Synthesis and Biological Evaluation of 1,2,4-Triazinylphenylalkylthiazolecarboxylic Acid Esters as Cytokine-Inhibiting Antedrugs with Strong Bronchodilating Effects in an Animal Model of Asthma

Eddy J. Freyne,[†] Jean F. Lacrampe,[‡] Frederik Deroose,[†] Gustaaf M. Boeckx,[†] Marc Willems,[†] Werner Embrechts,[†] Erwin Coesemans,[†] Johan J. Willems,[†] Jerome M. Fortin,[‡] Yannick Ligney,[‡] Lieve L. Dillen,[§] Willy F. Cools,[§] Jan Goossens,[§] David Corens,[†] Alex De Groot,[†] and Jean P. Van Wauwe*[,§]

Medicinal Chemistry Department and Department of Inflammation, Johnson & Johnson Pharmaceutical Research and Development, a Division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium, and Campus de Maigremont BP 615, 27106 Val de Reuil, France

Received June 30, 2004

The influx of leukocytes (eosinophils, lymphocytes, and monocytes) into the airways and their production of proinflammatory cytokines contribute to the severity of allergic asthma. We describe here the synthesis and pharmacological evaluation of a series of triazinylphenylalkylthiazolecarboxylic acid esters that were designed to act as lung-specific antedrugs and inhibitors of the production of interleukin (IL)-5, a primary eosinophil-activating and proinflammatory cytokine. Closer examination of the hydroxypropyl ester, **15**, indicated its high metabolic stability ($t_{1/2}$ > 240 min) in human lung S9 fraction but rapid conversion ($t_{1/2}$ = 15 min) into the pharmacologically inactive carboxylic acid by human liver preparations. In stimulated human whole blood cultures, **15** reduced not only the production of IL-5 (IC_{50} = 78 nM) but also the biosynthesis of the monocyte chemotactic proteins MCP-1 (IC_{50} = 220 nM), MCP-2 (IC_{50} = 580 nM), and MCP-3 (IC_{50} = 80 nM). In vivo, intratracheal administration of **15** (6 mg/animal) to allergic sheep, either before (−4 h) or after (+1.5 h) the pulmonary allergen challenge, completely abrogated the late-phase airway response and reduced the bronchial hyperreactivity to inhaled carbachol.

Introduction

Allergic asthma is a chronic lung disease characterized by airflow obstruction, symptoms of cough, breathlessness and chest tightness, increased airway responsiveness together with evidence of airway inflammation.[1] Inflammatory phenomena include denudation of the airway epithelium, edema of the submucosa, smooth muscle hypertrophy, and the infiltration or activation of inflammatory cells, in particular eosinophils and T lymphocytes.[2] The activated T lymphocytes belong to the Th-2 cell subpopulation and display a cytokine production profile that includes interleukin (IL)-4, IL-5, monocyte chemotactic protein (MCP)-1, MCP-2, and MCP-3. IL-4 induces the synthesis of IgE antibodies,[3] whereas IL-5 is responsible for growth, differentiation, activation, and survival of eosinophils.[4] MCP-1, MCP-2, and MCP-3 attract or activate basophils, monocytes, or eosinophils in the airways.[5] Thus, suppression or antagonism of proallergic cytokines may represent a viable approach for the treatment of asthma[6,7] Through compound library screening using the phytohemagglutinin (PHA)-activated human whole blood assay,[8] we identified a series of 1-phenyl-substituted 6-azauracil derivatives with micromolar IL-5 inhibiting efficacy.[9]

Subsequent targeted chemical synthesis resulted in the characterization of R146225 (Figure 1, **1**) as a nanomolar potent, orally active IL-5 inhibitor capable of reducing the pulmonary infiltration of eosinophils in *Cryptococcus*-challenged mice.[10]

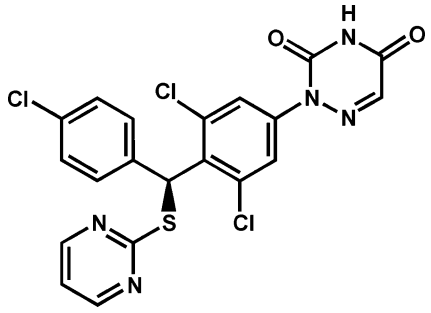


Figure 1. R146225 (**1**).

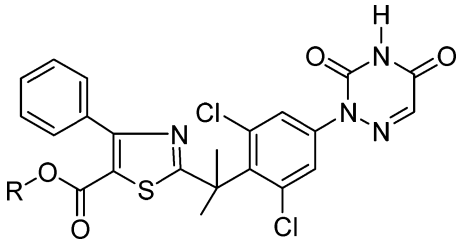
However, chronic dosing of **1** to pregnant rats and rabbits induced signs of teratogenesis and efforts to develop this compound were halted. However, convinced of the intrinsic antiasthmatic properties of compound **1**, we sought to eliminate the teratogenic side effect by designing 1-substituted 6-azauracil-containing antedrugs, i.e., compounds that exert their desired topical effect in the lung (target tissue) but are rapidly converted into inactive/nontoxic metabolites after entry into the systemic circulation.[11−14]

Synthetic efforts aimed at combining the 1-substituted 6-azauracil pharmacophore with "labile" carboxylic ester functionalities[14−16] led to the synthesis of a series of triazinylphenylalkylcarboxylic acid esters with lung-specific antedrug characteristics.

* Author to whom correspondence should be addressed. Phone: + 32-14 602464. Fax: + 32-14 605403. E-mail: jvwauwe@prdbe.jnj.com.
[†] Medicinal Chemistry Department, Belgium.
[‡] Medicinal Chemistry Department, France.
[§] Department of Inflammation, Belgium.

10.1021/jm049479m CCC: $30.25
Published on Web 12/29/2004

Table 1. Metabolic Stability of Compounds in Human Lung and Liver S9 Fractions and Their IL-5 Inhibitory Effects



compound	R	synthesis method	metabolic stability (% conversion at 60 min) human lung	metabolic stability (% conversion at 60 min) human liver	IL-5 inhibition (% inhibition) 10^{-6} M	IL-5 inhibition (% inhibition) 10^{-7} M
13	ethyl	A	17	29	94	67
14	isopropyl	A	1	10	94	85
15	$CH_2-CH_2-CH_2-OH$	A	8	76	83	58
16	*t*-butyl	A	0	7	89	79
17	H	B	0	0	0	0
18	methyl	B	47	49	93	84
19	*n*-propyl	B	23	68	96	69
20	*n*-butyl	B	18	86	88	51
21	*n*-pentyl	B	32	72	91	54
22	CH_2-cprop	B	27	87	92	56
23	isobutyl	B	27	80	90	56
24	$CH_2-CH=CH_2$	B	23	68	96	69
25	$CH_2-CH=C(Me)_2$	B	6	65	73	55
26	$CH_2-C\equiv CH$	B	20	55	90	87
27	CH_2-CH_2-Ph	B	24	94	88	62
28	CH_2CN	B	34	61	91	74
29	CH_2-CF_3	B	36	38	92	75
30	$CH_2-C\equiv C-Me$	C	3	1	100	96
31	CH_2-CH_2-OH	C	0	1	93	66
32	CH_2-CH_2-OPh	C	21	73	83	70
33	$CH_2(CO)Me$	C	19	64	85	57

