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DISCOVERY AND OPTIMIZATION OF INDOLE PYRROLOTHIAZOLE PAF ANTAGONISTS

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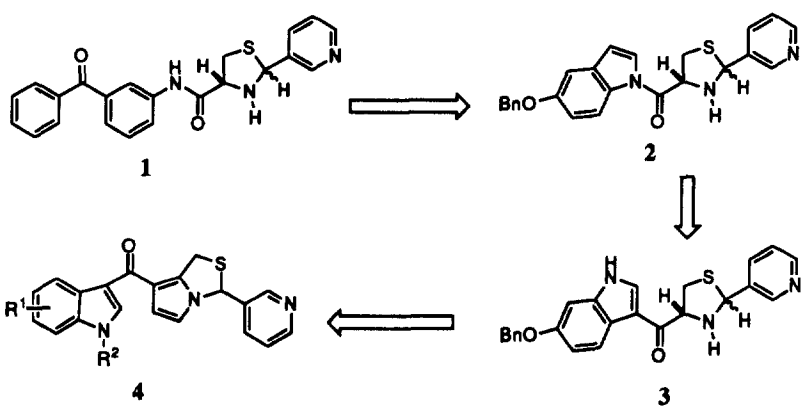
Abstract: 3-(3-Pyridinyl)-7-(indol-3-ylcarbonyl)-1*H*-3*H*-pyrrolo[1,2-c]thiazoles represent a new class of platelet activating factor antagonists. This series was discovered by combining the indole portion of a previous thiazolidine series with the known 3-pyridinyl-pyrrolothiazole pharmacophore. Optimization of the indole substituents resulted in the identification of **8i** as one of the most potent PAF antagonists yet described.

Platelet Activating Factor (PAF) is an endogenous phospholipid inflammatory mediator which exhibits a wide spectrum of biological activities *via* stimulation of specific G-protein coupled receptors found on a variety of cell types.[1] The biological effects induced by PAF include bronchoconstriction, vascular permeability, hypotension, and platelet degranulation. It has been implicated as an important mediator in inflammatory diseases including asthma, allergic rhinitis, septic shock, pancreatitis, and ischemia reperfusion injury.[2]

Our initial work in the PAF antagonists field centered on the thiazolidine amides such as **1**.[3] A large boost in binding potency was realized upon incorporating the amide nitrogen atom of **1** as part of an indole ring (Figure 1). An additional increase in *in vitro* potency was gained by introducing a hydrophobic substituent at the 5-position of the indole ring as exemplified by 1-acyl indole **2**. While these compounds were more potent PAF antagonists, they suffered from hydrolysis of the *N*-acyl bond and thus were short-lived *in vivo*. This problem was circumvented by inverting the indole ring giving the isosteric 3-acyl indoles (e.g., **3**) which increased *in vitro* potency after repositioning of the lipophilic substituent to the indole 6-position. It is interesting to note that with both the 1-acyl and the 3-acyl indoles, lipophilic substitution opposite to the

Figure 1

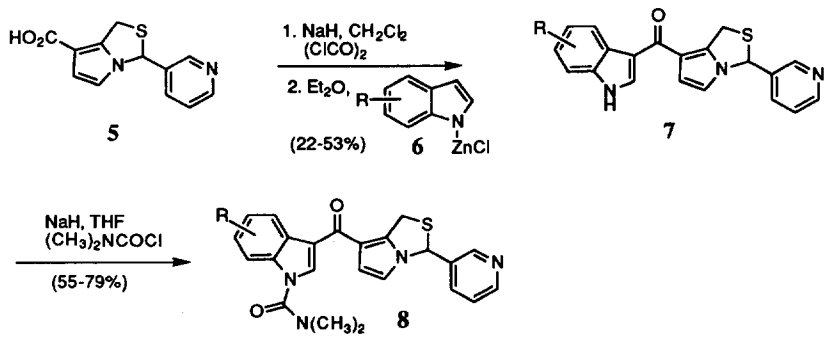


1 2 3 4

carbonyl group provided the greatest binding potency. While these 3-acyl indole thiazolidine PAF antagonists were potent *in vitro* they lacked an extended duration of *in vivo* activity, perhaps due to thiazolidine ring fragmentation.[3,4] An effort was therefore initiated to discover a suitable replacement for the thiazolidine heterocycle. The preparation of non-equilibrating bicyclic thiazolidines has previously been described as a potential solution to this problem.[5] Another approach involved combining the pyrrolothiazole ring system described by researchers at Rhône-Poulenc[6] with the 3-acylated indole portion of the Abbott thiazolidine PAF antagonists. The resulting 3-acyl indole pyrrolothiazole PAF antagonists **4** are the subject of this communication.

