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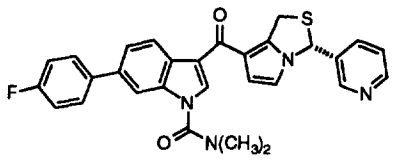
SYNTHESIS AND EVALUATION OF WATER SOLUBLE INDOLE PYRROLOTHIAZOLE PAF ANTAGONISTS

George S. Sheppard*, Steven K. Davidsen, George M. Carrera, Jr., Daisy Pireh, James H. Holms, H. Robin Heyman, Douglas H. Steinman, Michael L. Curtin, Richard G. Conway, David A. Rhein, Daniel H. Albert, Paul Tapang, Terrance J. Magoc and James B. Summers

Immunosciences Research Area, Department 47J, AP-10, Abbott Laboratories, Abbott Park, Illinois 60064.

Abstract 3-(3-Pyridinyl)-7-(indol-3-ylcarbonyl)-1*H*-3*H*-pyrrolo[1,2-c]thiazoles represent a class of potent, orally active PAF antagonists; however, the lead compounds in this series suffered from a lack of aqueous solubility. To overcome this limitation, a number of strategies were examined to achieve improved solubility, involving the incorporation of polar substituents and the use of prodrugs.

Platelet Activating Factor (PAF) is an endogenous phospholipid inflammatory mediator which is hypothesized to be involved in a number of pathologic states having an inflammatory component.[1] Clinical evaluations of PAF antagonists for the treatment of asthma, allergic rhinitis, septic shock, pancreatitis, and cardiac reperfusion injury have been undertaken, but to date there are no approved therapeutic agents having this mechanism of action. Research at Abbott Laboratories has focused on finding an agent to test the role of PAF in septic shock. This goal required the discovery of a potent, novel PAF antagonist having sufficient water solubility to be administered intravenously.

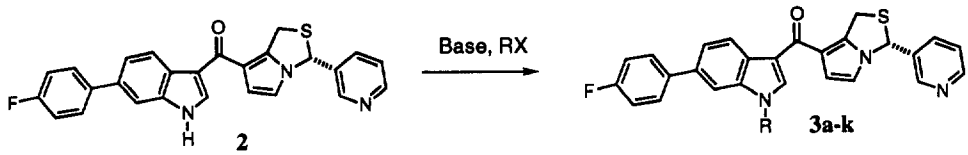


1

As described in the preceding letters, **1** represented a promising lead structure, having excellent affinity for the PAF receptor (K_i = 3.8 nM measured versus [3H]-PAF in rabbit platelet membranes[2]), as well as outstanding activity in a number of animal models of shock.[3] Unfortunately, its aqueous solubility[4] (<1 μg/mL at pH7) was insufficient for intravenous administration. A study was therefore undertaken to search for related compounds which would maintain the PAF antagonist activity of the lead structure, while improving aqueous solubility to an acceptable level, initially defined as 1 mg/ml at pH of 4-9.[5]

Our initial approach was to append charged or polar groups to accessible sites on the indole portion of the parent structure to improve its solubility. Although the *N*-dimethylcarbamoyl substituent was believed to be important to the activity of **1**, the indole nitrogen atom provided an easily functionalized site to examine, so a number of modifications were surveyed. The *N*-alkylated compounds **3e-k** were prepared

by reaction of an anion of **2** with the appropriate alkylating agents, in some cases followed by deprotection or refunctionalization (eq 1).[6] Likewise, acylated compounds were prepared either by direct acylation (**1**, **3b**, **3d**), or by acylation with 4-nitrophenylchloroformate, followed by amine introduction (**3a**, **3c**).[6]



(1)

As can be seen in Table 1, introduction of polar groups did, in some cases, significantly improve upon the solubility of the parent structure. In particular, the sulfonic acid derivatives **3c**, **3d**, and **3e** showed dramatic increases in solubility. Unfortunately, with the exception of **3j**, all the analogs containing polar indole nitrogen substituents suffered unacceptable losses in binding affinity. Consistent with earlier observations, substituents other than the dimethylcarbamoyl group led to a loss in activity *in vivo* as well.[7]

