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5-Lipoxygenase-activating protein inhibitors. Part 3: 3-{3-*tert*-Butylsulfanyl-1-[4-(5-methoxy-pyrimidin-2-yl)-benzyl]-5-(5-methyl-pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid (AM643)—A potent FLAP inhibitor suitable for topical administration

Nicholas Stock *, Christopher Baccei, Gretchen Bain, Charles Chapman, Lucia Correa, Janice Darlington, Christopher King, Catherine Lee, Daniel S. Lorrain, Pat Prodanovich, Angelina Santini, Kevin Schaab, Jilly F. Evans, John H. Hutchinson, Peppi Prasit

Amira Pharmaceuticals, 9535 Waples St., Suite 100, San Diego, CA 92121, USA

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ABSTRACT

AM643 (compound **6**, 3-{3-*tert*-butylsulfanyl-1-[4-(5-methoxy-pyrimidin-2-yl)-benzyl]-5-(5-methyl-pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid) was identified as a potential candidate for formulation as a topical agent for the treatment of skin disorders involving leukotriene production. Dermal application of **6** using a prototypical vehicle in a murine ear arachidonic acid model showed significant reduction in the concentrations of leukotrienes in mouse skin with concomitant reduction in ear swelling.

5-Lipoxygenase-activating protein (FLAP) in conjunction with 5-lipoxygenase (5-LO) converts membrane derived arachidonic acid (AA) to the leukotriene epoxide LTA_4.[1] Bifurcation of the metabolic pathway through either LTA_4 hydrolase or LTC_4 synthase produces the pro-inflammatory mediators LTB_4 and the cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4; see Fig. 1). LTB_4 binds to the GPCRs BLT1 and BLT2 eliciting neutrophil and eosinophil chemotaxis. The CysLTs activate at least two well defined GPCR's, namely $CysLT_1$[2] and $CysLT_2$,[3] the activation of the former leading to contraction of smooth muscle (bronchoconstriction), edema and eosinophil migration. The precise role of the $CysLT_2$ receptor remains unclear, but mice deficient in this receptor indicate a role for $CysLT_2$ in vascular permeability and inflammation.[4] Ongoing research also indicates the existence of several other CysLT receptors, namely GPR17,[5] $P2Y_{12}R$[6] and $CysLT_E$, the latter being first discovered in murine skin.[7] Dermal application of LTB_4 or the CysLTs to human skin produces a prolonged wheal and flare reaction, indicating a potential pathological role for leukotrienes in skin disorders.[8] The discovery that LTE_4 elicits a similar response to LTC_4 and LTD_4 in this setting may point to the role of the recently discovered $CysLT_E$ receptor as a therapeutic target that is yet to be realized.[9] Significant evidence exists to implicate abnormal LT production in the pathology of various skin diseases, including psoriasis,[10] atopic dermatitis, urticaria and others. For instance, LTs have been shown to be elevated in atopic dermatitis patients[11] and treatment with either Zileuton,[12] a 5-lipoxygenase (5-LO) inhibitor, or Montelukast,[13] a $CysLT_1$ antagonist, have shown beneficial effects. Patients with urticaria have been successfully treated with both $CysLT_1$ receptor antagonists and Zileuton.[14] Zafirlukast, another $CysLT_1$ antagonist, has also demonstrated

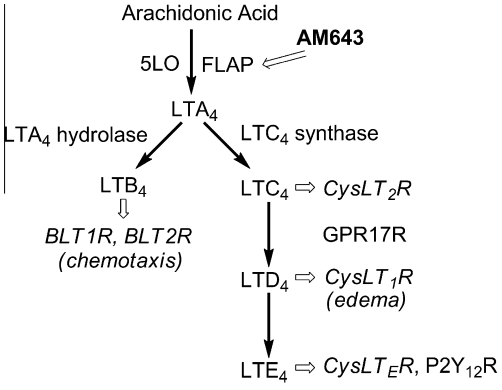

Figure 1. The arachidonic acid/leukotriene pathway.