As shown in Scheme 1, the synthesis of indole pyrrolothiazoles was achieved by indole 3-acylation with the pyrrolothiazole acid **5**.[7] Exclusive 3-acylation of indoles has been described by Bergman and Venemalm and involves reaction of the indole zinc anion with acid chlorides.[8] Thus, treatment of acid **5** with NaH and oxalyl chloride in methylene chloride afforded the acid chloride, which was added to an ether suspension of the zinc salt of indole **6**, prepared by methyl magnesium bromide addition to the indole followed by addition of zinc chloride. The resulting 3-(3-pyridinyl)-7-(indol-3-ylcarbonyl)-1*H*,3*H*-pyrrolo[1,2-c]thiazoles **7** were obtained in 22-53% yield after flash chromatography and recrystallization.

Scheme 1



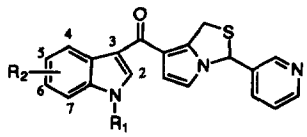
The substituted indoles **6** were commercially available or prepared by known methods.[3,9] Substituted indole pyrrolothiazoles that were prepared by this method contain substituents such as alkyl, aryl, alkoxy, aryloxy, and halogen.

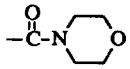
The indole *N*-substituted analogs were usually prepared by sodium hydride deprotonation of the 3-acyl indole nitrogen followed by treatment with alkylating or acylating agents. For example, reaction of **7** with NaH in THF followed by addition of dimethylcarbamoyl chloride afforded **8** in 55-79% yield after recrystallization. In some cases, acylation with 4-nitrophenylchloroformate followed by displacement of 4-nitrophenol with the appropriate amine proved to be more efficient.

In vitro antagonism of PAF was assessed by measuring the ability of test compounds to displace [^{3}H]PAF from rabbit platelet membrane PAF receptors.[3,10] As can be seen in Table 1, acylation of the indole nitrogen produced compounds which were less potent than the unsubstituted analog **7a**. This was also the case for single substitution on the indole phenyl ring (**7b-7e**). The combination of substituents on the indole nitrogen

and on the indole phenyl ring also produced compounds with inferior potency except for analog **8c** which is substituted at the 6-position with a lipophilic group. This result is consistent with the previously investigated thiazolidine series[3], where the presence of a lipophilic group opposite the connecting carbonyl provided the

Table 1: Initial Indole Substitution



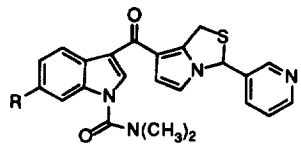
Compound	R_1	R_2	PAF receptor K_i (nM)
7a	H	H	53
7b	H	4-OH	327
7c	H	5-OBn	180
7d	H	6-OBn	64
7e	H	7-OBn	90
8a	$CON(CH_3)_2$	H	95
8b	$CON(CH_3)_2$	5-OBn	120
8c	$CON(CH_3)_2$	6-OBn	17
8d	$CON(CH_3)_2$	7-OBn	170
8e	$CON(CH_3)_2$	6-Cl	89
9	CO_2tBu	H	110
10		H	225

most potent analogs. This is also consistent with several proposed models of the PAF receptor which have indicated that many PAF antagonists possess a common lipophilic group that provides hydrophobic interactions with the receptor.[11]

While **8c** possessed reasonable binding potency, its activity *in vivo* was somewhat disappointing (Table 2). Additional 6-substituted, *N*-carbamoyl indole analogs were therefore prepared in order to optimize both PAF receptor binding activity and *in vivo* activity. Compounds were administered both orally and intravenously, and *in vivo* activity determined by inhibition of PAF-induced cutaneous vascular permeability in the rat.[3,10] As can be seen from Table 2, although there are exceptions, the more lipophilic 6-substituents generally provided greater potency in the receptor binding assay. A notable exception is the 6-pyridyl analog **8l** which possessed good binding affinity but not unexpectedly displayed poor *in vivo* activity. The *para*-fluorophenoxy analog **8k** was found very potent in the receptor binding assay with a K_i of 1.7 nM.