Chemistry

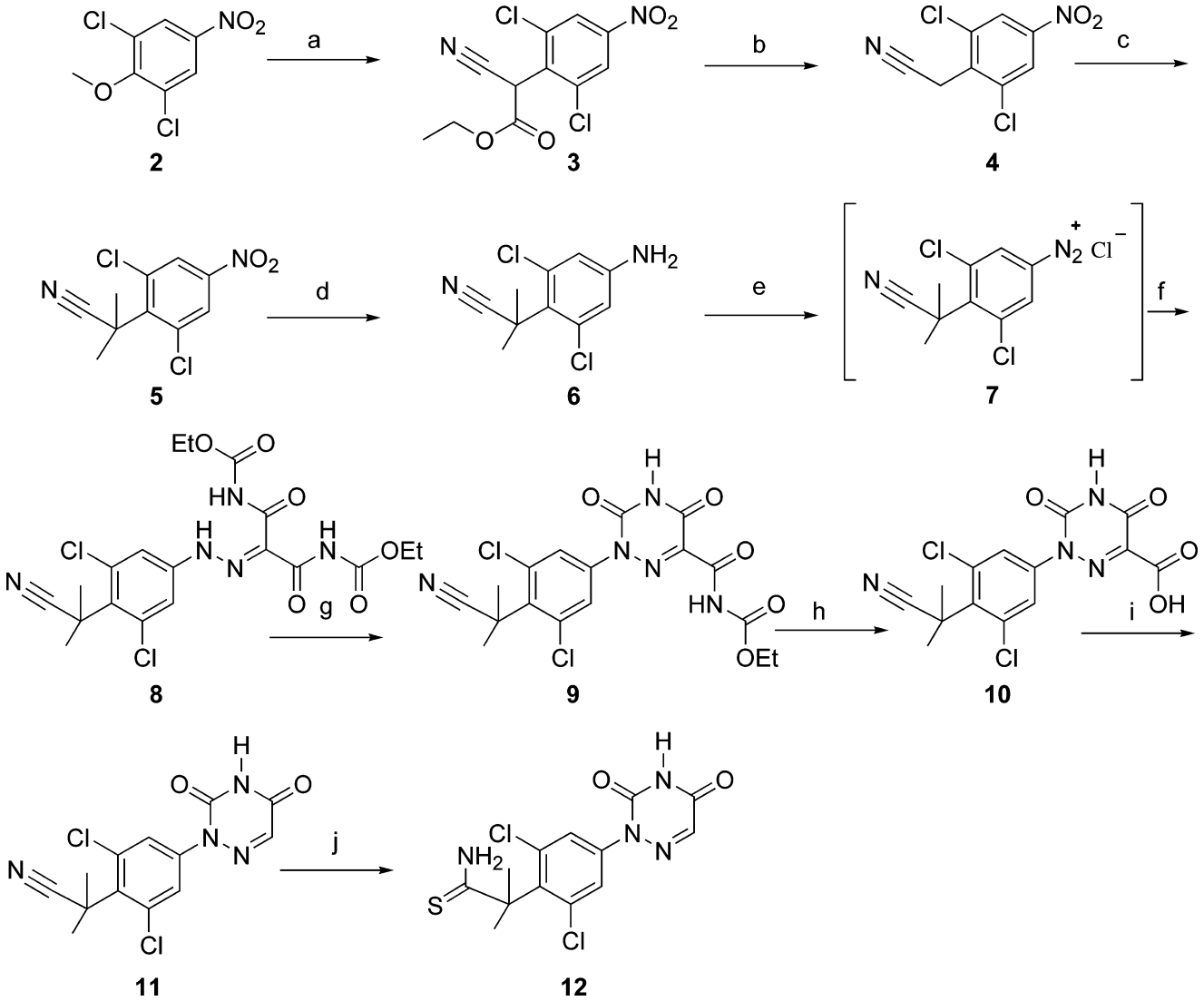
Further extension of our chemical program around **1** resulted in the discovery of triazinylphenyl-alkylthiazole derivatives as a novel family of nonchiral IL-5 inhibitors that served as a template for the synthesis of potential antedrugs.[17] The title compounds are compiled in Table 1, and the general procedures for their total syntheses are outlined in Schemes 1 and 2. The pivotal intermediate, thioamide **12** (Scheme 1), was prepared from the commercially available 2,6-di-chloro-4-nitroanisole in 10 steps. From 2,6-dichloro-4-nitroanisole **2**, selective displacement of the methoxy group was achieved with the sodium salt of ethyl cyanoacetate in dimethylformamide (DMF) at 5 °C giving **3**. The ethoxycarbonyl group was next removed in a mixture of dimethyl sulfoxide (DMSO)/H_2O using LiCl at 165 °C to produce compound **4**. Double methylation of **4** was obtained using MeI in tetrahydrofuran (THF) in the presence of benzyltriethylammonium chloride (BTEAC) and 50% NaOH at 50 °C affording **5**. Conversion to the corresponding aniline **6** was carried out by catalytic hydrogenation with Raney Ni in MeOH at room temperature. This aniline derivative was used immediately for the construction of the triazine-3,5(2*H*,4*H*)-dione ring. The synthesis of such a 6-azauracil ring had been reported.[18] We used an adapted procedure of **1**[9] and our anticoccidial product Clinacox.[19] In this method, 4-amino-2,6-dichloro-α,α-dimethylbenzeneacetonitrile (**6**) was converted to the diazonium salt **7** using an aqueous $NaNO_2$ solution in a mixture of HOAc and concentrated HCl at 10 °C. Compound **7** was not isolated, but after NaOAc was added, subsequently coupled further with the symmetrical malonyldiurethane to the intermediate **8**. Ring cyclization to **9** was carried out using KOAc in HOAc at 120 °C and without isolation was further hydrolyzed with concentrated HCl at reflux to the corresponding acid **10**. Decarboxylation to give the 6-azauracil derivative **11**, was done in thioglycolic acid at 100 °C. Finally, conversion of the nitrile **11** to the pivotal intermediate thioamide **12** was completed in pyridine/diisopropylethylamine (DIPEA), ensuring a constant flow of H_2S through the solution at 80 °C.

For the synthesis of the target compounds **13**−**33** (Scheme 2), three methods were elaborated. Following method A, the ester derivatives **13**−**15** were formed via direct cyclization of compound **12** with the appropriate α-bromo-*β*-oxo-benzenepropionate esters[17,20] **34**−**36** in DMF. For compound **15** (R = *t*-Bu), **12** was first condensed with *tert*-butyl α-bromo-*β*-oxo-benzenepropionate **37** in CH_3CN in the presence of K_2CO_3. The actual cyclization to the ester derivative **16** was carried out in refluxing *t*-BuOH. Conversion to the acid **17** was proven to go smoothly in CF_3COOH at room temperature. Following method B, compound **17** was alkylated directly with the appropriate alkyl halides in DMF using $NaHCO_3$ as a base at a temperature of 70 °C, giving the corresponding ester variations **18**−**28**. Finally, in method C, the acid **17** was activated using CDI in DMF at 40 °C. Then, the reactive imidazolylcarbonyl intermediate initially formed was treated further with the appropriate alcohol and DBU affording compounds **29**−**33**.

Pharmacology

Compounds were tested in vitro for their metabolic stability and inhibitory activity on IL-5 production. Metabolic stability was assessed in human lung and liver S9 fractions and expressed as percent of compound

Scheme 1. Synthesis of the Key Thioamide Intermediate **12**[a]



[a] Reagents and conditions: (a) NaH, $NCCH_2COOEt$, N_2 atm, DMF, 5 °C to room temperature; (b) LiCl, $DMSO/H_2O$ (5:2), 165 °C; (c) 50% NaOH, MeI, BTEAC, THF, 50 °C, 6 h; (d) H_2 (3 bar), Raney Ni (5%), MeOH, room temperature; (e) $NaNO_2$, H2O, HOAc, conc HCl, 10 °C; (f) NaOAc, CH2(CONHCOOEt)2, 10 °C; (g) KOAc, HOAc, 120 °C, 3 h; (h) conc HCl, reflux, 4 h; (i) $HSCH_2COOH$, 100 °C, 4 h; (j) H_2S, DIPEA, pyridine, 80 °C.