* Corresponding author. Tel.: +1 858 228 4666; fax: +1 858 228 4766.
E-mail address: nick.stock@amirapharm.com (N. Stock).

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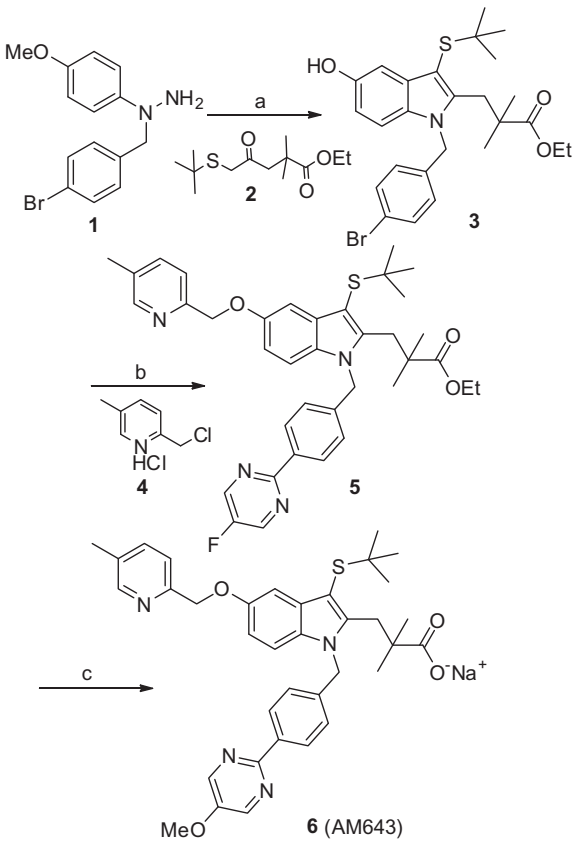
efficacy in capsular contracture, a post-operative fibrotic skin disorder.[15] There is also evidence that inhibition of LTs may be beneficial in the treatment of acne.[16]



Scheme 1. Reagents and conditions: (a) (i) toluene, AcOH, NaOAc, **2**, rt (55%); (ii) $AlCl_3$, *t*-BuSH, DCM, 0 °C to rt (93%); (b) (i) Bis(pinacolato)diboron, Pd(dppf)Cl_2·DCM (cat), KOAc, *p*-dioxane, 85 °C (88%); (ii) 2-chloro-5-fluoropyrimidine, K_2CO_3, DME, H_2O, Pd(PPh_3)$_4$ (cat), 80 °C (85%); (iii) **4**, Cs_2CO_3, MeCN, 45 °C (84%); (c) (i) LiOH·H_2O, THF, MeOH, H_2O, 65 °C (98%); (ii) NaOH (1 equiv), EtOH, H_2O, lyophilization (quant).

Table 1
Selected in vitro IC_{50} data for compound **6**[a]

Assay	IC_{50}
FLAP binding	2 nM
hLA	0.6 nM
hWB (15 min)	161 nM
hWB (5 h)	81 nM
mWB (15 min)	26 nM
mWB (4 h)	4 nM
rWB (15 min)	78 nM
rWB (4 h)	11 nM
CYP 3A4	8.8 μM
CYP 2D6	>30 μM
CYP 2C9	4.5 μM
COX-1/2	>100 μM

[a] FLAP binding: inhibition of ^{3}H-ligand binding to FLAP membranes ($n = 6$); hLA; inhibition of LTB_4 synthesis following ionophore (A23817) challenge in human leukocytes; hWB: inhibition of LTB_4 synthesis following ionophore challenge (A23817) in human blood after either 15 min or 5 h compound pre-incubation ($n = 7$, whereby each n is the average from two different donors); mWB (mouse whole blood) data is from a single experiment. rWB (15 min) is the average of six experiments and the 4 h data is the average of 10 experiments.

