New *N*-(Pyridin-4-yl)-(indol-3-yl)acetamides and Propanamides as Antiallergic Agents

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A series of new *N*-(pyridin-4-yl)-(indol-3-yl)alkylamides **44**−**84** has been prepared in the search of novel antiallergic compounds. Synthesis of the desired ethyl (2-methyindol-3-yl)acetates **1**−**4** was achieved by indolization under Fischer conditions; Japp-Klingemann method followed by 2-decarboxylation afforded the ethyl (indol-3-yl)alkanoates **17**−**25**. Amidification was successfully carried out by condensation of the corresponding acids or their *N*-aryl(methyl) derivatives with 4-aminopyridine promoted by 2-chloro-1-methylpyridinium iodide. Efforts to improve the antiallergic potency of the title series by variation of the indole substituents (R^1, R^2, R) and the length of the alkanoic chain (n = 1, 2, 3) led to the selection of *N*-(pyridin-4-yl)-[1-(4-fluorobenzyl)indol-3-yl]acetamide **45**, out of 41 compounds.This amide was 406-fold more potent than astemizole in the ovalbumin-induced histamine release assay, using guinea pig peritoneal mast cells, with an IC_{50} = 0.016 μM. Its inhibitory activity in IL-4 production test from Th-2 cells was identical to that of the reference histamine antagonist (IC_{50} = 8.0 μM) and twice higher in IL-5 assay: IC_{50} = 1.5 and 3.3 μM, repectively. In vivo antiallergic activity evaluation confirmed efficiency of **45** in sensitized guinea pig late phase eosinophilia inhibition, after parenteral and oral administration at 5 and 30 mg/kg, respectively. Its efficiency in inhibition of microvascular permeability was assessed in two rhinitis models; ovalbumin and capsaicin-induced rhinorrhea could be prevented after topical application of submicromolar concentrations of **45** (IC_{50} = 0.25 and 0.30 μM); and it also exerted significant inhibitory effect in the first test after iv and oral administration, with ID_{50} = 0.005 and 0.46 mg/kg.

Introduction

Two of the cytokines, secreted by T-cells in response to antigen stimulation, are considered to have a particular important role in atopic allergic disease: interleukin 4 (IL-4) and interleukin 5 (IL-5).

IL-4 and, to a lesser extent, IL-13 play a crucial role in the regulation of immunoglobulin E (IgE) synthesis by B-lymphocytes, in normal conditions and in allergic diseases. The activation effect of IL-4, which requires physical contact between (CD_4^+)T-cells and B-cells, is promoted by IL-5 and IL-6 but inhibited notably by interferons (IFN)-α and -γ and transforming growth factor β.[1,2] Binding of antigens to specific IgE on mast cells or basophiles leads to the cross-linking of IgE receptors, resulting in cell activation and the release of numerous inflammatory mediators, such as cytokines (IL-4, IL-5, TNFα), leukotrienes, and histamine.[3] These mediators contribute to bronchoconstriction and recruitement of inflammatory leucocytes and lymphocytes in atopic disorders.

There is now convincing evidence that activation of allergen-specific helper (CD_4^+) T-lymphocytes of the Th-2 subset induces release of cytokines, including interleukins 3 and 5 and granulocyte macrophage colony-stimulating factor (GM-CSF), with IL-5 as the most likely to be a specific stimulator of human eosinophil maturation, proliferation, and function.[4−9]

Eosinophils produce major basic protein (MBP) and eosinophil cationic protein (ECP) which are cytotoxic and may contribute to the denudation of epithelium seen in asthma.[4] Moreover eosinophil granules have the capacity to generate considerable quantities of leukotrienes (LTD_4),[8] notably after IgE-dependent stimuli[10] and of platelet activating factor (PAF), which in turn induce potent eosinophil chemotaxis.[11]

Approaches in the discovery of leukotriene modulators (LT) have resulted in several new classes of compounds: redox inhibitors of 5-LO,[12] indole, quinolylmethoxy or quindole-containing inhibitors of five lipoxygenase activating protein (FLAP),[13] styrylquinoline-containing antagonists of peptidyl LTD_4 receptor, and LTB_4 receptor antagonists.[14] But evidence to date indicates that LT modulators can provide symptomatic relief of inflammatory conditions rather than decrease process attenuation or reversal.[14] Dual cyclooxygenase and lipoxygenase inhibitors such as BW755C efficiently inhibit long-lasting ovalbumin-induced eosinophil recruitment in rats,[15] but none are in clinical development and targeted for the treatment of asthma. Among PAF antagonists, WEB2086, a potent and specific compound, was experimented in treatment of asthma; but unfortunately it suffered from a short duration of action.[12] Allergen-induced eosinophilia could also be inhibited by phosphodiesterase (PDE) inhibitors; CDP840, a potent

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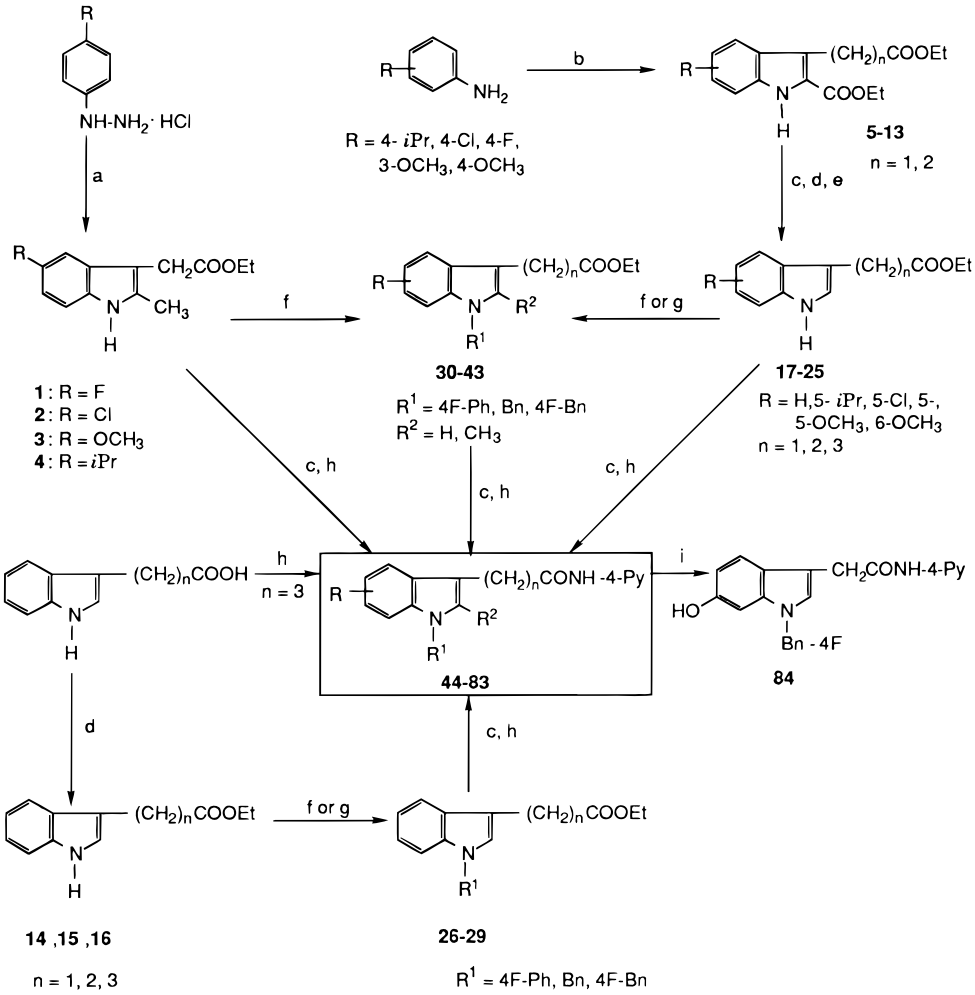
Scheme 1[a]



[a] Reagents and conditions: (a) (1) ethyl levulinate, CH_3COONa, 1 M CH_3COOH; (2) H_2SO_4, EtOH, reflux, 3 h; (b) (1) 12 M HCl, $NaNO_2$, 0 °C, 0.5 h; (2) diethyl 2-acetylglutarate ($n = 1$), ethyl 2-oxocyclopentane carboxylate ($n = 2$), EtOH, 6 M KOH, 0 °C, then room temperature for 2 h; (3) H_2SO_4, EtOH, reflux, 12 h; (c) 6 M NaOH, EtOH, reflux, 1 h; (d) 1 M HCl−EtOH, reflux, 1.5 h; (e) $Cu_2Cr_2O_5$, quinoline, reflux, 2 h; (f) NaH, DMSO, R^1CH_2Cl, room temperature for 12 h; (g) 4-iodofluorobenzene, Cu bronze, K_2CO_3, PhBr, reflux, 24 h; (h) 2-chloro-1-methylpyridinium iodide, TEA, 4-aminopyridine, CH_2Cl_2, reflux, 2 h; (i) with **83**, BBr_3, CH_2Cl_2, −80 °C, 1 h, then room temperature for 12 h.