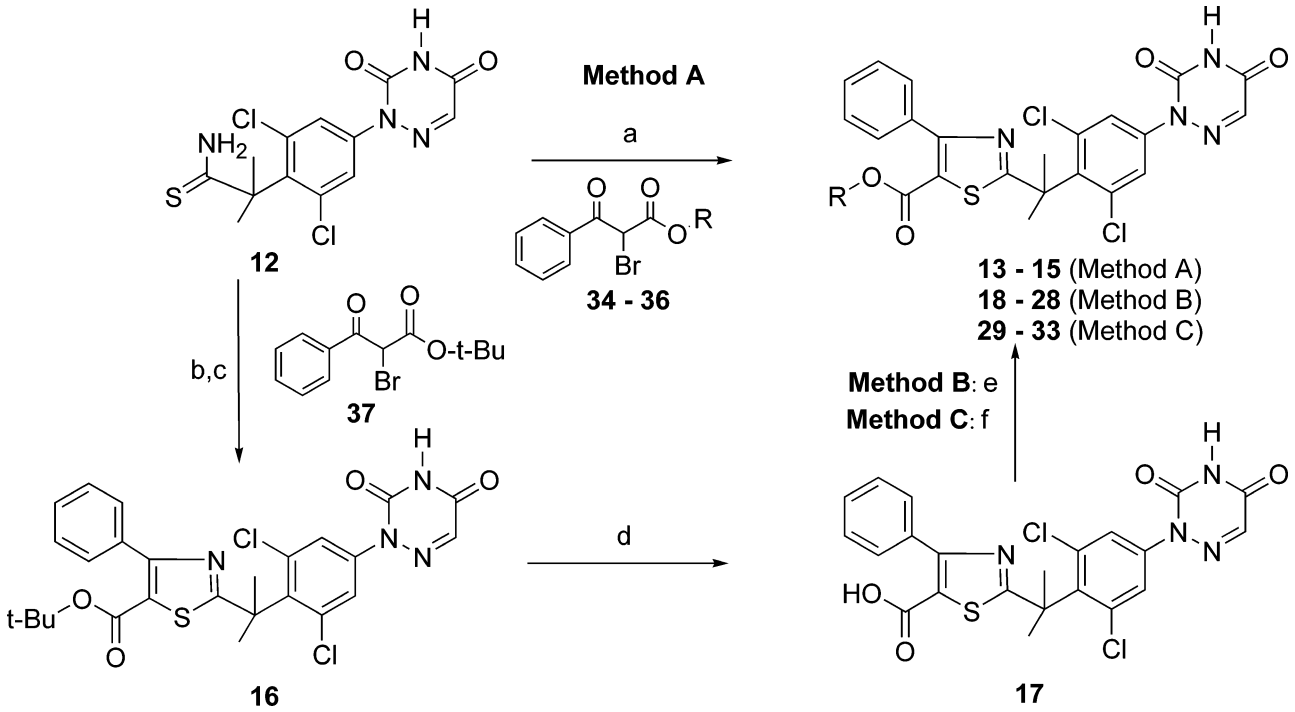
converted after a 60 min incubation at 37 °C, whereas IL-5 production was determined by ELISA in the supernatant of human whole blood cultures stimulated by PHA for 48 h.

As presented in Table 1, most esters were rather resistant to metabolic conversion when incubated with lung S9 fraction. Conversion rates below 10% were obtained for compounds **14**, **15**, **16**, **25**, **30**, and **31**. By contrast, when incubated with liver S9 fraction, several compounds, such as **15**, **20**, **21**, **22**, **23**, **25**, **27**, and **32** were readily (>70% conversion) metabolized. Taken together, only compounds **15** and **25** could be identified as compounds having a metabolic stability that was high in lung and low in liver S9 fractions. Because none of the tested esters was converted for more than 10% when incubated with fresh human plasma (data not shown), we used PHA-stimulated human whole blood cultures to assess their effect on the production of IL-5. As listed in Table 1, when tested at 10^{-7} M, all esters suppressed by more than 50% the production of this cytokine. However, the strongest IL-5 production inhibitors **14**, **16**, **18**, **26**, **28**, **29**, **30**, and **32** invariably displayed unfavorable antedrug characteristics. Taken together, these data led us to select **15** for additional experiments to document its potential as a lung-directed antedrug.

First, time course studies using human samples to determine the metabolic stability of **15** indicated that its metabolic half-life ($t_{1/2}$) exceeded 240 min in plasma and lung S9 fraction but was as short as 15 min in liver S9 preparation. Additional stability experiments that were done using tissues from sheep (the species to be used for in vivo evaluation) indicated that **15** is metabolically stable in sheep plasma and lung ($t_{1/2}$ > 240 min) but rather labile ($t_{1/2}$ = 60 min) in liver preparations, comparable to the human data.

Additionally, a 60 min incubation of **15** with human liver S9 fraction and subsequent LC/MSMS analysis of the reaction mixture identified the carboxylic acid **17** as the sole metabolic product. This compound had no effect on the whole blood production of IL-5 (Table 1), indicating that **15** was metabolized to a pharmacologically inactive product.

Scheme 2. Synthesis of Target Compounds **13**−**33**[a]



[a] Reagents and conditions: (a) method A, PhCOCH(Br)COOR **34**−**36**, $HO(CH_2)_3OH$/DMF or EtOH/DMF or K_2CO_3, CH_3CN; (b) PhCOCH(Br)COO*t*-Bu **37**, K_2CO_3, CH_3CN, room temperature; (c) *t*-BuOH, reflux; (d) CF_3COOH, room temperature, 4 h; (e) method B, acid **17**, appropriate RX (X = Br, I), $NaHCO_3$, DMF, 70 °C; (f) method C: acid **17,** appropriate ROH, CDI, DBU, DMF, 40 °C.

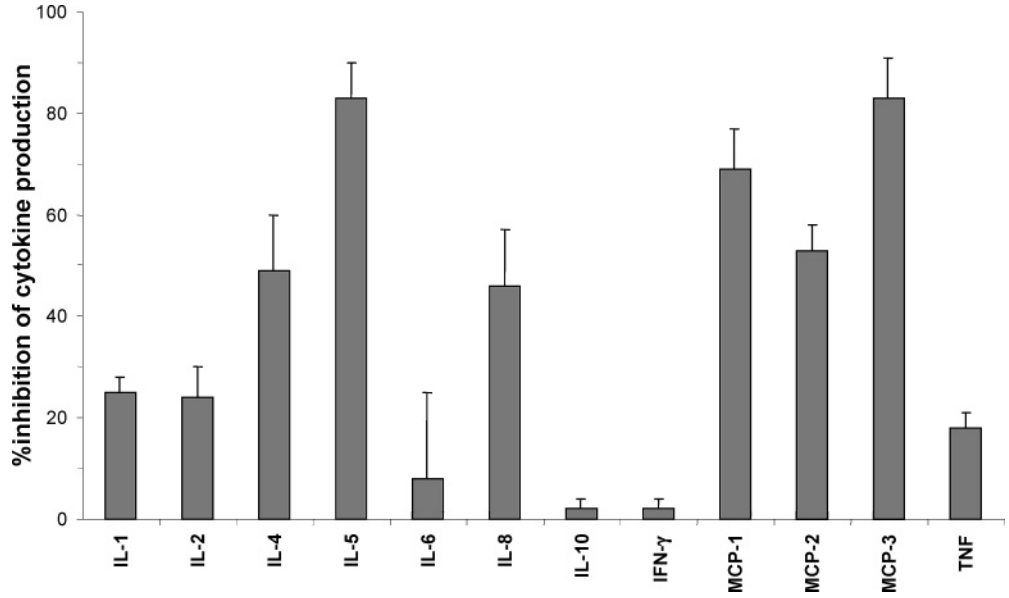


Figure 2. Effect of **15** (10^{-6} M) on cytokine protein production in PHA-stimulated human whole blood.

The breadth of cytokine inhibitory activity of **15** was then examined by determining the compound's effect on the PHA-induced whole blood production of other T-cell-derived cytokines besides IL-5. As shown in Figure 2, when tested at a concentration of 10^{-6} M, **15** not only reduced the production of IL-5 (83 ± 7% inhibition) but also suppressed the synthesis of IL-4 (49 ± 11% inhibition), IL-8 (46 ± 11% inhibition), MCP-1 (69 ± 8% inhibition), MCP-2 (53 ± 5% inhibition), and MCP-3 (83 ± 8% inhibition).