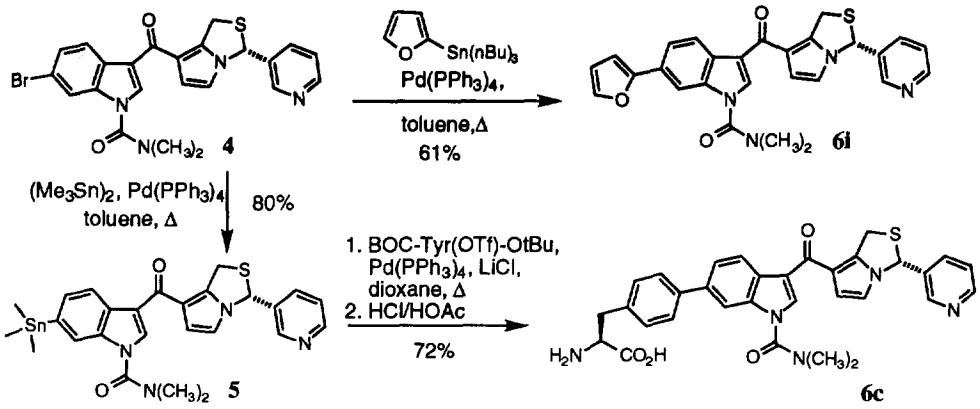
Table 1. Substitution at the Indole Nitrogen.

R	Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)	R	Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)
1 $CON(CH_3)_2$	3.8	<1 @pH7	**3f** $CH_2CH_2CO_2H$*	160	200 @ pH9
3a $CONHCH_2CO_2H$	130	48 @ pH9	**3g** CH_2(2-tetrazole)*	19	150 @ pH7
3b $CON(CH_2CH_2)_2NCH_3$	28	84 @ pH7	**3h** $CH_2CH_2NH_2$*	38	41 @ pH4
3c $CONHCH_2CH_2SO_3H$*	680	740 @ pH7	**3i** $CH_2CH_2NHSO_2CH_3$*	10	<1 @ pH7
3d $CO(2\text{-}C_6H_4SO_3K)$	300	4500 @ pH7	**3j** $CH_2CH_2SO_2NH_2$*	3.3	<1 @ pH7
3e $CH_2CH_2CH_2SO_3K$	100	1800 @ pH9	**3k** CH_2CO_2H*	18	490 @ pH7

* Data obtained for racemic compound.

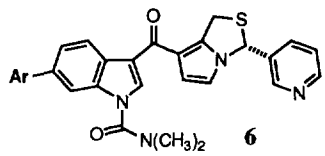
As described in a preceding letter, previous studies had indicated that an indole 6-phenyl substituent was associated with increased *in vivo* activity in this series of compounds. We therefore chose to examine incorporation of 6-aryl substituents bearing polar groups and 6-heteroaryl substituents to improve aqueous solubility. Palladium-catalyzed cross coupling reactions were used to efficiently prepare a number of analogs, as indicated by the representative syntheses shown in Scheme 1.[6] For example, coupling of available arylstannanes (**6i**) or boronic acids (**6a**) with bromoindole **4** provided the desired analogs directly. To avoid the need for preparation of a series of arylboronic acids and/or arylstannanes, conversion of bromoindole **4** to stannane **5** was carried out. This versatile intermediate was then coupled with a variety of commercially available aryl halides and triflates derived from commercially available phenols.

Scheme 1.



As indicated in Table 2, incorporation of polar functionality at the 6-aryl position provided only modest gains in aqueous solubility, which were accompanied by a drop in biological activity. Since it appeared that the indole lipophilic group of **1** was highly optimized, we then turned to the pyridyl-pyrrolothiazole ketone portion of the structure.