As part of our ongoing research into the development of FLAP inhibitors[17], we sought to develop a potent FLAP inhibitor suitable for topical application as a treatment modality for dermal diseases.

AM643 (**6**), sodium 3-{3-*tert*-butylsulfanyl-1-[4-(5-methoxy-pyrimidin-2-yl)-benzyl]-5-(5-methyl-pyridin-2-yl-methoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionate) was identified as a potent and selective inhibitor of the FLAP protein as part of the discovery program that led to the identification of AM103 and AM803, two oral FLAP inhibitors that have entered clinical trials in humans.[18] Compound **6** was synthesized according to Scheme 1. Fischer indolization of the substituted hydrazine **1**, with the thioketone **2** followed by demethylation of the indole afforded indole–phenol **3**. Conversion of the 4-bromophenyl moiety to the corresponding pinacol-boronate (Pd(dppf)Cl_2, bis(pinacolato)diboron, KOAc, dioxane) permitted Suzuki coupling of 2-chloro-5-fluoropyrimidine under standard conditions and alkylation of the phenol with 5-methyl-2-chloromethyl pyridine **4**[18] yielded the elaborated indole **5**. Hydrolysis of the hindered geminal-dimethyl ester in the presence of methanol, with simultaneous displacement of the fluorine on the pyrimidine with a methoxy group, followed by conversion to the sodium carboxylate salt gave **6** in 31% overall yield.

Table 1 summarizes the in vitro data for **6**. The FLAP binding assay was performed using human polymorphonuclear leukocyte (PMN) derived membranes and the human leukocyte (hLA) assay measures inhibition of LTB_4 synthesis in isolated human leuko-

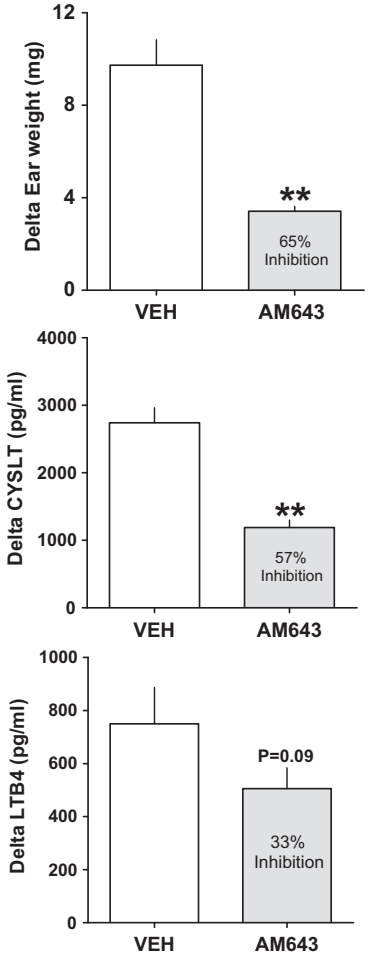


Figure 2. Oral administration of AM643 (30 mg/kg) decreased AA (4 mg/ear)—induced ear swelling and tissue CysLT biosynthesis. There was a trend toward decrease in LTB_4 but this did not reach statistical significance. Bars represent the means ± SEM of $n = 3$ mice per group $^{**}P < 0.01$ versus vehicle treated mice, two tailed *t*-test.

cytes after calcium ionophore stimulation. To measure the degree of protein shift in the presence of blood proteins, the whole blood (xWB, x = h, human; x = m, mouse; x = r, rat) assay measures LTB_4 inhibition following ionophore challenge after both a 15 min and either a 4 or 5 h pre-incubation period in blood.[19]