Concentration−response experiments indicated that **15** inhibited the production of IL-5, MCP-1, MCP-2, and MCP-3 with IC_{50} values of 78, 220, 580, and 80 nM, respectively. However, the compound barely affected the generation of IL-1 (24 ± 4% inhibition), IL-2 (23 ± 7% inhibition), IL-6 (8 ± 17% inhibition), IL-10 (3 ± 8% inhibition), IFN-γ (2 ± 2% inhibition), or TNF (18 ± 6%).

Finally, we examined the antiasthmatic effects of **15** in a model of *Ascaris suum* antigen-induced airway responses in conscious allergic sheep. In the vehicle trial, the antigen challenge enhanced the specific lung resistance (SR_L expressed in liters × cm H_2O/(L/s)) from 0.98 ± 0.02 to peak and late phase responses of 4.04 ± 0.81 and 2.09 ± 0.09, respectively. Pretreatment (4 h before the *Ascaris* challenge) with 6 mg of aerosolized **15** significantly reduced the late-phase bronchoconstric-

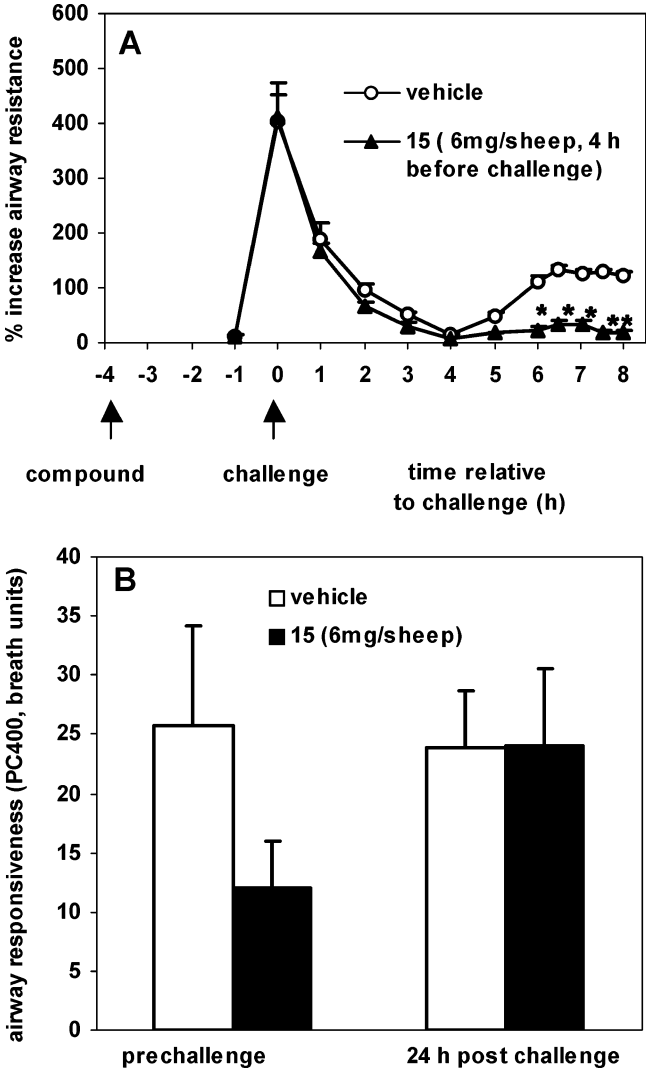


Figure 3. Effect of aerosolized **15** (6 mg, administered at 4 h before allergen exposure) on airway responses in allergen-challenged *Ascaris*-sensitive sheep. (A) Early- and late-phase bronchoconstriction was assessed as the percentage of increase of specific lung resistance (SR_L) over a 8 h period after antigen challenge (mean ± SE, $n = 5$; * $p < 0.01$ vs vehicle). (B) Airway hyperreactivity was determined by the change in the cumulative carbachol dose, expressed as breath units needed to induce a 400% increase in SR_L measured 24 h after the antigen challenge.

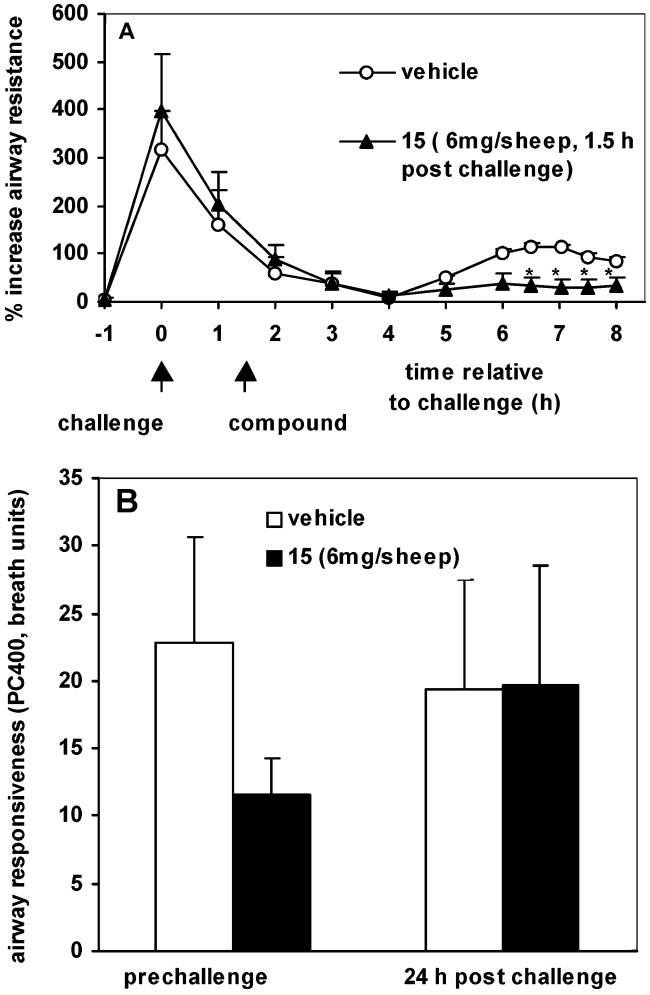


Figure 4. Effect of aerosolized **15** (6 mg, administered 1.5 h after allergen exposure) on (A) early- and late-phase bronchoconstriction and (B) airway responsiveness in allergen-challenged *Ascaris*-sensitive sheep ($n = 5$). Bronchoconstriction was measured as the percentage of increase of specific lung resistance (SR_L) over a 8 h period after antigen challenge (mean ± SE, $n = 5$; * $p < 0.01$ vs vehicle), and airway responsiveness (expressed in cumulative breath units of inhaled carbachol to induce a 400% increase in specific lung resistance) was measured before and 24 h postantigen challenge.

tion, although it had no effect on the early-phase reaction (Figure 3A). Also, as shown in Figure 3B, **15** completely blocked the development of airway hyperreactivity, as evidenced by its ability to prevent the fall in the PC_{400} values, i.e., the provocative dose of inhaled carbachol that caused a 400% increase of SR_L.

Of importance, delaying the administration of **15** to 1.5 h after the antigen challenge still produced marked suppressive effects on the late-phase response and airway responsiveness (Figure 4A and B).

To assess the extent of systemic exposure, sheep ($n = 5$) received an intratracheal administration of aerosolized **15** (6 mg) and blood was drawn at 5, 10, 20, 60, 120, and 240 min later. Plasma concentrations of **15** reached a maximal value of 28.4 ± 4.5 ng/mL (5×10^{-8} M) at 5 min after treatment decreasing rapidly to less than 2 ng/mL at 60 min. Levels of the carboxylic acid metabolite **17** peaked to 17.2 ± 3.1 ng/mL at 10 min and became virtually undetectable (<5 ng/mL) at the 120 min time point.