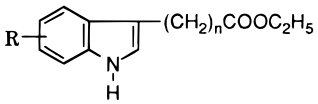
novel stereoselective inhibitor of PDE IV,[16] which is predominant in eosinophils, prevents allergen-induced bronchospasm; but the clinical use of these compounds could be limited by gastrointestinal side effects, such as increased acid secretion, nausea, and vomiting.[17] Glucocorticoids are highly effective against eosinophil pulmonary infiltration,[18,19] but they often produce significant side effects, especially when administered by oral route in severe asthma. The selective regulation of IL-4 and IL-5 may therefore offer an alternative approach to the control of eosinophilic bronchial inflammation in asthma.

In previous works, we reported on the synthesis and biological evaluation of *N*-(4,6-dimethylpyridinyl-2-yl)-heteroaryl carboxamides and acetamides as a novel type of nonacidic antiinflammatory agents.[20] Although the corresponding indolylalkylamides exhibited only moderate anti-edematous effect, to our surprise, results of pharmacological screening tests revealed that some of these compounds significantly inhibited IL-4, IL-5 biosynthesis and/or histamine release. These data and the fact that indole constitutes the central core of numerous potent LTD_4 antagonists,[21−23] PAF antagonists,[24] or inhibitors of FLAP,[12] prompted us to carry out an extensive pharmacomodulation in this series. We report here the results of in vitro and in vivo investigations in the *N*-(pyridin-4-yl)-(indol-3-yl)alkylamides **44**−**84** (Table 3).

Chemistry

Preparation of the target (indol-3-yl)alkanoates under Fischer conditions is depicted in Scheme 1. The intermediate phenylhydrazones were prepared by condensation of the corresponding substituted phenylhydrazines with ethyl levulinate (method A) or under Japp-Klingemann reaction conditions[25] (method B) from substituted anilines and diethyl 2-acetylglutarate ($n = 1$) or ethyl 2-oxocyclopentane carboxylate ($n = 2$) as keto compounds.[26] The phenylhydrazones were cyclized without purification to yield ethyl (2-methylindol-3-yl)acetates **1**−**4** and the indole diesters **5**−**13,** respectively. Indolization of 3-methoxyphenylhydrazone afforded a mixture of 4-and 6-methoxyindole derivatives (1:8) which were separated by silica gel chromatography. The hydrolysis of the diesters **5**−**13**, followed by monoesterification and C-2 decarboxylation by the couple Cu_2-

Table 1. Physical and Synthetic Data for Ethyl (Indol-3-yl)alkanoates **17**−**25**



no.	R	*n*	yield (%)[a]	formula	mp (°C) solvent
17	5-OCH_3	1	16	$C_{13}H_{15}NO_3$	90[b]
18	5-OCH_3	2	28	$C_{14}H_{17}NO_3$	74[b]
19	5-Cl	1	15	$C_{12}H_{12}ClNO_2$	90[b]
20	5-Cl	2	22	$C_{13}H_{14}ClNO_2$	75[b]
21	5-F	1	18	$C_{12}H_{12}FNO_2$	76[b]
22	5-F	2	25	$C_{13}H_{14}FNO_2$	54[b]
23	5-*i*-Pr	1	14	$C_{15}H_{19}NO_2$	46[b]
24	5-*i*-Pr	2	17	$C_{16}H_{21}NO_2$	41[b]
25	6-OCH_3	1	12	$C_{13}H_{15}NO_3$	55[c]

[a] Overall yield after the four-step sequence (steps b−e, Scheme 1). [b] Petroleum ether. [c] Diisopropyl ether.

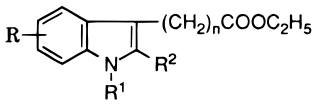
Cr_2O_5/quinoline, afforded the desired ethyl (indol-3-yl)-alkanoates **17**−**25** (Table 1). Ethyl (indol-3-yl)alkanoates **14**, **15**, **16** (n = 1, 2, 3) were obtained by refluxing the corresponding commercially available acids in HCl ethanol (Scheme 1). *N*-substitution of indole esters **1**−**4** and **14**−**25** was carried out either with 4-iodofluorobenzene under Ullmann conditions, using Cu/K_2CO_3 in refluxing bromobenzene (method C), or with benzyl halogenides after metalation with NaH in DMSO (method D), affording the esters **26**−**43** in fair yields (Table 2). These esters failed to undergo aminolysis with 4-aminopyridine, even in the presence of a catalytic amount of 2-hydroxypyridine.[27] On the contrary, activation of the corresponding acids, resulting from alkaline hydrolysis of the esters, by reaction with 2-chloro-1-methylpyridinium iodide (method E) afforded the target *N*-(pyridin-4-yl)-(indol-3-yl)alkylamides **44**−**46** and **48**−**83** in moderate to excellent yields; the N-unsubstituted 4-(indol-3-yl) butanamide **47** was obtained by the same method, starting from the corresponding commercially available acid (Table 3). Last, demethylation of the methoxy compound **83** was carried out by BBr_3 (method F), in a 71% yield, leading to the 6-hydroxy derivative **84**.

Structure−Activity Relationships

Antiallergic activity was first evaluated by three in vitro assays: inhibition of IL-4 and IL-5 production in Th-2 cells and inhibition of ovalbumin-induced histamine release in guinea pig peritoneal mast cells. Results expressed in percent inhibition for IL-4 and IL-5 and by IC_{50} for histamine antagonism are shown in Table 3. IC_{50} toward IL-4 and IL-5 inhibition are displayed in Table 4 for the most potent compounds. Among these, actetamide **45** was selected for further evaluation in three complementary assays: inhibition of ionomycin-induced histamine release, inhibition of acetylcholine hyperreactivity of rat trachea induced by tetraethylammonium chloride, and inhibition of carbachol-induced contraction of isolated guinea pig trachea.

IL-4 Inhibition. It was previously established that amides issued from 2- and 3-aminopyridines were regularly less active than their 4-pyridinyl congeners: none of them exhibited an inhibition percentage greater than 35% at 10 μM; this statement prompted us to limit pharmacomodulation to the last subseries.

Table 2. Physical and Synthetic Data for *N*-Substituted Ethyl (Indol-3-yl)alkanoates **26**−**43**

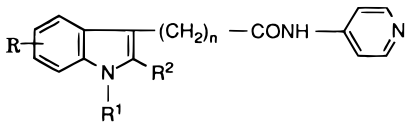


no.	*n*	R	R^1	R^2	method yield (%)	formula	mp (°C) solvent
26	1	H	4-FPh	H	C 79	$C_{18}H_{16}FNO_2$	96[a]
27	1	H	4-FBn	H	D 60	$C_{19}H_{18}FNO_2$	62[a]
28	2	H	4-FBN	H	D 75	$C_{20}H_{20}FNO_2$	56[a]
29	3	H	4-FBn	H	D 66	$C_{21}H_{22}FNO_2$	46[a]
30	1	5-OCH_3	4-FPh	H	C 60	$C_{19}H_{18}FNO_3$	98[a]
31	2	5-OCH_3	Bn	H	D 70	$C_{21}H_{23}NO_3$	61[a]
32	2	5-OCH_3	4-FBn	H	D 72	$C_{21}H_{22}FNO_3$	63[a]
33	1	5-Cl	4-FBn	H	D 69	$C_{19}H_{17}ClFNO_2$	65[a]
34	1	5-F	4-FPh	H	C 85	$C_{18}H_{15}F_2NO_2$	110[a]
35	1	5-F	Bn	H	D 69	$C_{19}H_{18}FNO_2$	65[a]
36	2	5-F	Bn	H	D 70	$C_{20}H_{20}FNO_2$	61[a]
37	1	5-F	4-FBn	H	D 72	$C_{19}H_{17}F_2NO_2$	62[c]
38	2	5-F	Bn	H	D 80	$C_{20}H_{19}F_2NO_2$	69[c]
39	1	5-F	Bn	CH_3	D 52	$C_{20}H_{20}FNO_2$	85[c]
40	2	5-*i*-Pr	Bn	H	D 65	$C_{23}H_{27}NO_2$	66[c]
41	2	5-*i*-Pr	4-FBn	H	D 68	$C_{23}H_{26}FNO_2$	58[c]
42	1	5-*i*-Pr	4-FBn	CH_3	D 42	$C_{23}H_{26}FNO_2$	81[c]
43	1	6-OCH_3	4-FBn	H	D 42	$C_{20}H_{20}FNO_3$	102[a]

[a] Diisopropyl ether. [c] Petroleum ether.