Table 2. Substitution at the Indole 6-Position

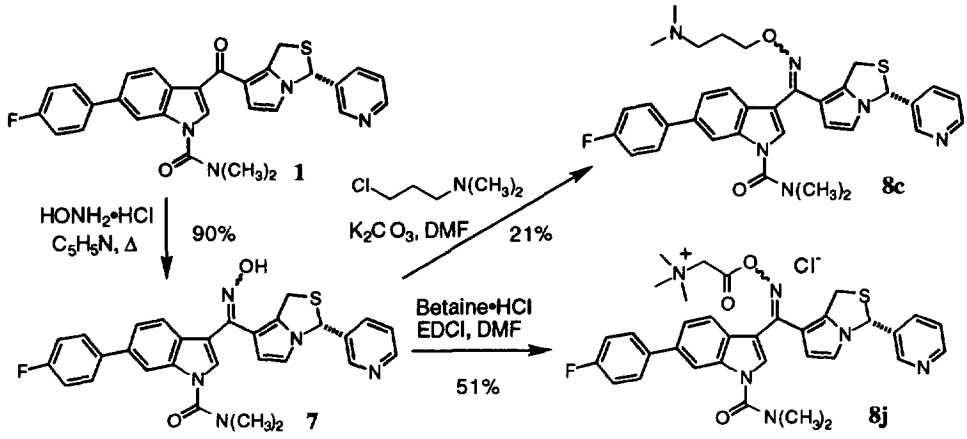


Ar		Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)	Ar		Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)
1	4-F-C_6H_4	3.8	<1 @ pH7	**6e**	4-$HOCH_2C_6H_4$	16	11 @ pH7
6a	3-NH_2-C_6H_4*	110	230 @ pH7	**6f**	3-pyridyl*	10	15 @ pH7
6b	4-SO_2NH_2-C_6H_4	45	<1 @ pH7	**6g**	2-thaizolyl	71	6 @ pH7
6c	4-(2'(*S*)-$C_6H_4CH_2$ $CH(NH_2)CO_2H$	54	190 @ pH4	**6h**	5-uricyl	240	200 @ pH7
6d	4-H_2O_3PO-C_6H_4	250	130 @ pH7	**6i**	2-furyl	39	150 @ pH7

* Data obtained for racemic compound.

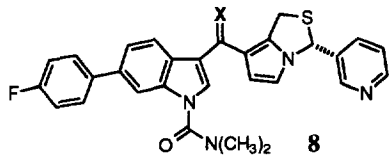
A number of simple ketone derivatives were prepared directly from **1**. Oxime formation was found to provide a mixture of isomers **7** which had equivalent activity to the parent ketone, both *in vitro* and *in vivo*. While this modification did not improve upon the aqueous solubility, it did provide a new point of attachment for solubilizing groups. A similar approach has been reported to be successful for the solubilization of the pyrrolothiazole-containing PAF antagonist RP-59227 (Tulopafant).[8] Accordingly, a number of functionalized oximes bearing polar groups were prepared by direct oxime formation (**7**, **8a**), alkylation of **7** (**8b-d**), and acylation of **7** (**8e-j**) as shown for representative examples in Scheme 2.[6]

Scheme 2.



As was found previously for the substituted indole analogs, a loss of receptor binding was seen upon introduction of polar functionality (Table 3). This is in contrast to the results reported in reference 8, where basic solubilizing groups were accommodated in a related series of PAF antagonists. Despite the disappointing biological activities, improved solubilities were obtained by the introduction of ionizable acidic or basic groups into the molecule. This led us to evaluate the use of oxime esters **8h-j** as potential solubilizing prodrugs of the biologically active oxime. While improved solubilities and *in vivo* activity comparable to the parent compound were achieved by this approach,[9] the acylated oximes were found to be unstable with respect to both hydrolysis and Beckmann rearrangement to less active compounds on standing in aqueous solution.[10]