Conclusions

We have synthesized a series of 1,2,4-triazinylphenylalkylcarboxylic acid esters that inhibit the production of IL-5 and have the potential to act as lung-specific antedrugs. On the basis of its favorable metabolic and pharmacological properties, the hydroxypropyl ester **15** was selected for more detailed evaluation. This compound showed high metabolic stability ($t_{1/2}$ > 240 min) in human lung S9 samples but was rapidly ($t_{1/2}$ = 15 min) converted by human liver S9 fraction to the pharmacologically inactive carboxylic acid **17**. Furthermore, when profiled for its cytokine specificity, **15** was found to suppress at submicromolar potency the human whole blood production of IL-5 and MCP-1, -2, and -3 and, to a lesser extent, the biosynthesis of IL-4 and IL-8, while the generation of several other cytokines (IL-

1, IL-2, IL-6, IL-10, IFN-γ, and TNF) was barely affected.

As each of these cytokines can trigger proallergic activities,[3−5,21] inhibition of their production may explain the antiallergic effect of **15** in the *Ascaris* sheep model of asthma. Indeed, intratracheal administration of aerosolized **15** reduced the antigen-induced late-phase bronchoconstriction and development of airway responsiveness. The compound abrogated these late phase reactions without affecting the immediate bronchial response, in line with the noninvolvement of cytokines during the early stage of the bronchoconstriction reaction. An intriguing feature is that compound **15** generated its effect when administered both before (prophylactic mode) and after (curative mode) the antigen challenge. This curative activity appears to be exceptional in this sheep model and has not been reported for a number of antiallergic drugs, including the corticosteroid budenoside[22] and the mast cell tryptase inhibitor APC 366.[23]

Additonally, the intratracheal administration of **15** resulted in short-lived and minimal plasma exposure, supporting its characterization as a compound with potential use in the topical treatment of allergic disorders.

Experimental Section

All of the air and moisture sensitive reactions were conducted under a nitrogen atmosphere. All of the reagents and starting materials were obtained from commercial sources and used without further purification. THF, DMF, CH_2Cl_2, $CHCl_3$, toluene, and pyridine were dried over 3 Å molecular sieves. DIPE refers to diisopropyl ether, DIPA refers to *N*,*N*-diisopropylethylamine, DBU refers to 1,8-diazabicyclo[5.4.0]undec-7-ene, CDI refers to 1,1′-carbonyldiimidazole, NBS refers to *N*-bromosuccinimide, and BTEAC refers to *N*,*N*,*N*′-benzyltriethylammonium chloride. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400, DPX 400, and DPX 360 spectrometers. DMSO-d_6 was used as solvent, unless otherwise mentioned. The chemical shifts are expressed in δ, parts per million (ppm), downfield from internal tetramethylsilane (TMS). The peak patterns are shown as the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Melting points were determined on a Kofler or Buchi B545 instrument and are uncorrected. Elemental analyses were performed on a Carlo-Erba EA1110 analyzer. Analytical results were within ±0.4% of the theoretical values unless otherwise noted.

Silica gel thin-layer chromatography was performed on precoated plates Kieselgel 60F_{254} (E. Merck, AG, Darmstadt, Germany). Silicagel chromatography was performed with Kieselgel 60 (0.063−0.200 mm) (E. Merck, AG, Darmstadt, Germany).

Ethyl 1,3-Dichloro-α-cyano-4-nitrobenzeneacetate (3). To a cooled (5 °C), stirred solution of ethyl cyanoacetate (122.4 g, 1.08 mol) in DMF (1 L), NaH dispersion (60% mineral oil) (40.5 g, 1.08 mol) was added in portions under a N_2 atmosphere. After the addition was completed, the mixture was stirred further at 5 °C for 1 h until evolution of hydrogen had stopped. Nitro derivative **2** (200 g, 0.9 mol, commercially available) was added, and the mixture was allowed to warm to room temperature overnight under stirring. The reaction mixture was poured onto ice and adjusted to pH = 3 with concentrated HCl. The resulting precipitate was filtered off, washed with water, and taken up in CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting solid was stirred up in *n*-hexane and dried in vacuo overnight (60 °C) to give **3**: 261.3 g (96%).

2,6-Dichloro-4-nitrobenzeneacetonitrile (4). To a stirred mixture of nitro derivative **3** (2 g, 6.6 mmol) and LiCl (0.254 g, 6.0 mmol), DMSO (5 mL) and water (2 mL) were added at room temperature. The reaction flask was placed in an oil bath that was preheated at 165 °C. The reaction mixture was stirred for 30 min at 165 °C (CO_2 gas evolution). The dark red-brown reaction solution was poured out into ice−water, and this mixture was extracted DIPE (3×). The combined organic extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo on a water bath at 40 °C, yielding an off-white solid. The compound was crystallized from DIPE (25 mL), filtered off, washed with DIPE and *n*-hexane, and dried in vacuo (50 °C) to give the pure title compound **4**: 1.04 g (68%), mp 106 °C. Anal. ($C_8H_4Cl_2N_2O_2$) C,H,N.

2,6-Dichloro-α,α-dimethyl-4-nitrobenzeneacetonitrile (5). To a solution of compound **4** (577.6 g, 2.51 mol), methyl iodide (1,069 g, 7.33 mol), and BTEAC (57 g, 0.251 mol) in THF (5 L), 50% NaOH (960 mL) was added in a dropwise manner. The mixture was stirred vigorously at 50 °C for 6 h. The reaction mixture was poured out on ice−water and extracted with DIPE. The combined organic extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. The residual solid was purified by column chromatography on silica gel (30%EtOAc/70% hexane). The fractions containing pure product were combined and concentrated in vacuo to dryness. The resulting solid was stirred up in *n*-hexane, filtered off, and dried in vacuo to give **5**: 457 g (70%).

4-Amino-2,6-dichloro-α,α-dimethylbenzeneacetonitrile (6). A mixture of 2,6-dichloro-α,α-dimethyl-4-nitrobenzeneacetonitrile (**5**; 17.1 g, 66 mmol) in MeOH (200 mL) was hydrogenated under pressure (3 bar) for 1 h, using Raney Ni (5%) at room temperature. The catalyst was filtered through Celite and washed with MeOH, and the filtrate was concentrated in vacuo to afford **5**: 15.0 g (>95%). The compound was immediately used further in the preparation of **10**.

2-[3,5-Dichloro-4-(1-cyano-1-methylethyl)phenyl]-2,3,4,5-tetrahydro-3,5-dioxo-1,2,4-triazine-6-carboxylic Acid (10). A solution of 4-amino-2,6-dichloro-α, α-dimethylbenzeneacetonitrile (**6**; 77.6 g, 0.34 mol) in HOAc (700 mL) and concentrated HCl (102 mL) was stirred under a nitrogen atmosphere at 10 °C for 30 min. A solution of $NaNO_2$ (24.8 g, 0.36 mol) in H_2O (50 mL) was added dropwise over 30 min maintaining the temperature around 10 °C forming the corresponding diazonium salt **7**. After an additional 80 min at 10 °C, a mixture of NaOAc (83.64 g, 1.02 mol) and malonyldiurethane (92.1 g, 0.374 mol) was added in one portion. After 40 min, the reaction mixture was poured out on ice−water (2 L). The precipitate was filtered off, washed with water, and dissolved in CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure affording diethyl *N*,*N*′-[2-[[3,5-dichloro-4-(1-cyano-1-methylethyl)phenyl]hydrazono]-1,3-dioxo-1,3-propanediyl]dicarbamate (**8**): 135 g (84%). The compound was dissolved in HOAc (1 L) and treated with KOAc (27.25 g, 0.28 mol), and the reaction mixture was refluxed for 3 h at 120 °C forming ethyl [[2-[3,5-dichloro-4-(1-cyano-1-methylethyl)phenyl]-2,3,4,5-tetrahydro-3,5-dioxo-1,2,4-triazin-6-yl]carbonyl]carbamate (**9**). Subsequently, concentrated HCl (70 mL, 0.84 mol) was added, and after an additional stirring for 4 h at reflux, the mixture was allowed to warm to room temperature. After being stirred at ambient temperature over the weekend, the reaction mixture was poured out on ice and extracted with CH_2Cl_2. The organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo to give the title compound **10**: 100,6 g (98%, over two steps from **8**).