Among the three types of substituents introduced at indolic nitrogen (4-fluorophenyl, benzyl, and 4-fluorobenzyl), generally speaking, the last one exerted the most favorable influence; nevertheless the 4-fluorophenyl derivative **67**, contrary to **70**, was active and equipotent to **45** (Table 3). Tentative increase or emergence of activity by methylation at C-2 remained unsuccessful in 9 compounds out of 10 experimented; only **74** was more active than its nonmethylated counterpart **70.** Introduction of different substituents at C-5 (OCH_3, Cl, F, *i*-Pr) exerted a detrimental effect (**44** vs **51** and **45** vs **61**, **70**, **78**); on the contrary, activity was maintained by a methoxy group fixed at C-6 in **83** (IC_{50} = 9.8 μM). Increasing of the alkanoate chain of indolyl acetamide **45** (leading to **46**, n = 2) reduced IL-4 inhibition, and no marked increase of this activity was observed in **48** (n = 3); so further pharmacomodulation at this level was limited to n = 1 and 2. Replacement of the acetamide chain by a propanamide one did not increase IL-4 inhibition, except in the 5-F and 5-Cl subseries: compounds **58** and **69**. Nevertheless, even in the acetamide subseries, inhibition remained moderate and only 4 compounds (**45**, **67**, **74**, **83**) exhibited IC_{50} in the range of 8−10 μM (Table 4). Although these amides showed 3 orders of magnitude weaker activity against IL-4 ex-

Table 3. *N*-(Pyridin-4-yl)-*ω*-(indol-3-yl)alkylamides **44**–**84**



					IL-4	IL-5	histamine
no.	*n*	R	R^1	R^2	% inhibition[a] at 10 μM	% inhibition[a] at 10 μM	IC_{50}[b] (μM)
44	1	H	4-FPh	H	31	48	0.302
45	1	H	4-FBn	H	63	100	0.016
46	2	H	4-FBn	H	2.9	22	0.220
47	3	H	H	H	20	NA	2.9
48	3	H	4-FBn	H	31	42	0.51
49	1	5-OCH_3	H	H	NA	NA	>10
50	2	5-OCH_3	H	H	NA	26	NT
51	1	5-OCH_3	4-FPh	H	24	22	NT
52	1	5-OCH_3	Bn	H	33	38	2.80
53	2	5-OCH_3	Bn	H	NA	NA	3.900
54	2	5-OCH_3	4-FBn	H	NA	NA	NT
55	1	5-OCH_3	H	CH_3	NA	NA	NT
56	1	5-OCH_3	4-FBn	CH_3	NA	22	5.00
57	1	5-Cl	H	H	NA	NA	4.500
58	2	5-Cl	H	H	26	25	1.460
59	1	5-Cl	Bn	H	39	49	NT
60	2	5-Cl	Bn	H	NA	NA	0.74
61	1	5-Cl	4-FBn	H	NA	54	0.230
62	2	5-Cl	4-FBn	H	NA	41	1.30
63	1	5-Cl	H	CH_3	NA	NA	>10
64	1	5-Cl	4-FBn	CH_3	28	26	NT
65	1	5-F	H	H	NA	NA	>10
66	2	5-F	H	H	NA	25	2.210
67	1	5-F	4-FPh	H	51	71	NT
68	1	5-F	Bn	H	NA	48	0.060
69	2	5-F	Bn	H	27	42	0.420
70	1	5-F	4-FBn	H	NA	43	NT
71	2	5-F	4-FBn	H	NA	37	0.380
72	1	5F	H	CH_3	NA	NA	10.13
73	1	5-F	Bn	CH_3	NA	41	0.480
74	1	5F	4-FBn	CH_3	49	61	0.40
75	1	5-*i*-Pr	H	H	33	29	4.92
76	2	5-*i*-Pr	H	H	NA	28	3.120
77	2	5-*i*-Pr	Bn	H	NA	25	6.000
78	1	5-*i*-Pr	4-FBn	H	NA	23	NT
79	2	5-*i*-Pr	4-FBn	H	NA	35	5.340
80	1	5-*i*-Pr	H	CH_3	NA	NA	2.800
81	1	5-*i*-Pr	Bn	CH_3	NA	34	7.54
82	1	5-*i*-Pr	4-FBn	CH_3	NA	NA	6.900
83	1	6-OCH_3	4-FBn	H	51	76	0.330
84	1	6-OH	4-FBn	H	29	37	0.940
	astemizole				66	74	*b*

[a] Mean of three individual experiments each performed in duplicate (±SEM); NA, no activity; NT, not tested. [b] IC_{50} (μM) of reference drugs. Astemizole: 6.5 ± 1.3; Terfenadine: 4.7 ± 0.1. Cyclosporin A: 1.1 ± 0.4.

Table 4. IL-4 and IL-5 Production Inhibition of Compounds **45**, **61**, **67**, **74**, and **83**

no.	IL-4 IC_{50} (μM)	IL-5 IC_{50} (μM)
45	8.00	1.50
61	>10	8.80
67	9.70	4.56
74	10.20	7.87
83	9.80	2.28
astemizole	8.00	3.30
terfenadine	4.95	3.96
dexamethasone	0.0047	0.0035
cyclosporin A	0.073	0.242

pression than dexamethasone (IC_{50} = 4.7 nM) and cyclosporin A (IC_{50} = 73 nM), they were as active as astemizole (IC_{50} = 8.0 μM).

IL-5 Inhibition. The stucture–activity profile of these *N*-pyridinyl alkylcarboxamides does not markedly vary between IL-4 and IL-5 assays. Nevertheless, they inhibited more regularly and more efficiently IL-5 production: 13 acetamides (instead of 5) possessed percent inhibition greater than 40%, at 10 μM (Table 3). The presence of a methyl group at C-2 induced the same deleterious effect, except in **74**. Introduction of methoxy and isopropyl groups at C-5 resulted in a marked decrease of activity; on the other hand, the 5-Cl, 5-F, and 6-OCH_3 derivatives **59**, **61**, **74**, and **83**, although less active than the non-homocycle-substituted congener **45**, exerted significant activity at 10 μM, and the 5-F derivative **67** was nearly twice as active as its 5-H counterpart **44**. Although 2 orders of magnitude less active than dexamethasone and cyclosporin A (Table 4), [1-(4-fluorobenzyl)indol-3-yl]acetamides **45** and **83** showed higher potency than astemizole: IC_{50} = 1.50, 2.28, and 3.30 μM, respectively.

Histamine Release Inhibition. Among the 32 amides tested in the ovalbumin-induced histamine

Table 5. Inhibition of Late Phase Eosinophilia in Bronchoalveolar Lavage (BAL) of Guinea Pigs after Parenteral or Oral Administration of **45** and Zardaverine

			% inhibition	
administration route	compd	dose (mg/kg)	2 h before challenge	4 h after challenge
IP	**45**	10	59	NI
IP	**45**	5	NI[a]	41
IP	zardaverine	5	NI	45
PO	**45**	30	35	28
PO	zardaverine	10	67	60

[a] NI: no inhibition.

release assay, 12 compounds possessed IC_{50} in the submicromolar range (Table 3). In line with precedent observations about optimal chain length, propanamide **46** and butyramide **48** were 14-fold and 32-fold less efficacious than acetamide **45**; the same phenomenon was observed, in the 5-Cl and 5-F subseries, by comparison of propanamides **62** and **69** with their respective acetamides **61** and **68**. As observed for IL-4 and IL-5 inhibition, removal of the ether functionality of **83**, leading to phenol **84**, exerted a deleterious effect: IC_{50} = 0.330 and 0.940 μM. Comparison with astemizole points to the very high potency of acetamides **45** and **68** which were 406-fold and 108-fold more efficient than the reference histamine antagonist: IC_{50} = 0.016 and 0.060 and 6.5 μM, respectively.

The interest of **45**, as histamine release inhibitor, was confirmed by its high activity in the ionomycin-induced histamine release inhibition: IC_{50} = 0.011 μM. In the same conditions, IC_{50} of disodium cromoglycate (DSCG), used as a reference drug, was >1 μM.

Inhibition of Acetylcholine Hyperreactivity. Inhibition by **45** of tetraethylammonium chloride (TEA)-induced acetylcholine hyperreactivity was carried out in rat tracheal segments. It exerted a potent inhibitory effect at a very low micromolar concentration: IC_{50} = 0.021 μM, i.e., at a 60-fold lower concentration than verapamil (IC_{50} = 1.3 μM).