Table 2 also indicates that a nonpolar aryl group at the indole 6-position is required for potent *in vivo* activity. The 6-phenyl (**8h**) and 6-*para*-fluorophenoxy (**8k**) analogs were exceptionally potent ($ED_{50} \leq 0.03$ mg/kg, *iv*). The *para*-fluorophenyl substituted analog **8i** was the most potent of the series with an ED_{50} equal

Table 2: 6-Substituted Indole Pyrrolothiazole Analogs



Compound	R	Receptor Binding K_i (nM)	Rat Skin Permeability ED_{50} (mg/kg, po)	ED_{50} (mg/kg, iv)
8a	H	95	34% @ 30 mg/kg	ND†
8c	OBn	17	3.3	ND
8e	Cl	89	1.4	42% @ 1 mg/kg
8f	Br	15	1.5	41% @ 1 mg/kg
8g	Bn	13	ND	0.09
8h	Ph	23	0.06	0.03
8i*	4-F-Ph	3.8	0.06	0.006
8j*	4 F-PhS	17	ND	0.27
8k*	4-F-PhO	1.7	ND	0.03
8l	3-Pyr	11	58% @ 10 mg/kg	ND

* data obtained for the R enantiomer (see reference 7). †not determined

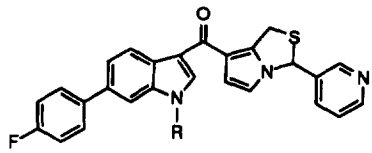
to 0.006 mg/kg when given intravenously. The thioether analog **8j** was surprisingly less active *in vivo* with an ED_{50} = 0.27 mg/kg, *iv*.

An reinvestigation of indole nitrogen substitution was undertaken with analogs containing the 6-(4-fluorophenyl)indole functionality to determine whether the dimethylcarbamoyl group was the optimal substituent. As shown in Table 3, several analogs were found to possess potent activity in the receptor binding assay as well as the *in vivo* assay. For example, compound **11g** containing the homologated dimethylcarbamoyl group was very potent with a binding constant of < 1 nM and an ED_{50} of 0.009 mg/kg, *iv*. However, none of these indole nitrogen substituted analogs were superior to the dimethylcarbamoyl substituted analog **8i** in the rat skin permeability assay.

As shown in the previous tables, several analogs in the indole pyrrolothiazole series demonstrated *in vitro* and *in vivo* biological activity similar to compound **8i** (e.g., **11c** and **11g**); however, it was the extended duration of action that distinguished **8i** as the superior compound in this class. Following a 0.1 mg/kg intravenous dose in the rat, **8i** inhibited the PAF-induced increase in skin permeability by more than 50% for 16 hours. Active metabolites of **8i** may contribute to this long duration of action. An account of the metabolic profile of **8i** is reported in the following letter of this journal.

Additional pharmacological testing of **8i** was performed to further evaluate its biological activity.[10,12] Its affinity for PAF receptors from rabbit platelet membranes was determined to be competitive, reversible, and stereoselective (*S* enantiomer K_i = 760 nM). A survey of thirty-eight other receptors, ion channels, and enzymes showed no inhibitory activity due to **8i** at concentrations up to 10 μM.[13] PAF-induced cellular

Table 3: Optimization of Indole *N*-Substitution



11

Compound	R	Receptor Binding K_i (nM)	Rat Skin Permeability ED_{50} (mg/kg, po)	ED_{50} (mg/kg, iv)
8i*	$CON(CH_3)_2$	3.8	0.06	0.006
7f	H	75	6.40	ND†
11a	$CONHCH_3$	10	0.12	ND
11b	$CONHCH_2CH_3$	19	ND	0.18
11c	$CONH_2$	6.0	0.06	0.01
11d*	CH_3	7.0	ND	0.09
11e*	CH_2CH_2OH	5.0	0.76	0.10
11f	$CH_2CH_2NH_2$	38	1.30	ND
11g*	$CH_2CON(CH_3)_2$	0.7	0.12	0.009
11h	CH_2CH_2COOH	160	5.10	0.10
11i	$SO_2N(CH_3)_2$	940	5.50	ND
11j	$CONHNH_2$	25	0.57	0.04

*data obtained for the R enantiomer (see reference 7). †not determined

responses, such as the release of serotonin, β-thromboglobulin, and platelet factor 4, were found to be potently and selectively inhibited by **8i**. In other *in vivo* animal models and models of endotoxic shock, the administration of **8i** produced potent inhibitory activity with long duration.

In summary, the indole pyrrolothiazole class of PAF antagonists was discovered by combining the indole portion of the Abbott thiazolidine series with the known 3-pyridinyl-pyrrolothiazole pharmacophore. Substituents at the 1- and 6-position of the indole were necessary for potent biological activity. The 6-(4-fluorophenyl) analog **8i** was identified as one of the most potent and long-lived PAF antagonists yet described.[10] Based on its exceptional biological profile, **8i** was the subject of a number of additional studies, two of which follow this communication.[14]

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14. For an account of the development of a prodrug form of **8i** see: 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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