2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)-α,α-dimethylbenzeneacetonitrile (11). A stirred suspension of **10** (111.6 g, 0.28 mol) in thioglycolic acid (250 mL) was heated for 4 h at 100 °C, then allowed to cool to room temperature, and stirred further overnight. The reaction mixture was poured out on ice and extracted three times with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and filtered, and the solvent was removed in vacuo. Toluene was added and concentrated under reduced pressure (3×). The residual material was purified by short column chromatogra-

phy on silica gel using solvent (98% CH_2Cl_2/2% CH_3OH). The pure fractions were collected, and the solvent was removed in vacuo. The residual product was stirred up in DIPE, filtered off, and washed with DIPE in vacuo at 50 °C for 16 h yielding **11** (39.3 g, 44%).

2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl]-α,α-dimethylbenzeneethane-thioamide (12). A stirred solution of **11** (56 g, 0.172 mol) and DIPEA (67 g, 0.662 mol) in pyridine (500 mL) was heated at 80 °C. At this temperature, H_2S gas was bubbled through the reaction mixture for 24 h. After an additional stirring at room temperature over the weekend, the solvent was removed in vacuo. The crude material was purified by HPLC chromatography on silicagel (Novasep column, diameter 80 mm; silicagel 20−45 μm) using 93%CH_2Cl_2/7% MeOH as solvent system affording thioamide **12**: 24 g (40%).

1-Methylethyl α-Bromo-*β*-oxo-benzenepropionate (35). To a solution of 1-methylethyl *β*-oxo-benzenepropionate[24](3 g, 1.5 mmol) in CH_2Cl_2 (75 mL) was added a solution of Br_2 (0.67 mL, 1.3 mmol) in CH_2Cl_2 (5 mL) in a dropwise manner at 10 °C. The reaction mixture was stirred further for an additional 1 h and then treated with 10% K_2CO_3 solution. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated in vacuo giving the crude bromide **35** (3.6 g, 87%), which was used subsequently in the synthesis of compound **14**.

Ethyl α-Bromo-*β*-oxo-benzenepropionate (34) and 1,1-Dimethylethyl α-Bromo-*β*-oxo-benzenepropionate (37). Bromide **34**[20] and **37** were synthesized from the commercially available ethyl *β*-oxo-benzenepropionate and 1,1-dimethylethyl *β*-oxo-benzenepropionate,[25] respectively, according to the method for the preparation of **35**. Compound **34** and **37** were immediately used further in the synthesis of **13** and **16**, respectively.

3-Hydroxypropyl α-Bromo-*β*-oxo-benzenepropionate (36). To a vigorously stirred solution of 3-hydroxypropyl-*β*-oxo-benzenepropionate[26] (26.0 g, 0.097 mol) in $CHCl_3$ (250 mL), NBS (20.3 g, 0.114 mol) was added in portions over 2 h at room temperature under a nitrogen atmosphere. After an additional stirring for 2 h, the reaction mixture was treated with an aqueous $NaHCO_3$ solution. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residual bromide **36** (30.1 g, 85%) was immediately used further in the preparation of compound **15**.

Representative Procedure for the Synthesis of the Target Compounds (13−15, 16). Method A. Compounds **13**−**15** and **16** were synthesized via direct ring closing reaction of the thioamide **12** with the appropriate α-bromo-*β*-oxo-benzenepropionate ester derivatives **34**−**37**.

3-Hydroxypropyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (15). To a stirred solution of bromide **36** (30.1 g, 0.1 mol) in DMF (300 mL) under a N_2 atmosphere, 1,3-propane-diol (30 mL) and subsequently thioamide **12** (35.9 g, 0.1 mol) were added. The mixture was stirred at ambient temperature overnight and then for an additional 3.5 h at 70 °C. The reaction mixture was partially concentrated in vacuo, poured out in ice−water, and extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo giving a residual solid that was purified by chromatography on a short column of silica gel (99% CH_2Cl_2/1% MeOH to 98% CH_2Cl_2/2% MeOH). After an additional purification via HPLC on silica gel (100% CH_2Cl_2 to 97.5% CH_2Cl_2/2.5% MeOH), the compound was obtained as a white solid **15**: 20.9 g (37%), mp 137 °C. Anal. ($C_{25}H_{22}Cl_2N_4O_5S$) C, H, N.

Ethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (13). Compound **13** was synthesized from **12** according to method A using an EtOH/DMF mixture (2/1,v/v) (27%), mp 80 °C. Anal. ($C_{24}H_{20}Cl_2N_4O_4S$) C, H, N.

1-Methylethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (14). Compound **14** was synthesized from **10** according to method A using CH_3CN as solvent and K_2CO_3 as base (30%), mp 215 °C. Anal. ($C_{25}H_{22}Cl_2N_4O_4S$) C, H, N.

1,1-Dimethylethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (16). Compound **16** was synthesized from **12** according to method A, in which the initial reaction with *tert*-butyl α-bromo-*β*-oxo-benzenepropionate **37** was performed in CH_3CN using K_2CO_3 as base, and the subsequent ring closing reaction was carried out in refluxing *t*-BuOH to afford **16** (50%), mp 130 °C. Anal. ($C_{26}H_{24}Cl_2N_4O_4S$) C, H, N.

Representative Procedure for the Synthesis of the Target Compounds (18−28). Method B: Propyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)-phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (19). A mixture of compound **16** (29 g, 0.0518 mol) in CF_3-COOH (200 mL) was stirred at room temperature for 4 h. The reaction mixture was poured out on ice, and the precipitate was washed with water and then taken up in CH_2Cl_2. The organic layer was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residual solid was purified by column chromatography on silica gel (20−45 μm; 97% CH_2Cl_2/3% MeOH/0.1% AcOH). The pure fractions were combined and concentrated in vacuo, and the residual solid was recrystallized (CH_3CN) to afford 2-[1-[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylic acid **(17)**: 25.0 g (95%), mp >250 °C.

To a mixture of the acid **17** (2 g, 4 mmol) and $NaHCO_3$ (0.33 g, 4 mmol) in DMF (8 mL), a mixture of *n*-propyl bromide (0.48 g, 4 mmol) in DMF (2 mL) was added at room temperature and heated for 6 h at 70 °C. The reaction mixture was poured out on ice, acidified with HCl (3 N), and extracted with EtOAc. The organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residual product was purified by column chromatography on silica gel (15−40 μm; 99.25% CH_2Cl_2/0.75% MeOH). The pure fractions were combined and concentrated in vacuo, and the residue was crystallized (diethyl ether/DIPE) to yield compound **19:** 0.56 g (54%), mp 172 °C. Anal. ($C_{25}H_{22}Cl_2N_4O_4S$) C, H, N.

Methyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (18). Compound **18** was synthesized from **17** and methyl iodine according to method B (8%), mp 175 °C. Anal. Calcd for ($C_{23}H_{18}Cl_2N_4O_4S$): C, 53.39; H, 3.51; N, 10.83. Found: C, 52.39; H, 3.38 N, 10.38.

Butyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (20). Compound **20** was synthesized from **17** and *n*-butyl bromide according to method B (11%), mp 130 °C. Anal. ($C_{26}H_{24}Cl_2N_4O_4S$) C, H, N.

Pentyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (21). Compound **21** was synthesized from **17** and *n*-pentyl bromide according to method B (23%), mp 134 °C. Anal. ($C_{27}H_{26}Cl_2N_4O_4S$) C, H, N.

Cyclopropylmethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (22). Compound **22** was synthesized from **17** and cyclopropylmethyl bromide according to method B (15%), mp 100 °C. Anal. ($C_{26}H_{22}Cl_2N_4O_4S$) C, H, N.

2-Methylpropyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (23). Compound **23** was synthesized from **17** and isobutyl bromide according to method B (17%), mp 149 °C. Anal. Calcd for ($C_{26}H_{20}Cl_2N_4O_4S$): C, 55.82; H, 4.32; N, 10.01. Found: C, 55.63; H, 4.09; N, 9.93.