Inhibition of Carbachol-Induced Contraction. Relaxation of carbachol-precontracted guinea pig trachea, induced by **45**, was very moderate at the high concentration of 100 μM (36%); higher concentrations (up to 500 μM) did not cause further increase in the relaxatory activity of **45**. This weak inhibitory effect on the carbachol-induced contraction can be considered as unspecific.

Biological Properties of Compound 45

In vivo efficacy of **45** in allergic disorders was confirmed by carrying out two assays: inhibition of late phase eosinophilia in actively sensitized and challenged guinea pigs and inhibition of rhinitis in sensitized and challenged rats or induced by capsaicin in naive rats.

Inhibition of allergically induced late phase eosinophilia. The results are summarized in Table 5. Compound **45** significantly decreased the eosinophil number in bronchoalveolar lavage of ovalbumin-treated guinea pigs, after parenteral and oral administration. After ip administration of 5 mg/kg, percent inhibition, 4 h after challenge, was comparable to that of zardaverine: 41 and 45%, respectively. Although less potent than zardaverine after oral administration, **45** induced significant inhibition (~30%) 2 h before and 4 h after challenge, at a dose of 30 mg/kg.

Table 6. In Vivo Activity of **45** in Rhinitis Model: Inhibition of Ovalbumin-Induced Rhinitis in Anesthetized Rats after Topical, Intravenous, or Oral Administration of **45**

administration route	compd	IC_{50} (μM)	ID_{50} (μg/kg)
topical application (nasal perfusion)	**45**	0.25	
	antazoline	1.26	
	mepyramine	7.41	
IV	**45**		5[a]
PO			460[b]

[a] Administered 30 min before challenge. [b] Administered 60 min before challenge.

Inhibition of Allergically Induced or Capsaicin-Induced Rhinitis. On the first rhinitis model, **45** possessed a very potent inhibitory activity after topical administration: IC_{50} = 0.25 μM, (Table 6). With regard to this mainly histamine-mediated phenomenon, it was markedly more efficient than were classical antihistamines such as antazoline and mepyramine, whose IC_{50} were 5-fold and 30-fold higher than that of **45**. Its inhibitory activity was confirmed after iv administration at a very low dose: ID_{50} = 5 μg/kg. Although markedly less active per os, due likely to poor oral bioavailability, its ID_{50} remained nevertheless inferior to 0.5 mg/kg. This effect, in line with in vitro data, indicates that **45** could also be able to inhibit histamine release in vivo.

Surprisingly, **45** was roughly 10-times more potent than morphine in the inhibition of the capsaicin-induced increase in nasal vascular permeability of rats, after topical application: IC_{50} = 0.3 and 4.1 μM, respectively. Capsaicin stimulates the sensory c fibers, resulting in the release of substance P (SP) which is responsible for the increased vascular leakage. Morphine inhibits the capsaicin-induced SP release by stimulation of its presynaptic receptor located on c fiber terminals. Such a mechanism for **45** might be excluded because there is large evidence that capsaicin does not influence the histamine content in the guinea pig nasal mucosa.[28]

Conclusion

We prepared a series of *N*-(4-pyridinyl)-(indol-3-yl) acetamides and propanamides and conducted the structure−activity relationship studies on their inhibitory activities toward IL-4 and IL-5 production in Th-2 cells and histamine release from mast cells. The results may be summarized as follows: (1) inhibitory activity was increased by placement of 4-fluorophenyl or (4-fluoro)-benzyl groupings at indolic nitrogen; (2) generally speaking, the presence of a methyl group at the C-2 position of indole was detrimental; (3) the incorporation of an acetamide chain at C-3 provided the optimal potency, and (4) introduction of halogen, isopropyl, methoxy, or hydroxy groups at C-5 or C-6 failed to improve potency.

Indolylacetamide **45** is an extremely strong inhibitor of the allergically induced histamine release from peritoneal mast cells; morever, it was able to inhibit the IL-4 and IL-5 biosynthesis with IC_{50} values in low micromolar ranges. In keeping with its in vitro profile, **45** potently inhibited the mainly histamine-mediated increase of allergically induced vascular permeability in the nasal cavity of actively sensitized rats and the allergen-induced late phase eosinophilia in actively

sensitized guinea pigs. Although no direct study of the mechanism of action of compound **45** was carried out up to now, it could be assumed that this amide is liable to down regulate Ca^{2+} concentration in lymphocytes, insofar as enhancing intracellular Ca^{2+} concentration with the ionophor (A23187) induces an incrase of IL-4 production.[29] In the same way, histamine release inhibition could be linked to a decrease in intracellular Ca^{2+} concentration via interaction with the chloride channel, like DSCG.[30] Finally, literature data dealing with TEA-induced acetylcholine hyperreactivity,[31] as well as capsaicin−induced rhinitis[32] could also suggest the involvement of acetamide **45** in the regulation of Ca^{2+}-activated ion channels.

The present results suggest that **45** may prove to be a valuable candidate as lead structure for further synthetic work aimed at reducing IL-4 and IL-5 release and thereby IgE biosynthesis production. This conclusion is in agreement with recent literature data on the high IgE biosynthesis suppressive activity of *N*(α-carboxyphenyl)phenylacetamides.[33]

Experimental Section

A. Chemical Synthesis. Melting points were determined in open glass capillaries and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 250 spectrometer (250 MHz) (Bruker, Wissenbourg, France), using DMSO-d_6 as solvent; chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. IR spectra were run with KBr pellets with a Beckman IR 4230 grating infrared spectrophotometer (Beckman, Gagny, France). Microanalyses were performed on a Perkin-Elmer CHN 240 apparatus. Elemental analyses were within 0.4% of the calculated value. Silica gel 60 (E. Merck, 230−400 mesh) was used for preparative column chromatography. Diethyl ether was freshly distilled from sodium benzophenone. Reagents were obtained commercially (Aldrich Chemical Co.) and used without further purification. Chemical yields reported are unoptimized specific examples of one preparation. The following ethyl (5-substituted-2-methylindol-3-yl)acetates have been previously described: 5-F,[34] 5-Cl,[35] and 5-OCH_3.[36] The ethyl (indol-3-yl)alkanoates (n = 1, 2, 3) **14**, **15**, and **16** were obtained by HCl−ethanol esterification of the corresponding acids, purchased from Aldrich Chemical Co. The acids of the following ethyl esters have been previously synthesized starting from β-ketoesters or ethyl γ-oxobutyrate: **17**,[37] **18**,[38] **20**,[38] **22**,[39] **25**[37] and **19**,[40] **21**.[41]

Ethyl (5-Isopropyl-2-methylindol-3-yl)acetate (4). 4-Isopropylphenylhydrazine hydrochloride (7.3 g, 39.1 mmol) was dissolved on heating in a solution of sodium acetate (5.9 g, 43 mmol) in 1 M CH_3COOH (80 mL). Ethyl levulinate (6.7 g, 46.9 mmol) was added, and after cooling the precipitated hydrazone was filtered, washed with water, and dried in vacuo to provide 10.2 g of crude hydrazone. The hydrazone was then added to a solution of sulfuric acid (10 mL) in absolute ethanol (100 mL) and heated at reflux for 3 h. The mixture was concentrated to a volume of 50 mL, the pH was adjusted to pH 6 with 1 M NaOH, and the mixture was extracted with dichloromethane (2 × 100 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on silica gel, eluting with dichloromethane to give **4** (7.6 g, 75%) as beige crystals: mp 38 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 7.0 Hz), 1.27 (d, 6H, J = 7.0 Hz), 2.37 (s, 3H), 2.98 (m, 1H, J = 7.0 Hz), 3.68 (s, 2H), 4.04 (q, 2H, J = 7.0 Hz), 6.96 (dd, 1H, J = 8.7 Hz, 2.1 Hz), 7.22 (d, 1H, J = 8.7 Hz), 7.28 (d, 1H, J = 2.1 Hz), 10.71 (m, 1H). Anal. ($C_{16}H_{21}NO_2$) C, H, N.