The data demonstrates that compound **6** is a potent inhibitor of LTB_4 biosynthesis in blood with a significant potency increase after prolonged incubation.[20] In human blood at 15 min, **6** exhibits an IC_{50} of 161 nM against LTB_4 production and at equilibrium after 5 h, possesses an IC_{50} of 81 nM. Compound **6** shows no inhibition of either the COX-1 or 2 enzymes at high concentrations (>100 μM). Cytochrome P450 inhibition is minimal and is anticipated to be of nominal consequence in a dermal application. One of the differentiating factors of compound **6** from other FLAP inhibitors studied was that the sodium salt proved highly soluble in a prototypical, nonirritating, dermal vehicle consisting of 75% propylene glycol, 15% transcutol and 10% water, thus allowing facile dermal application in animal models.

To demonstrate the effectiveness of AM643 (**6**) in inhibiting LT biosynthesis in vivo we utilized a murine topical AA model. In this model the application of AA to the ears of mice produces an immediate erythema and edema response with subsequent increase in ear weight.[21] Each mouse is dosed topically on one ear with AA in ethanol (4 mg in 40 μL) and the other ear solely with 40 μL ethanol as a control. After a 30 min period ear samples are collected using a 6 mm dermal punch, weighed and assayed for LT concentrations. As a benchmark study, oral administration of **6** to mice (30 mg/kg in 0.5% methocel) 4 h prior to AA treatment, showed significant inhibition of ear edema in the AA treated ear with concomitant reduction in both LTB_4 and CysLT concentrations, although the LTB_4 inhibition did not reach statistical significance (Fig. 2). Analysis of plasma samples from these mice indicated sufficient concentration of compound **6** at 4.5 h post-dose (av 1.5 μM, $n = 4$) to completely inhibit FLAP and hence LT biosynthesis (mWB LTB_4 IC_{50} at 4 h = 4 nM). A significant component of the edema and erythema is due to metabolism of the applied arachidonic acid through the cyclooxygenase-1 (COX-1) pathway thereby producing pro-inflammatory prostaglandins. The levels of inhibition achieved with the FLAP inhibitor **6** are therefore in line with expectations for complete leukotriene synthesis inhibition alone.

Topical administration of **6** showed a dose dependent decrease in CysLTs and LTB_4 in the mouse ear and also a reduction in edema as measured by the change in ear weight. At the 0.1% dose concentration of **6**, CysLTs were reduced by 37%, LTB_4 was reduced 55% and the change in ear weight by 61%, demonstrating a similar effect to that seen with systemic dosing (see Fig. 3) Higher concentrations of compound **6** (1% and 10%) produced no significant improvement over the 0.1% dose (data not shown).

To assess the potential for dermal irritancy of AM643 (**6**), we employed an abraded rat skin model. The study comprised administering 2 mL of a 10 mg/mL solution of **6** (~40 μM) in the aforementioned vehicle to 20% of the body area of healthy male rats. The shaved skin was abraded prior to compound administration then occluded for 24 h after which the dosage site was cleaned of