2-Propenyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (24). Compound **24** was synthesized from **17** and allyl bromide according to method B (15%), mp 172 °C. Anal. ($C_{25}H_{20}Cl_2N_4O_4S$) C, H, N.

3-Methyl-2-butenyl 2-[1-[2,6-dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (25). Compound **25** was synthesized from **17** and 3,3-dimethylallyl bromide according to method B (11%), mp 90 °C. Anal. Calcd for ($C_{27}H_{24}Cl_2N_4O_4S$): C, 56.75; H, 4.23; N, 9.80. Found: C, 56.56; H, 4.33; N, 9.36.

2-Propynyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (26). Compound **26** was synthesized from **17** and propargyl bromide according to method B (19%), mp 180 °C. Anal. ($C_{25}H_{18}Cl_2N_4O_4S$) C, H, N.

2-Phenylethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (27). Compound **27** was synthesized from **17** and phenethyl bromide according to method B (17%), mp 146 °C. Anal. Calcd for ($C_{30}H_{24}Cl_2N_4O_4S$): C, 59.31; H, 3.98; N, 9.22. Found: C, 58.86; H, 3.88; N, 9.19.

Cyanomethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (28). Compound **28** was synthesized from **17** and bromoacetonitrile according to method B (12%). Anal. ($C_{24}H_{17}Cl_2N_5O_4S$) C, H, N.

Representative Procedure for the Synthesis of the Target Compounds (29−33). Method C: Phenoxyethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (32). A mixture of the acid **17** (1.35 g, 2.7 mmol) and CDI (0.57 g, 3.5 mmol) in DMF (10 mL) was stirred for 1 h at 40 °C. Phenoxyethanol (0.74 g, 5.4 mmol) in DBU (0.4 g, 2.7 mmol) was added, and the reaction mixture was stirred further for 3 h at 40 °C. The mixture was poured out on ice and acidified with HCl (3 N). The precipitate was filtered off, washed with water, and taken up in CH_2Cl_2. The organic solution was washed with water, dried ($MgSO_4$), and concentrated in vacuo. The residual solid was purified by chromatography on silica gel (99%CH_2Cl_2/1%MeOH). Compound **32** was obtained from *n*-pentane: 1.2 g (72%), mp 80 °C. Anal. ($C_{30}H_{24}Cl_2N_4O_5S$) C, H, N.

2,2,2-Trifluoroethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (29). Compound **29** was synthesized from **17** and 2,2,2-trifluoroethanol according to method C (36%), mp 180 °C. Anal. Calcd for ($C_{24}H_{17}Cl_2F_3N_4O_4S$): C, 49.24; H, 2.93; N, 9.57. Found: C, 48.62; H, 2.97; N, 9.37.

2-Butynyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (30). Compound **30** was synthesized from **17** and 2-butyn-1-ol according to method C (49%), mp 184 °C. Anal. ($C_{26}H_{20}Cl_2N_4O_4S$) C, H, N.

2-Hydroxyethyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (31). Compound **31** was synthesized from **17** and ethylene glycol according to method C (17%), mp 174 °C. Anal. ($C_{24}H_{20}Cl_2N_4O_5S$) C, H, N.

2-Oxo-propyl 2-[1-[2,6-Dichloro-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)phenyl]-1-methylethyl]-4-phenyl-5-thiazolecarboxylate (33). Compound **33** was synthesized from **17** and 1-hydroxy-2-propanone according to method C (30%), mp 226 °C. Anal. ($C_{25}H_{20}Cl_2N_4O_5S$) C, H, N.

Pharmacology. Materials. For in vitro experiments, compounds were dissolved at 5 mM in DMSO and appropriately diluted with culture medium (RPMI 1640 supplemented with 2 mM glutamine, 100 U/mL penicillin and 10 μg/mL streptomycin). PHA was obtained from Murex (Dartford, U.K.). S9 preparations of human liver and lung were obtained from Gentest and Analytical Biological Services, respectively.

Human Lung and Liver S9 Incubations. Compounds (10^{-6} M) were incubated at 37 °C in 100 μL of phosphate buffered saline with human liver S9 fraction (10 μg protein/ml; obtained from BD Biosciences) or human lung S9 preparation (50 μg protein/ml; obtained from Analytical Biological Services). After 0, 15, and 60 min of incubation, 3 volumes of acetonitrile were added, and the samples were vortexed. After centrifugation, supernatants were evaporated, and the residues were dissolved in 100 μL of 50% acetonitrile containing 0.05% formic acid for analysis by LC-MSMS. Briefly, 10 μL samples were injected on a Symmetry C_{18} column (50 mm × 2.1 mm, 3.5 μm; Milford, MA. The mobile phase was 0.05% formic acid (solvent A). Solvent B consisted of acetonitrile with 0.05% formic acid. A shallow gradient at a flow rate of 0.5 mL/min was performed to ensure an elution time of 3−10 min. A split ratio of $^1/_5$ is applied after the photodiode array (PDA) detector allowing 100 μL/min to enter the mass spectrometer. Quantitative analysis was performed using the MRM transition of the parent ion to the daughter ion.

Cytokine Production. Blood from adult healthy volunteers was anticoagulated in heparin (12.5 U/mL) and cultured as described.[8] Briefly, blood samples were diluted 3-fold in culture medium, and 300 μL fractions were preincubated for 15 min in the presence or absence of test compound (10^{-6}−10^{-9} M). Then cultures were stimulated by the addition of 100 μL of PHA (final concentration of 5 μg/mL) and incubated at 37 °C for 24 or 48 h (in the case of IL-5) in a humidified 6% CO_2 atmosphere. Cell supernatants were then removed and stored at −20 °C. Cytokine levels were determined by ELISA using the Duoset antibodies (R&D Systems, Abingdon, U.K.) according to the manufacturer's instructions. Concentrations were calculated from standard curves using recombinant cytokines supplied by R&D Systems.

Antigen-Induced Airway Responses in Sheep. Airway responsiveness was assessed in a sheep model of atopic asthma as described.[22,23] Adult sheep (mean body weight 30 kg) that had shown positive allergic skin and airway responses to *Ascaris suum* antigen were used. Mean pulmonary flow resistance (R_L) was calculated from the analysis of 5−10 breaths by using the esophageal balloon catheter technique. Immediately after the determination of R_L, thoracic gas volume (V_{tg}) was measured in a constant-volume body plethysmograph to obtain the specific lung resistance ($SR_L = R_L \times V_{tg}$). Aerosols of *Ascaris suum* extract (82 000 protein nitrogen units/mL in phosphate buffered saline) were generated using a disposable medical nebulizer and directed into the animals via a plastic T-piece, which had one end connected to the inspiratory port of a Harvard respirator and the other end to the nasotracheal tube. *Ascaris* aerosols were delivered at a tidal volume of 500 mL and a rate of 20 breaths/min for 20 min. Aerosols of **15** (dissolved at 2 mg/mL in a solution containing 2 mg/mL tromethamine, 100 mg/mL hydroxypropyl-β-cyclodextrin, 30 mg/mL mannitol, and 3 mg/mL *N*-methyl-glucamine (final pH 8.5)) and of carbachol (0.25, 0.5, 1, 2, and 4% w/v in phosphate buffered saline) were aerosolized and delivered by the same nebulizer system.

Airway responsiveness was assessed from cumulative dose−response curves to nebulized carbachol by measuring SR_L immediately after inhalation of buffer (phosphate buffered saline) and after each consecutive administration of 10 breaths of increasing concentrations of carbachol (0.25, 0.5, 1, 2, and 4% w/v in buffer). The cumulative carbachol dose (in breath units), that increases SR_L by 400% over the postbuffer value (i.e., PC_{400}) was calculated by interpolation from the dose−response curve. One breath unit (BU) was defined as one breath of an aerosol solution containing 1% w/v carbachol.