General Procedures for Preparation of Ethyl ω-(indol-3-yl)alkanoates. Ethyl 3-(5-Isopropylindol-3-yl)propanoate (24) (Method B). Step 1: Ethyl 3-(2-Ethoxycarbonyl-5-isopropylindol-3-yl)propanoate (10). 4-Isopropylaniline (9.1 g, 67.5 mmol) was dissolved in 12 M HCl (35 mL) and cooled to 0 °C. $NaNO_2$ (5.1 g, 73.9 mmol) in H_2O (30 mL) was added dropwise so that the temperature remained below 3 °C. Separately was prepared a mixture of ethyl 2-oxocyclopentane carboxylate (10.5 g, 67.5 mmol) in ethanol (75 mL), ice (125 g), and 4 M KOH (60 mL). The solution of diazonium salt was added to this mixture with stirring. After 2 h, the mixture was extracted with diethyl ether (3 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to leave a dark oil which was added to a solution of sulfuric acid (10 mL) in absolute ethanol (100 mL). After refluxing for 12 h, the mixture was concentrated and dichloromethane (200 mL) was added, washed with 10% Na_2CO_3 and brine. The organic layer was dried (Na_2SO_4), concentrated, and purified by chromatography on silica gel, eluting with dichloromethane to give **10** (6.2 g, 28%) as white crystals: mp 73 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, 3H, J = 7.1 Hz), 1.28 (d, 6H, J = 6.9 Hz), 1.38 (t, 3H, J = 7.1 Hz), 2.61 (t, 2H, J = 7.8 Hz), 2.99 (m, 1H, J = 6.9 Hz), 3.32 (t, 2H, J = 7.8 Hz), 4.04 (q, 2H, J = 7.1 Hz), 4.37 (q, 2H, J = 7.1 Hz), 7.21 (d, 1H, J = 8.5 Hz), 7.37 (d, 1H, J = 8.5 Hz), 7.49 (s, 1H), 11.48 (s, 1H). Anal. ($C_{19}H_{25}NO_4$) C, H, N.

Step 2: 3-(2-Carboxy-5-isopropylindol-3-yl)propanoic acid. Diester **10** (6.2 g, 18.8 mmol) was dissolved in ethanol (150 mL). 6 M NaOH (20 mL) was added, and the mixture was refluxed for 1 h. After evaporation to a volume of 50 mL, 1 M HCl was added, up to pH 6 and the mixture was extracted with ethyl acetate (3 × 50 mL). The organic layers were dried (Na_2SO_4) and concentrated to provide the 3-(2-carboxy-5-isopropylindol-3-yl)propanoic acid (4.9 g, 95%) as white crystals: mp 181 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (d, 6H, J = 6.9 Hz), 2.53 (t, 2H, J = 7.3 Hz), 2.99 (m, 1H, J = 6.9 Hz), 3.28 (t, 2H, J = 7.3 Hz), 7.18 (dd, 1H, J = 8.6 Hz, 1.4 Hz), 7.35 (d, 1H, J = 8.6 Hz), 7.51 (d, 1H, J = 1.4 Hz), 11.35 (s, 1H), 12.51 (m, 2H). Anal. ($C_{15}H_{17}NO_4$) C, H, N.

Step 3: Ethyl 3-(2-Carboxy-5-isopropylindol-3-yl)propanoate. A solution of 3-(2-carboxy-5-isopropylindol-3-yl)-propanoic acid (4.9 g, 17.8 mmol) in 1 M HCl in ethanol (50 mL) was refluxed for 1.5 h. After evaporation to a volume of 20 mL, the mixture was cooled and filtered. The crystals were washed with diisopropyl ether to give ethyl 3-(2-carboxy-5-isopropylindol-3-yl)propanoate (4.3 g, 79%) as white crystals: mp 186 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (t, 3H, J = 7.1 Hz), 1.27 (d, 6H, J = 6.8 Hz), 2.56 (t, 2H, J = 7.5 Hz), 2.98 (m, 1H, J = 6.8 Hz), 3.29 (t, 2H, J = 7.5 Hz), 4.02 (q, 2H, J = 7.1 Hz), 7.18 (d, 1H, J = 8.6 Hz,), 7.35 (d, 1H, J = 8.6 Hz), 7.48 (s, 1H), 11.53 (s, 1H), 13.01 (s, 1H). Anal. ($C_{17}H_{21}NO_4$) C, H, N.

Step 4: Ethyl 3-(5-Isopropylindol-3-yl)propanoate (24). To a solution of ethyl 3-(2-carboxy-5-isopropylindol-3-yl)-propanoate (4.3 g, 14.1 mmol) in quinoline (80 mL), copper chromite (0.8 g, 2.6 mmol) was added and the mixture was refluxed under N_2 for 2 h. After cooling, diethyl ether (200 mL) was added and the mixture was filtered. The filtrate was washed with 2 M HCl (3 × 100 mL) and brine, then dried (Na_2SO_4), filtered, concentrated, and purified by chromatography on silica gel, eluting with dichloromethane to provide **24** (3.0 g, 81%) as beige crystals: mp 41 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, J = 7.1 Hz), 1.27 (d, 6H, J = 6.9 Hz), 2.63 (t, 2H, J = 7.8 Hz), 2.95 (t, 2H, J = 7.8 Hz), 4.11 (q, 2H, J = 7.1 Hz), 7.02 (dd, 1H, J = 8.7 Hz, 1.7 Hz), 7.23 (d, 1H, J = 1.7 Hz), 7.30 (d, 1H, J = 8.4 Hz), 7.36 (s, 1H), 10.84 (s, 1H). Anal. ($C_{16}H_{21}NO_2$) C, H, N.

Compounds **17**−**23** and **25** were prepared in a similar manner; see Table 1.

General Procedure for Preparation of Ethyl *N*-(4-Fluorophenyl)-(indol-3-yl)acetates (Method C). Ethyl [1-(4-Fluorophenyl)-5-fluoroindol-3-yl]acetate (34). To a solution of ethyl (5-fluoroindol-3-yl)acetate **21** (3.9 g, 17.6 mmol) in bromobenzene (70 mL) was added 4-iodofluorobenzene (4.3 g, 19.3 mmol), anhydrous K_2CO_3 (4.9 g, 35.2 mmol), and Cu bronze (2 g). The mixture was refluxed under N_2 for 24 h. After cooling and filtration, the filtrate was concentrated and purified by chromatography on silica gel, eluting with dichloromethane to provide **34** (4.7 g, 85%) as beige crystals: mp 110 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.1 Hz),

3.86 (s, 2H), 4.15 (q, 2H, J = 7.1 Hz), 7.09 (dd, 1H, J = 9.2 Hz, 2.6 Hz), 7.41−7.67 (m, 6H), 7.70 (s, 1H). Anal. ($C_{18}H_{15}F_2NO_2$) C, H, N.

Compounds **26** and **30** were prepared in a similar manner; see Table 2.

General Procedure for Preparation of Ethyl *N*-Benzylated *ω*-(Indol-3-yl)alkanoates (Method D). Ethyl [1-(4-Fluorobenzyl)-6-methoxyindol-3-yl]acetate (43). To a suspension of NaH (0.3 g, 12.5 mmol) in dry DMSO (40 mL), under N_2, at room temperature, was carefully added a solution of ethyl (6-methoxyindol-3-yl)acetate **25** (2.3 g, 9.7 mmol) in DMSO (40 mL), and the mixture was stirred for 0.5 h. 4-Fluorobenzyl chloride (1.4 g, 9.7 mmol) was added, and reaction was completed overnight. H_2O (300 mL) was added, and the mixture was extracted with diethyl ether (3 × 80 mL). The organic layers were washed with brine, dried (Na_2SO_4), concentrated, and purified by chromatography on silica gel, eluting with dichloromethane to provide **43** (1.4 g, 42%) as beige crystals: mp 102 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, J = 7.1 Hz), 3.72 (s, 2H), 3.78 (s, 3H), 4.10 (q, 2H, J = 7.1 Hz), 5.35 (s, 2H), 6.72 (dd, 1H, J = 8.6 Hz, 2.2 Hz), 7.04 (d, 1H, J = 2.2 Hz), 7.17 (dd, 2H, J = 8.8 Hz), 7.26 (s, 1H), 7.30 (dd, 2H, J = 8.8 Hz, 5.7 Hz), 7.42 (d, 1H, J = 8.6 Hz). Anal. ($C_{20}H_{20}FNO_3$) C, H, N.

Compounds **27, 28, 29, 31, 32, 33**, and **35**−**42** were prepared in a similar manner; see Table 2.