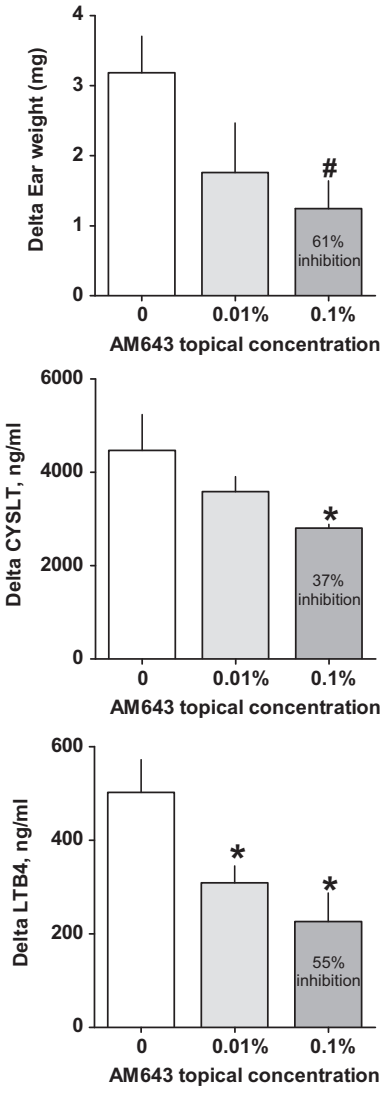


Figure 3. Topical administration of AM643 (**6**) (0.01% and 0.1%) led to a concentration dependent decreased AA (4 mg/ear)–induced ear swelling and tissue leukotriene biosynthesis. Bars represent the means ± SEM of $n = 3$ mice per group. $^{*}P < 0.05$ versus vehicle treated mice, Dunnett's post hoc comparisons following ANOVA; $^{\#}P < 0.05$ versus vehicle treated mice, *t*-test.

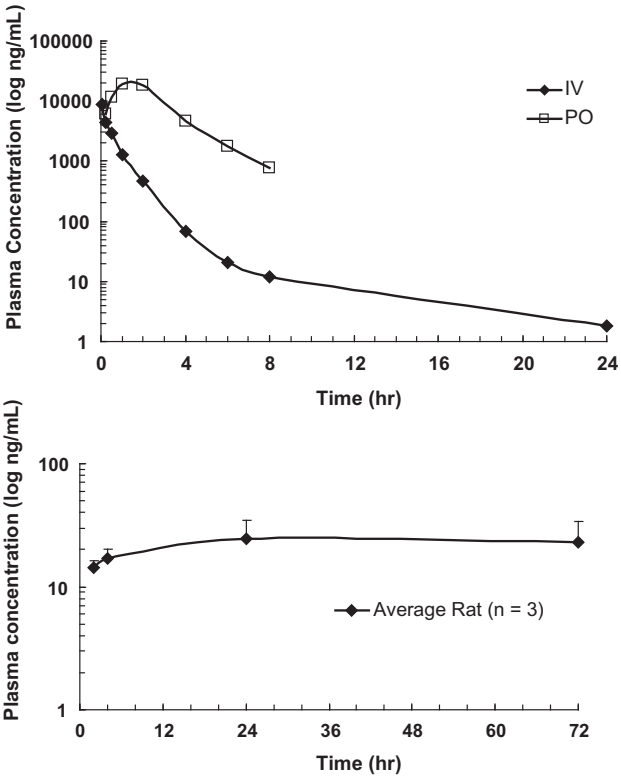


Figure 4. Pharmacokinetics of **6**. Top: 10 mg/kg in 0.5% methocel (po) and 1 mg/kg in 10% EtOH/40% PEG400/50% water (iv); bottom: plasma concentration from rat after topical dosing of **6** (see text).

residual material and the animals observed for a period of 72 h. Dermal irritancy scoring was conducted following the modified Draize method[22] and blood samples were drawn to assess the degree of systemic compound exposure following dermal application. Topical administration of **6** in this manner showed no findings of erythema or edema (a Draize score of zero) and no change in body weight over the 72 h test period. Of note is the finding that small, but detectable levels of compound were present in the plasma of the animals at all time points. Compound **6** possesses a terminal $t½$ of approximately 2 h in rat following intravenous dosing, and a comparison of the dermal and oral rat pharmacokinetics (see Fig. 4) indicate that a continued slow leaching of compound through the skin occurs in rat. The presence of low concentrations of **6** in the rat plasma after topical dosing also demonstrates that the compound in this preliminary vehicle is suitably penetrant.

In conclusion, we have identified a potent FLAP inhibitor that demonstrates robust efficacy in a murine arachidonic acid ear swelling model upon topical administration. Furthermore, AM643 (**6**) possesses no apparent potential for irritancy in an abraded rat skin study and shows a long residence time in rodent skin after topical application. Additional studies and optimization of a suitable dermal vehicle for clinical development will be published in due course.

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