Baseline airway responsiveness was determined in five sheep 2−4 days before start of vehicle or compound treatment. On the challenge day, baseline SR_L measurements were repeated, and the animals were treated with aerolized compound or vehicle either 0.5, 2, 4, or 16 h before or 1.5 h after the challenge with *Ascaris suum* antigen. Every sheep served as its own control and was treated with vehicle as well as with test compound in experiments that were separated by at least 14 days. Changes in SR_L were monitored hourly from 1 to 6 h after challenge and on the half-hour from 6 $^1/_2$ to 8 h after challenge. The compound's effect on airway responsiveness was measured 24 h after *Ascaris* challenge.

Acknowledgment. We thank Mireille Vanderhallen, Ann Dierckx, Freddy Lauwers, and Iolona Medaer for their administrative assistance.

Supporting Information Available: NMR or detailed elemental analysis data on compounds **3**−**6** and **10**−**33**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sears, M. R. Descriptive epidemiology of asthma. *Lancet* **1997**, *350*, 1−4.
(2) Costa, J. J.; Weller, P. F.; Galli, S. J. The cells of the allergic response. *J. Am. Med. Assn.-JAMA* **1997**, *278*, 1815−1822.
(3) Ryan, J. J. Interleukin-4 and its receptor: Essential mediators of the allergic response. *J. Allergy Clin. Immunol*. **1997**, *99*, 1−5.
(4) Egan, R. W.; Umland, S. P.; Cuss F. M.; Chapman, R. W. Biology of interleukin-5 and its relevance to allergic diseases. *Allergy*. **1996**, *51*, 71−81.
(5) Baggiolini, M.; Dahinden, C. J. CC chemokines in allergic inflammation. *Immunol Today* **1994**, *15*, 127−133.
(6) Barnes, P. J. Therapeutic strategies for allergic diseases. *Nature* **1999**, *402*, B31−B38.
(7) Van Wauwe, J. Interleukin-5 as a potential target for asthma treatment.*Drug News Perspect.* **2000**, *13*, 197−204.
(8) Van Wauwe, J.; Aerts, F.; Walter, H.; de Boer, M. Cytokine production by phytohemagglutinin-stimulated human blood cells: Effects of corticosteroids, T cell immunosuppressants and phosphodiesterase IV inhibitors. *Inflamm. Res.* **1995**, *44*, 400−405.
(9) Lacrampe, J. F.; Freyne, E. J.; Venet, M. G.; Boeckx G. M. Novel IL-5 inhibiting 6-azauracil derivatives. WO Patent 99 02505, 1999. Freyne, E. J.; Boeckx G. M.; Van Wauwe, J. P.; Diels G. S. Interleukin-5 inhibiting 6-azauracil derivatives. WO Patent 99 02504, 1999. Freyne, E. J.; Lacrampe J. F.; Deroose F. D.; Venet, M. G. IL-5 inhibiting 6-azauracil derivatives. EP Patent 1114046, 2003.
(10) Van Wauwe, J. P.; Aerts, F.; Cools, M.; Deroose, F.; Freyne, E.; Goossens, J.; Hermans, B.; Lacrampe, J.; Van Genechten, H.; Van Gerven, F.; Van Nyen, G. Identification of R146225 as a novel, orally active inhibitor of interleukin-5 biosynthesis. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 655−661.
(11) Lee, H. J.; You, Z.; Ko, D. H.; McLean, H. M. Advances in prodrugs and antedrugs. *Curr. Opin. Drug Discovery Dev*. **1998**, *1*, 235−244.
(12) Procopiou, P. A.; Biggadike, K.; English, A. F.; Farrell, R. M.; Hagger, G. N.; Hancock, A. P.; Haase, M. V.; Irving, W. R.; Sareen, M.; Snowden, M. A.; Solanke, Y. E.; Tralau-Stewart, C. J.; Walton, S. E.; Wood, J. A. Novel glucocorticoid antedrugs possessing a 17β-(γ-lactone) ring. *J. Med. Chem.* **2001**, *44*, 602−612.
(13) Bodor, N.; Buchwald, P. Retrometabolism-based drug design and targeting. In *Burger's Medicinal Chemistry and Drug Discovery. Vol. 2, Drug Discovery and Drug Development*, 6th ed.; Abraham D. J., Ed.; Wiley: New York, 2003; Vol. 2, pp 533−608.
(14) You, Z.; Heiman, A. S.; Hudson, C. E.; Lee, H. J. Effect of chirality at C-20 of methyl 11β, 17α, 20-trihydroxyl-3-oxo-1,4-pregnadien-21-oate derivatives on antiinflammatory activity. *Steroids* **2002**, *67*, 353−359.
(15) Park, K.-K.; Ko D.-H.; You, Z.; Lee, H. J. Metabolism of steroid antiinflammatory antedrugs in vitro: methyl 3, 20-dioxo-11β, 17α, 21-trihydroxy-1,4-pregnadiene-16α-carboxylate; its 9α-fluorinated, and their 21-*O*-acyl derivatives. *Steroids* **2003**, *68*, 315−319.
(16) Park, K.-K.; Ko D.-H.; You, Z.; Cooperwood, J. S.; Park, J.; Lee, H. J. Chirality effect of C-20 on metabolism of methyl 11β, 17α, 20-trihydroxy-3-oxo-1,4-pregnadien-21-oate derivatives. *Steroids* **2004**, *69*, 87−91.
(17) Freyne, E. J.; Deroose, F. D.; Lacrampe, J. F.; Embrechts W. C.; Fortin J. M. IL-5 inhibiting 6-azauracil derivatives. WO Patent 00 37451, 2000. Lacrampe, J. F.; Freyne E. J.; Deroose F. D.; Fortin, J. M.; Coesemans, E. Interleukin-5 inhibiting 6-azauracil derivatives. WO Patent 01 10866, 2001.
(18) Caroll, R. D.; Miller, M. W.; Mylari, B. L.; Chappel, L. R.; Howes Jr, H. L.; Lynch, M. J.; Lynch, J. E.; Gupta, S. K.; Rash, J. J.; Koch, R. C. Anticoccidial derivatives of 6-azauracil. 5. Potentiation by benzophenone side chains. *J. Med. Chem.* **1983**, *26*, 96−100.
(19) Boeckx, G. M.; Raeymaekers, A.; Sipido, V. Alpha-aryl-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)benzene-acetonitriles. US Patent 4,631, 278, 1986.
(20) Abignente, E.; de Caprariis, P.; Rimoli, M. G.; Avallone, L.; Gomez P. L.; Rossi, F.; D′Amico, M.; Calderaro, V.; Parrillo, C. Research on heterocyclic compounds. XXXI. Synthesis and inflammatory activity of 2-arylimidazo[1,2-a]pyrazine-3-carboxylic acids. *Eur. J. Med. Chem.* **1993**, *28*, 337−346.
(21) Erger, R. A.; Casale, T. B. Interleukin-8 plays a significant role in IgE-mediated lung inflammation. *Eur. Respir.* **1998**, *11*, 299−300.
(22) Abraham, W. M.; Lanes, S.; Stevenson, J. S.; Yerger, L. D. Effect of an inhaled glucocorticosteroid (budesonide) on post-antigen induced increases in airway responsiveness. *Bull. Eur. Physopathol. Respir.* **1986**, *22*, 387−392.
(23) Clark, J. M.; Abraham, W. M.; Fishman, C. E.; Forteza, R.; Ahmed, A.; Cortes, A. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. *Am. J. Respir. Crit. Care Med.* **1995**, *152*, 2076−2083.
(24) Toshiaki, K.; Masato, T. Acylation of active methylene compounds via palladium-complex-catalyzed carbonylative cross-coupling of organic halides. *Tetrahedron Lett.* **1986**, *27*, 4745−4748.
(25) Turner, J. A.; Jacks, W. S. Acylation of ester enolates by *N*-methoxy-*N*-methylamides: An effective synthesis of β-keto esters. *J. Org. Chem.* **1989**, *54*, 4229−4231.
(26) Li, A.-H.; Moro S.; Forsyth, N.; Melman, N.; Ji, X.; Jacobson, K. A. Synthesis, CoMFA analysis, and receptor docking of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A3 adenosine receptor antagonists. *J. Med. Chem.* **1999**, *42*, 706−721.

JM049479M