H, pyr-CH_2), 5.5 (br s, 1 H, NH), 4.90 (q, J = 7 Hz, 2 H, NEt), 5.0–4.7 (m, 2 H, $COOCH_2$), 4.7–4.4 (m, 2 H, $COOCH_2$), 3.6–3.0 (m, 6 H, RCH_2N), 2.69 (s, 3 H, CH_3), 1.73 (t, J = 7 Hz, 3 H, NEt), 1.7–0.7 (m, ~33 H). Anal. ($C_{33}H_{58}IN_3O_6S.3H_2O$) C H, N.

B. Biological Methods: Inhibition of Platelet Aggregation in Vitro. Platelet aggregation studies were carried out by the method of Born.[9] Blood was collected in 3.16% sodium citrate (1 vol for 9 vol of blood) by cardiac puncture from male New Zealand rabbits (2–2.5 kg of body weight). Platelet-rich plasma (PRP) was prepared by centrifuging of the blood at 250 g for 10 min at 4 °C. The PRP was diluted with platelet-poor plasma obtained by further centrifugation at 3000 g for 10 min. The platelet number was adjusted to 3.5×10^5 cells/mm^3. Platelet aggregation was induced by C18-PAF (1.5×10^{-8} M) and measured by using a dual-channel aggregometer Chrono-log 500. Activity of the inhibitors was expressed as the IC_{50} value, i.e. the concentrations required to inhibit platelet aggregatory response by 50%. The values shown in the tables were calculated by linear regression from a single experimental curve with no less than four data points, each point being the mean of the percent inhibition at a given concentration obtained from one to three independent experiences.

Inhibition of PAF-Induced Hypotension in Normotensive Rats. Hypotension studies were performed as described by Baranes.[10] Male Sprague–Dawley rats, weighing 180–220 g, were anesthetized with sodium pentobarbital (50 mg/kg ip). Blood pressure was recorded from the left carotid artery with a Beckman

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pressure transducer coupled to a Beckman R611 polygraph. Right and left femoral veins were catheterized to inject PAF (0.5 μg/kg) or the test compound. Test compounds were administered by intravenous injection (1 mL/kg, dissolved in saline) 3 min before PAF injection. Control animals received only the vehicle. Blood pressure was monitored and percent inhibition of PAF-induced hypotension with respect to controls was calculated. The results were expressed as ID_{50} values, i.e. the dose of the test compound required to inhibit PAF-induced hypotension by 50%. The results were calculated by linear regression from a single experimental curve with no less than four points, each point being the mean of the percent inhibition at a given dose obtained from two or more independent experiences.

Statistics. Statistical analyses of pharmacological data (i.e. IC_{50} and ID_{50} values with their 95% confidence limits) were made by using a standard pharmacology program implemented on an IBM PC.[11]

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Registry No. 1 (I^-), 131729-40-3; 1 (Cl^-), 100488-87-7; **3**, 136408-39-4; **4a**, 136408-61-2; **4b**, 136408-62-3; **4c**, 134472-16-5; **4d**, 136408-63-4; **4e**, 136408-64-5; **4f**, 136408-65-6; **5a**, 136408-66-7; **5b**, 136408-67-8; **5c**, 134472-00-7; **5d**, 136408-68-9; **5e**, 136408-69-0; **5f**, 136408-70-3; **6a**, 136408-71-4; **6b**, 136408-72-5; **6c**, 134472-01-8; **6d**, 136408-73-6; **6e**, 136408-74-7; **6f**, 136408-75-8; **7e**, 136408-76-9; **8e**, 136408-77-0; **9**, 134471-90-2; **10**, 134471-93-5; **11**, 134471-91-3; **12**, 134472-12-1; **13**, 134471-85-5; **14**, 134471-87-7; **15**, 136408-40-7; **16**, 134472-08-5; **17**, 134472-05-2; **18**, 134505-53-6; **19**, 134471-75-3; **20**, 134472-11-0; **21**, 136408-41-8; **22**, 136408-42-9; **23**, 136434-36-1; **24**, 134472-02-9; **25**, 136408-43-0; **26**, 136408-44-1; **27**, 136408-45-2; **28**, 136408-47-4; **29**, 136408-48-5; **30**, 136408-49-6; **31**, 136408-50-9; **32**, 136408-51-0; **33**, 136408-52-1; **34**, 136408-53-2; **35**, 136408-54-3; **36**, 134471-99-1; **37**, 136408-55-4; **38**, 136408-56-5; **39**, 136408-57-6; **40**, 136408-58-7; **41**, 136408-60-1; **43**, 131730-72-8; **44**, 131730-85-3; **45**, 131730-92-2; $HO(CH_2)_2S(CH_2)_2OH$, 111-48-8; $CH_3(CH_2)_{16}NCO$, 7418-01-1; $PhOC(O)O(CH_2)_2S(CH_2)_2OC(O)OPh$, 136434-37-2; pyridine, 110-86-1; 2-picolylamine, 3731-51-9; *n*-octadecylamine, 124-30-1; 1,3-propanediol, 504-63-2.

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Fluorinated Colchicinoids: Antitubulin and Cytotoxic Properties

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The synthesis of B-ring and C-ring trifluoroacetamide-substituted colchicinoids and fluoro-substituted colchicineethylamides is presented. The B-ring trifluoroacetamido-substituted analogues exhibit moderate enhancement of potency compared to the nonfluorinated analogues for tubulin assembly inhibition and cytotoxicity toward two wild type cell lines. The C-ring substituted fluoroethylamides have reduced relative potencies in the same systems due to the strong electron-withdrawing effect of the fluoro derivatives. The fluoro colchicinoids are much more cytotoxic toward drug-resistant cell lines than to the wild type cell lines. Their enhanced potency is probably due to an effect of the fluoro moiety on functions specific to resistant cells and/or their higher hydrophobicity that may result in higher intracellular drug content. This finding may suggest the application of designed fluorinated anticancer drugs to overcome acquired resistance which may develop after several regiments of treatment with a nonfluorinated chemotherapeutic agent.

Introduction

Colchicine (**1a**, Chart I) is a potent drug that interferes with microtubule assembly both in vitro and in vivo.[1] It forms a complex with tubulin which inhibits the microtubule assembly process.[2] Two partial binding sites on tubulin were observed for the colchicine molecule: one for the trimethoxyphenyl A-ring and one for the 2-methoxytropone C-ring.[3] The role of ring B in the binding process is still unclear.[4] NMR studies utilizing ^{13}C-labeled colchicine established the existence of a second low-affinity binding site.[5] ^{13}C and ^1H NMR studies of drug-biological substrate binding have several major drawbacks, among them buffer or medium background signals, substrate signals, and low sensitivity in the case of ^{13}C. On the other hand, ^{19}F NMR proved to be an effective tool for such studies,[6] and metabolites of fluorinated drugs in body fluids can be detected by NMR at concentrations as low as 10 μM.[7] We decided to explore the feasibility of using colchicinoids with fluorinated substituents for studies exploring the binding of cholchicine to tubulin,[8] cells, and tissues. The synthesis of colchicine analogues has afforded many compounds with improved cytotoxic properties; among them are several trifluoroacetamido-substituted analogues.[9,10]

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