General Procedure for Preparation of *N*-(Pyridin-4-yl)-*ω*-(indol-3-yl)alkanamides (Method E). *N*-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-6-methoxyindol-3-yl]acetamide (83). To a solution of [1-(4-fluorobenzyl)-6-methoxyindol-3-yl]acetic acid (1.2 g, 3.8 mmol) in dichloromethane (100 mL) were added triethylamine (1.0 g, 9.5 mmol), 2-chloro-1-methylpyridinium iodide (1.16 g, 4.5 mmol), and 4-aminopyridine (0.36 g, 3.8 mmol). The mixture was refluxed for 2 h and, after cooling, washed with H_2O, dried (Na_2SO_4), concentrated and purified by chromatography on silica gel, eluting with dichloromethane/ethanol (97:3) to provide **83** (1.1 g, 71%) as yellow crystals: mp 152 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 2H), 3.75 (s, 3H), 5.36 (s, 2H), 6.72 (dd, 1H, J = 8.6, 1.9 Hz), 7.04 (d, 1H, J = 1.9 Hz), 7.17 (dd, 2H, J = 8.8 Hz), 7.27 (s, 1H), 7.30 (dd, 2H, J = 8.8, 5.9 Hz), 7.49 (d, 1H, J = 8.6 Hz), 7.60 (d, 2H, J = 6.2 Hz), 8.43 (d, 2H, J = 6.2 Hz), 10.52 (s, 1H). Anal. ($C_{23}H_{20}FN_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-[1-(4-fluorophenyl)indol-3-yl]acetamide (44). Method E:** 67% yield; mp 152 °C; ^{1}H NMR (DMSO-d_6) δ 3.92 (s, 2H), 7.15−7.74 (m, 9H), 7.62 (d, 2H, J = 6.0 Hz), 8.45 (d, 2H, J = 6.0 Hz), 10.62 (s, 1H). Anal. ($C_{21}H_{16}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[1-(4-fluorobenzyl)indol-3-yl]acetamide (45). Method E:** 71% yield; mp 140−142 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 2H), 5.38 (s, 2H), 7.02 (dd, 1H, J = 7.2 Hz), 7.08−7.17 (m, 3H), 7.27 (dd, 2H, J = 8.8, 5.6 Hz), 7.43 (s, 1H), 7.45 (d, 1H, J = 8.5 Hz), 7.59 (d, 2H, J = 5.4 Hz), 7.61 (d, 1H, J = 8.1 Hz), 8.41 (d, 2H, J = 5.4 Hz), 10.53 (s, 1H). Anal. ($C_{22}H_{18}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-[1-(4-fluorobenzyl)indol-3-yl]propanamide (46). Method E:** 53% yield; mp 124 °C; ^{1}H NMR (DMSO-d_6) δ 2.77 (t, 2H, J = 7.2 Hz), 3.08 (t, 2H, J = 7.2 Hz), 5.36 (s, 2H), 7.03−7.16 (m, 4H), 7.22 (dd, 2H, J = 8.6 Hz, 5.4 Hz), 7.32 (s, 1H), 7.45 (d, 1H, J = 8.0 Hz), 7.60 (d, 2H, J = 6.0 Hz), 7.63 (d, 1H, J = 8.0 Hz), 8.45 (d, 2H, J = 6.0 Hz), 10.33 (s, 1H). Anal. ($C_{23}H_{20}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-4-(indol-3-yl)butyramide (47). Method E:** 71% yield; mp 91 °C; ^{1}H NMR (DMSO-d_6) δ 2.01 (m, 2H), 2.45 (t, 2H, J = 7.5 Hz), 2.77 (t, 2H, J = 7.5 Hz), 6.69 (dd, 1H, J = 7.9 Hz), 7.09 (dd, 1H, J = 7.9 Hz), 7.17 (d, 1H, J = 2.1 Hz), 7.37 (d, 1H, J = 7.9 Hz), 7.56 (d, 1H, J = 7.9 Hz), 7.60 (d, 2H, J = 6.3 Hz), 8.43 (d, 2H, J = 6.3 Hz), 10.30 (s, 1H), 10.82 (m, 1H). Anal. ($C_{17}H_{17}N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-4-[1-(4-fluorobenzyl)-indol-3-yl]butyramide (48). Method E:** 73% yield; mp 56 °C; ^{1}H NMR (DMSO-d_6) δ 2.01 (m, 2H), 2.46 (t, 2H, J = 7.4 Hz), 2.78 (t, 2H, J = 7.4 Hz), 5.37 (s, 2H), 7.03 (dd, 1H, J = 8.0 Hz), 7.09−7.19 (m, 3H), 7.28 (dd, 2H, J = 8.5, 5.6 Hz), 7.33 (s, 1H), 7.44 (d, 1H, J = 8.0 Hz), 7.57−7.60 (m, 3H), 8.43 (d, 2H, J = 6.3 Hz), 10.30 (s, 1H). Anal. ($C_{24}H_{22}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(5-methoxyindol-3-yl)acetamide (49). Method E:** 50% yield; mp 166 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 3H), 3. 81 (s, 2H), 6.76 (dd, 1H, J = 8.7 Hz, 2.5 Hz), 7.13 (d, 1H, J = 2.5 Hz), 7.26 (s, 1H), 7.28 (d, 1H, J = 8.7 Hz), 7.63 (dd, 2H, J = 5.0, 1.5 Hz), 8.44 (d, 2H, J = 5.0, 1.5 Hz), 10.54 (s, 1H), 10.83 (m, 1H). Anal. ($C_{16}H_{15}N_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-3-(5-methoxyindol-3-yl)propanamide (50). Method E:** 52% yield; mp 140−141 °C; ^{1}H NMR (DMSO-d_6) δ 2.76 (t, 2H, J = 7.9 Hz), 3.03 (t, 2H, J = 7.9 Hz), 3.78 (s, 3H), 6.74 (dd, 1H, J = 8.7, 2.4 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.12 (d, 1H, J = 2.2 Hz), 7.25 (d, 1H, J = 8.7 Hz), 7.63 (d, 2H, J = 5.0 Hz), 8.45 (d, 2H, J = 5.0 Hz), 10.42 (s, 1H), 10.65 (m, 1H). Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-[1-(4-fluorophenyl)-5-methoxyindol-3-yl]acetamide (51). Method E:** 79% yield; mp 218 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 3H), 3.87 (s, 2H), 6.87 (dd, 1H, J = 8.9, 2.5 Hz), 7.23 (d, 1H, J = 2.5 Hz), 7.39−7.61 (m, 6H), 7.61 (d, 2H, J = 6.0 Hz), 8.45 (d, 2H, J = 6.0 Hz), 10.60 (s, 1H). Anal. ($C_{22}H_{18}FN_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-(1-benzyl-5-methoxyindol-3-yl)acetamide (52). Method E:** 30% yield; mp 145 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 3H), 3. 81 (s, 2H), 5.38 (s, 2H), 6.78 (dd, 1H, J = 8.8, 2.5 Hz), 7.15 (d, 1H, J = 2.5 Hz), 7.20−7.36 (m, 6H), 7.41 (s, 1H), 7.62 (d, 2H, J = 6.2 Hz), 8.44 (d, 2H, J = 6.2 Hz), 10.56 (s, 1H). Anal. ($C_{23}H_{21}N_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-3-(1-benzyl-5-methoxyindol-3-yl)propanamide (53). Method E:** 60% yield; mp 135 °C; ^{1}H NMR (DMSO-d_6) δ 2.75 (t, 2H, J = 7.0 Hz), 3.04 (t, 2H, J = 7.0 Hz), 3.78 (s, 3H), 5.32 (s, 2H), 6.76 (dd, 1H, J = 9.0, 2.5 Hz), 7.10−7.32 (m, 8H), 7.61 (d, 2H, J = 4.5 Hz), 8.44 (d, 2H, J = 4.5 Hz), 10.32 (s, 1H). Anal. ($C_{24}H_{23}N_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-3-[1-(4-fluorobenzyl)-5-methoxyindol-3-yl]propanamide (54). Method E:** 81% yield; mp 144 °C; ^{1}H NMR (DMSO-d_6) δ 2.75 (t, 2H, J = 7.0 Hz), 3.05 (t, 2H, J = 7.0 Hz), 3.78 (s, 3H), 5.32 (s, 2H), 6.76 (dd, 1H, J = 8.9, 2.1 Hz), 7.02−7.22 (m, 5H), 7.27 (s, 1H), 7.32 (d, 1H, J = 8.9 Hz), 7.60 (d, 2H, J = 4.5 Hz), 8.45 (d, 2H, J = 4.5 Hz), 10.32 (s, 1H). Anal. ($C_{24}H_{22}FN_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-(5-methoxy-2-methylindol-3-yl)acetamide (55). Method E:** 50% yield; mp 203 °C; ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 3.72 (s, 2H), 3.74 (s, 3H), 6.66 (dd, 1H, J = 8.2, 2.0 Hz), 7.07 (d, 1H, J = 2.0 Hz), 7.16 (d, 1H, J = 8.5 Hz), 7.61 (d, 2H, J = 4.5 Hz), 8.43 (d, 2H, J = 4.5 Hz), 10.48 (s, 1H), 10.72 (s, 1H). Anal. ($C_{17}H_{17}N_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-methoxy-2-methylindol-3-yl]acetamide (56). Method E:** 72% yield; mp 162 °C; ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 3.76 (s, 3H), 3.79 (s, 2H), 5.38 (s, 2H), 6.71 (dd, 1H, J = 8.7 2.5 Hz), 7.09−7.19 (m, 5H), 7.30 (d, 1H, 8.7 Hz), 7.61 (dd, 2H, J = 4.7 Hz, 1.5 Hz), 8.44 (dd, 2H, J = 4.7, 1.5 Hz), 10.54 (s, 1H). Anal. ($C_{24}H_{22}FN_3O_2$) C, H, N.