Table 3. Ketone Derivatives.*

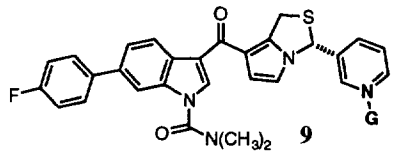


	X	Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)		X	Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)
1	O	3.8	<1 @ pH7	**8e**	$NOCON(CH_3)_2$	6	210 @ pH7
7	NOH	0.37	<1 @ pH7	**8f**	$NOCO(4\text{-}C_6H_4SO_3Na)$	53	3900 @ pH7
8a	$NOCH_2CO_2H$	74	900 @ pH9	**8g**	$NOCO(2\text{-}C_6H_4SO_3H)$	11	1600 @ pH7
8b	$NO(CH_2)_3(1\text{-}C_4H_8N)$	450	4300 @ pH4	**8h**	$NOCOCH_2CH_2CO_2H$	ND†	310 @ pH9
8c	$NO(CH_2)_3N(CH_3)_2$	160	9100 @ pH4	**8i**	$NOCOCH_2N(CH_3)_2$	ND†	300 @ pH4
8d	$NO(CH_2)_2N(CH_3)_2$	200	1000 @ pH4	**8j**	$NOCOCH_2N(CH_3)_3Cl$	ND†	1900 @ pH7

* The oximes were obtained and tested as approximately equimolar mixtures of *E* and *Z* isomers. †Binding values were not obtained for these oxime ester prodrugs.

The final area examined involved functionalization of the pyridine nitrogen. A number of analogs were prepared *via* the ring opening of dinitrophenylpyridinium salts, as described for the synthesis of the *N*-oxide **9a** in the preceding letter. Unfortunately, formation of pyridinium ylides did not provide large increases in aqueous solubility (Table 4). In contrast, formation of a quaternary pyridinium salt provides an impressive degree of water solubility; however, the quaternized pyridinium compound **9e** lacked PAF antagonist activity. This result suggested the temporary incorporation of a charged pyridinium moiety in a solubilizing prodrug which would release the active pyridine species upon enzymatic hydrolysis.

Table 4. Functionalized Pyridine Derivatives.



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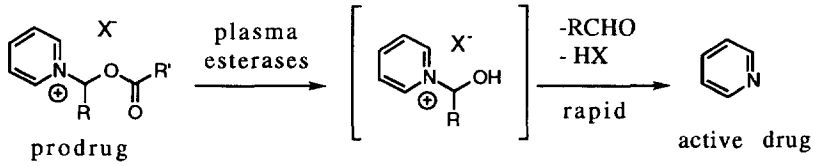
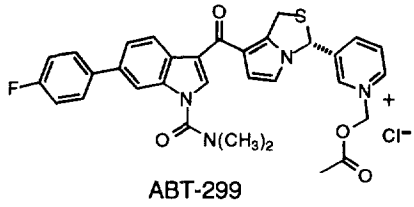
NG	Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)	NG	Receptor Binding K_i (nM)	Aqueous Solubility (μg/ml)
1 N:	3.8	<1 @ pH7	**9c** $NNCONH_2$	100*	12 @ pH7
9a NO	2.3	56 @ pH7	**9d** $NNCO\text{-}3\text{-}C_5H_4N$	120*	8 @ pH7
9b NNH	2000*	110 @ pH7	**9e** $N^+CH_3\ I^-$	4000*	2800 @ pH7

* Data obtained for racemic compound.



Accordingly, a series of *N*-acyloxyalkyl pyridinium salts was prepared and examined as potential prodrugs of **1**. Details of this study, which culminated in the selection of ABT-299 (aqueous solubility > 100 mg/mL) as a compound for clinical development, are described in a recent publication.[11]



ABT-299

In summary, a number of analogs of structure **1** incorporating polar substituents were prepared and evaluated for aqueous solubility and PAF antagonist activity. No analogs achieving the targeted level of aqueous solubility were found which maintained the desired level of PAF antagonist activity. These results dictated the use of a solubilizing prodrug of **1** to obtain an agent suitable for clinical evaluation.