***N*-(Pyridin-4-yl)-(5-chloroindol-3-yl)acetamide (57). Method E:** 40% yield; mp 200 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 2H), 7.10 (d, 1H, J = 9.0 Hz), 7.39 (s, 1H), 7.41 (d, 1H, J = 9.0 Hz), 7.60 (d, 2H, J = 4.5 Hz), 7.67 (s, 1H), 8.44 (d, 2H, J = 4.5 Hz), 10.53 (s, 1H), 11.20 (s, 1H) Anal. ($C_{15}H_{12}ClN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-(5-chloroindol-3-yl)propanamide (58). Method E:** 65% yield; mp 188 °C; ^{1}H NMR (DMSO-d_6) δ 2.75 (t, 2H, J = 7.0 Hz), 3.05 (t, 2H, J = 7.0 Hz), 7.10 (dd, 1H, J = 8.5 Hz, 1.5 Hz), 7.26 (s, 1H), 7.39 (d, 1H, J = 8.5 Hz), 7.60 (d, 2H, J = 4.5 Hz), 7.66 (d, 1H, J = 1.5 Hz), 8.45 (d, 2H, J = 4.5 Hz), 10.34 (s, 1H), 11.05 (s, 1H). Anal. ($C_{16}H_{14}ClN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(1-benzyl-5-chloroindol-3-yl)acetamide (59). Method E:** 62% yield; mp 138 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 2H), 5.43 (s, 2H), 7.12−7.53 (m, 8H), 7.60 (d, 2H, J = 5.0 Hz), 7.70 (s, 1H), 8.44 (d, 2H, J = 5.0 Hz), 10.56 (s, 1H). Anal. ($C_{22}H_{18}ClN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-(1-benzyl-5-chloroindol-3-yl)propanamide (60). Method E:** 70% yield; mp 160 °C; ^{1}H NMR (DMSO-d_6) δ 2.74 (t, 2H, J = 7.2 Hz), 3.06 (t, 2H, J = 7.2 Hz), 5.38 (s, 2H), 7.09−7.25 (m, 6H), 7.40 (s, 1H), 7.46 (d, 1H, J =

8.5 Hz), 7.58 (d, 2H, J = 6.0 Hz), 7.69 (d, 1H, J = 1.8 Hz), 8.44 (d, 2H, J = 6.0 Hz), 10.31 (s, 1H). Anal. ($C_{23}H_{20}ClN_3O$) C, H, N.