References and Notes

1. For a recent review, see: Summers, J. B.; Albert, D. H. *Adv. Pharmacol.* **1995**, *32*, 67.

2. For a description of this assay, see: Sheppard, G. S.; Pireh, D.; Carrera, G. M., Jr.; Bures, M. G.; Heyman, H. R.; Steinman, D. H.; Davidsen, S. K.; Phillips, J. G.; Guinn, D. E.; May, P. D.; Conway, R. G.; Rhein, D. A.; Calhoun, W. C.; Albert, D. H.; Magoc, T. J.; Carter, G. W.; Summers, J. B., Jr. *J. Med. Chem.* **1994**, *37*, 2011. The dose-response curves were generally measured twice, with the K_i reported as the mean of the observed values. Values differing by less than 2-3 fold cannot be reliably differentiated with this number of data points. For comparison, the widely studied heteroazepine PAF antagonist WEB-2086 displays a K_i of 98 nM under this protocol.

3. For a summary of the *in vivo* properties of **1**, see: Summers, J. B.; Albert, D. H.; Davidsen, S. K.; Conway, R. G.; Holms, J. H.; Magoc, T. J.; Luo, G.; Tapang, P.; Rhein, D. A.; Carter, G. W. *Adv. Prostaglandin Thromboxane Leukot. Res.* **1995**, *23*, 475.

4. Aqueous solubilities were measured as follows: Test compounds were suspended in the indicated aqueous buffer, targeting a final concentration of 20 mg/ml, sonicated, then equilibrated overnight at ambient temperature in the dark. The samples were centrifuged, and the supernatant analyzed by UV absorption relative to a standard solution of the same compound prepared by diluting a 40 mM DMSO solution to 1mM with methanol, followed by still further dilution to 20 μM with buffer solution containing 10 % methanol.

5. Compounds were initially evaluated for aqueous solubility at pH7. Most compounds containing basic or acidic functionality were also tested at pH4 and pH9, respectively. In some cases this follow up testing was not performed when the compounds in question were deemed uninteresting on the basis of their activity in the receptor binding or *in vivo* screening assays. The values reported are at the pH displaying the highest observed aqueous solubility.

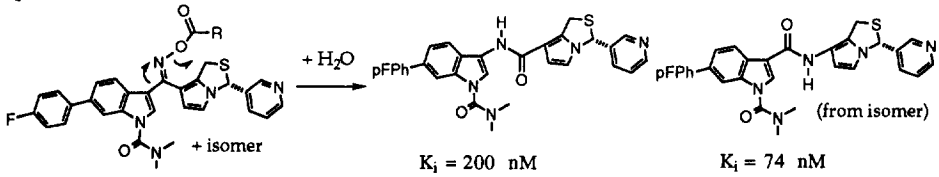
6. All tested compounds gave ^{1}H NMR spectra, mass spectra, and elemental analyses consistent with the indicated structures. Experimental procedures and spectral data may be found in Summers, J. B.; Davidsen, S. K.; Holms, J. H.; Pireh, D.; Heyman, H. R.; Martin, M. B.; Steinman, D. H.; Sheppard, G. S.; Carrera, G. M. PCT patent application WO 93/01813.

7. Albert, D., Tapang, P. personal communication.

8. Soler, F.; Floch, A.; Robaut, C.; Lavé, D.; Cavero, I. *Drugs Fut.* **1992**, *17*, 207.

9. The *in vivo* activity of the oxime esters **8h** and **8i** in a PAF-induced rat skin permeability assay was not significantly different from that observed for oximes **7** (ED_{50} i.v. = 0.015, 0.015, and 0.007 mg/kg, respectively; ED_{50} p.o. = 0.071, 0.082, and .125 mg/kg, respectively). The potency of **8j** in this assay was not evaluated because the compound produced lethality in 86% of the test animals at a dose of 25 mg/kg. A description of this assay may be found in reference 2.

10. The Beckmann rearrangement products were intentionally prepared, characterized and tested. The compounds coeluted on HPLC with breakdown products formed from oxime esters upon standing in aqueous solution.



11. Davidsen, S. K.; Summers, J. B.; Albert, D. H.; Holms, J. H.; Heyman, H. R.; Magoc, T. J.; Conway, R. G.; Rhein, D. A.; Carter, G. W. *J. Med. Chem.* **1994**, *37*, 4423.

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