N-(Pyridin-4-yl)-[5-chloro-1-(4-fluorobenzyl)indol-3-yl]acetamide (61). Method E: 51% yield; mp 150 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 2H), 5.42 (s, 2H), 7.13−7.21 (m, 3H), 7.30 (dd, 2H, J = 8.7 Hz, 5.6 Hz), 7.52 (d, 1H, J = 8.5 Hz), 7.54 (s, 1H), 7.61 (d, 2H, J = 4.5 Hz), 7.70 (d, 1H, J = 2.0 Hz), 8.44 (d, 2H, J = 4.5 Hz), 10.57 (s, 1H). Anal. ($C_{22}H_{17}ClFN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-[5-chloro-1-(4-fluorobenzyl)indol-3-yl]propanamide (62). Method E:** 56% yield; mp 58 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (t, 2H, J = 7.0 Hz), 3.05 (t, 2H, J = 7.0 Hz), 5.37 (s, 2H), 7.06 (dd, 2H, J = 8.6 Hz), 7. 12 (dd, 1H, J = 8.7 Hz, 2.0 Hz), 7.21 (dd, 2H, J = 8.6 Hz, 5.6 Hz), 7.40 (s, 1H), 7.48 (d, 1H, J = 8.7 Hz), 7.58 (d, 2H, J = 5.7 Hz), 7.68 (d, 1H, J = 2.0 Hz), 8.44 (d, 2H, J = 5.7 Hz), 10.30 (s, 1H). Anal. ($C_{23}H_{19}ClFN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(5-chloro-2-methylindol-3-yl)acetamide (63). Method E:** 63% yield; mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 3.75 (s, 2H), 7.01 (dd, 1H, J = 8.5, 2.0 Hz), 7.29 (d, 1H, J = 8.5 Hz), 7.59−7.62 (m, 3H), 8.44 (d, 2H, J = 4.5 Hz), 10.50 (s, 1H), 11.12 (s, 1H). Anal. ($C_{16}H_{14}ClN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[5-chloro-1-(4-fluorobenzyl)-2-methylindol-3-yl]acetamide (64). Method E:** 81% yield; mp 145 °C; ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 3.83 (s, 2H), 5.44 (s, 2H), 7.06−7.19 (m, 5H), 7.46 (d, 1H, J = 8.5 Hz), 7.61 (d, 2H, J = 5.2 Hz), 7.68 (s, 1H), 8.44 (d, 2H, J = 5.2 Hz), 10.55 (s, 1H). Anal. ($C_{23}H_{19}ClFN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(5-fluoroindol-3-yl)acetamide (65). Method E:** 50% yield; mp 200 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 2H), 6.94 (ddd, 1H, J = 8.7 Hz, 2.5 Hz), 7.34−7.41 (m, 3H), 7.60 (d, 2H, J = 6.2 Hz), 8.43 (d, 2H, J = 6.2 Hz), 10.53 (s, 1H), 11.09 (m, 1H). Anal. ($C_{15}H_{12}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-(5-fluoroindol-3-yl)propanamide (66). Method E:** 51% yield; mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 2.74 (t, 2H, J = 7.0 Hz), 3.03 (t, 2H, J = 7.0 Hz), 6.94 (ddd, 1H, J = 8.5, 1.5 Hz), 7.25 (s, 1H), 7.33−7.39 (m, 2H), 7.60 (d, 2H, J = 4.5 Hz), 8.44 (d, 2H, J = 4.5 Hz), 10.33 (s, 1H), 10.92 (s, 1H). Anal. ($C_{16}H_{14}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[5-fluoro-1-(4-fluorophenyl)indol-3-yl]-acetamide (67). Method E:** 68% yield; mp 186 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 2H), 7.09 (ddd, J = 9.2 Hz, 2.5 Hz), 7.42−7.67 (m, 6H), 7.62 (d, 2H, J = 6.1 Hz), 7.71 (s, 1H), 8.45 (d, 2H, J = 6.1 Hz), 10.61 (s, 1H). Anal. ($C_{21}H_{15}F_2N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(1-benzyl-5-fluoroindol-3-yl)acetamide (68). Method E:** 70% yield; mp 137 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 2H), 5.43 (s, 2H), 6.99 (dd, 1H, J = 8.8 Hz), 7.23−7.50 (m, 7H), 7.54 (s, 1H), 7.61 (d, 2H, J = 5.2 Hz), 8.45 (d, 2H, J = 5.2 Hz), 10.57 (s, 1H). Anal. ($C_{22}H_{18}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-(1-benzyl-5-fluoroindol-3-yl)propionamide (69). Method E:** 80% yield; mp 184 °C; ^{1}H NMR (DMSO-d_6) δ 2.75 (t, 2H, J = 6.9 Hz), 3.05 (t, 2H, J = 6.9 Hz), 5.39 (s, 2H), 6.97 (dd, 1H, J = 8.8 Hz), 7.14−7.44 (m, 8H), 7.60 (d, 2H, J = 5.5 Hz), 8.45 (d, 2H, J = 5.5 Hz), 10.32 (s, 1H). Anal. ($C_{23}H_{20}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[5-fluoro-1-(4-fluorobenzyl)indol-3-yl]-acetamide (70). Method E:** 60% yield; mp 156 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (s, 2H), 5.42 (s, 2H), 7.00 (ddd, 1H, J = 9.0, 2.5 Hz), 7.17 (dd, 2H, J = 8.9 Hz), 7.31 (dd, 2H, J = 8.9 Hz, 5.7 Hz), 7.40 (dd, 1H, J = 9.0, 2.5 Hz), 7.49 (dd, 1H, J = 9.0, 4.4 Hz), 7.54 (s, 1H), 7.61 (d, 2H, J = 4.5 Hz), 8.44 (d, 2H, J = 4.5 Hz), 10.56 (s, 1H). Anal. ($C_{22}H_{17}F_2N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-[5-fluoro-1-(4-fluorobenzyl)indol-3-yl]propanamide (71). Method E:** 58% yield; mp 131 °C; ^{1}H NMR (DMSO-d_6) δ 2.73 (t, 2H, J = 7.1 Hz), 3.03 (t, 2H, J = 7.1 Hz), 5.36 (s, 2H), 6.97 (dd, J = 8.7 Hz), 7.06 (dd, 2H, J = 8.9 Hz), 7.22 (dd, 2H, J = 8.9, 5.6 Hz), 7.38−7.57 (m, 3H), 7.59 (d, J = 6.2 Hz), 8.44 (d, 2H, J = 6.2 Hz), 10.31 (s, 1H). Anal. ($C_{23}H_{19}F_2N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(5-fluoro-2-methylindol-3-yl)acetamide (72). Method E:** 64% yield; mp 200 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 3.73 (s, 2H), 6.85 (ddd, 1H, J = 8.5 Hz, 2.4 Hz), 7.23−7.33 (m, 2H), 7.61 (d, 2H, J = 6.2 Hz), 8.44 (d, 2H, J = 6.2 Hz), 10.49 (s, 1H), 11.01 (m, 1H). Anal. ($C_{16}H_{14}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(1-benzyl-5-fluoro-2-methylindol-3-yl)acetamide (73). Method E:** 61% yield; mp 181 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H), 3.82 (s, 2H), 5.45 (s, 2H), 6.90 (ddd, 1H, J = 9.1, 2.4 Hz), 7.01−7.43 (m, 7H), 7.61 (d, 2H, J = 4.9 Hz), 8.44 (d, 2H, J = 4.9 Hz), 10.54 (s, 1H). Anal. ($C_{23}H_{20}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[5-fluoro-1-(4-fluorobenzyl)-2-methylindol-3-yl]acetamide (74). Method E:** 65% yield; mp 142 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3H), 3.81 (s, 2H), 5.44 (s, 2H), 6.91 (ddd, 1H, J = 8.9 Hz, 2.5 Hz), 7.04−7.44 (m, 6H), 7.61 (d, 2H, J = 6.0 Hz), 8.45 (d, 2H, J = 6.0 Hz), 10.54 (s, 1H) Anal. ($C_{23}H_{19}F_2N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(5-isopropylindol-3-yl)acetamide (75). Method E:** 61% yield; mp 175 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.26 (d, 6H, J = 7.0 Hz), 2.96 (m, 1H, J = 7.0 Hz), 3.78 (s, 2H), 7.02 (dd, 1H, J = 8.7, 1.5 Hz), 7.25 (d, 1H, J = 1.5 Hz), 7.30 (d, 1H, J = 8.7 Hz), 7.45 (s, 1H), 7.61 (dd, 2H, J = 5.0, 1.5 Hz), 8.44 (d, 2H, J = 5.0, 1.5 Hz), 10.52 (s, 1H), 10.84 (m, 1H). Anal. ($C_{18}H_{19}N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-(5-isopropylindol-3-yl)propanamide (76). Method E:** 64% yield; mp 134 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 6H, J = 6.9 Hz), 2.78 (t, 2H, J = 7.1 Hz), 2.99 (m, 1H, J = 6.9 Hz), 3.19 (t, 2H, J = 7.1 Hz), 6.84 (dd, 1H, J = 8.9, 2.1 Hz), 7.10 (d, 1H, J = 2.1 Hz), 7.12 (d, 1H, J = 2.1 Hz), 7.28 (d, 1H, J = 8.9 Hz), 7.60 (d, 2H, J = 5.8 Hz), 8.44 (d, 2H, J = 5.8 Hz), 10.40 (s, 1H), 10.71 (m, 1H). Anal. ($C_{19}H_{21}N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-(1-benzyl-5-isopropylindol-3-yl)propanamide (77). Method E:** 50% yield; mp 116 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (d, 6H, J = 6.9 Hz), 2.75 (t, 2H, J = 7.0 Hz), 2.96 (m, 1H, J = 6.9 Hz), 3.06 (t, 2H, J = 7.0 Hz), 5.33 (s, 2H), 7.01 (d, 1H, J = 8.5 Hz), 7.19−7.27 (m, 6H), 7.33 (d, 1H, J = 8.5 Hz), 7.42 (s, 1H), 7.61 (d, 2H, J = 5.4 Hz), 8.44 (d, 2H, J = 5.4 Hz), 10.33 (s, 1H). Anal. ($C_{26}H_{27}N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-5-isopropylindol-3-yl]acetamide (78). Method E:** 75% yield; mp 88 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (d, 6H, J = 7.0 Hz), 2.96 (m, 1H, J = 7.0 Hz), 3.80 (s, 2H), 5.37 (s, 2H), 7.04 (d, 1H, J = 8.5 Hz), 7.16 (dd, 2H, J = 8.7 Hz), 7.27−7.47 (m, 5H), 7.61 (d, 2H, J = 6.0 Hz), 8.44 (d, 2H, J = 6.0 Hz), 10.56 (s, 1H). Anal. ($C_{25}H_{24}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-3-[1-(4-fluorobenzyl)-5-isopropylindol-3-yl]propanamide (79). Method E:** 60% yield; mp 107 °C; ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 6H, J = 6.9 Hz), 2.78 (t, 2H, J = 7.1 Hz), 3.02 (m, 1H, J = 6.9 Hz), 3.20 (t, 2H, J = 7.1 Hz), 5.17 (s, 2H), 6.91 (d, 1H, J = 8.9 Hz), 7.12−7.42 (m, 5H), 7.44 (s, 1H), 7.61 (d, 2H, J = 5.5 Hz), 8.44 (d, 2H, J = 5.5 Hz), 10.42 (s, 1H). Anal. ($C_{26}H_{26}FN_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(2-methyl-5-isopropylindol-3-yl)acetamide (80). Method E:** 75% yield; mp 208 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (d, 6H, J = 7.0 Hz), 2.39 (s, 3H), 2.96 (m, 1H, J = 7.0 Hz), 3.73 (s, 2H), 6.92 (dd, 1H, J = 8.2, 2.0 Hz), 7.18 (d, 1H, J = 8.2 Hz), 7.37 (d, 1H, J = 2.0 Hz), 7.61 (d, 2H, J = 4.5 Hz), 8.44 (d, 2H, J = 4.5 Hz), 10.49 (s, 1H), 10.74 (s, 1H). Anal. ($C_{19}H_{21}N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-(1-benzyl-2-methyl-5-isopropylindol-3-yl)acetamide (81). Method E:** 70% yield; mp 164 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (d, 6H, J = 7.0 Hz), 2.38 (s, 3H), 2.95 (m, 1H, J = 7.0 Hz), 3.82 (s, 2H), 5.40 (s, 2H), 6.87 (dd, 1H, J = 8.5, 1.5 Hz), 7.05 (d, 1H, J = 8.5 Hz), 7.21−7.35 (m, 5H), 7.46 (d, 1H, J = 1.5 Hz), 7.62 (d, 2H, J = 4.7 Hz), 8.44 (d, 2H, J = 4.7 Hz), 10.55 (s, 1H). Anal. ($C_{26}H_{27}N_3O$) C, H, N.

***N*-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-2-methyl-5-isopropylindol-3-yl]acetamide (82). Method E:** 79% yield; mp 172 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (d, 6H, J = 7.0 Hz), 2.39 (s, 3H), 2.98 (m, 1H, J = 7.0 Hz), 3.81 (s, 2H), 5.41 (s, 2H), 6.86 (d, 1H, J = 8.4 Hz), 7.10−7.41 (m, 6H), 7.60 (d, 2H, J = 5.4 Hz), 8.44 (d, 2H, J = 5.4 Hz), 10.40 (s, 1H). Anal. ($C_{25}H_{24}FN_3O$) C, H, N.

Method F. *N*-(Pyridin-4-yl)-[1-(4-fluorobenzyl)-6-hydroxyindol-3-yl]acetamide (84). A solution of acetamide **83** (1.9 g, 4.9 mmol) in dichloromethane (45 mL) was cooled to

−80 °C under N_2. 1 M Boron tribromide in dichloromethane (12.25 mL) was added dropwise. After removal of the cooling bath, the mixture was stirred overnight. A saturated solution of $NaHCO_3$ was added slowly up to pH 7, cooling with an ice bath. The mixture was washed with H_2O, dried (Na_2SO_4), concentrated, and purified by chromatography on silica gel, eluting with dichloromethane/ethanol (95:5) to provide 6-hydroxy derivative **84** (0.77 g, 42%) as beige crystals: mp 213 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 2H), 5.27 (s, 2H), 6.59 (dd, 1H, J = 8.3 Hz, 1.9 Hz), 6.70 (d, 1H, J = 1.9 Hz), 7.17 (dd, 2H, J = 8.8 Hz), 7.21 (s, 1H), 7.22 (dd, 2H, J = 8.8, 5.9 Hz), 7.39 (d, 1H, J = 8.3 Hz), 7.59 (d, 2H, J = 6.1 Hz), 8.43 (d, 2H, J = 6.1 Hz), 9.01 (s, 1H), 10.51 (s, 1H). Anal. ($C_{22}H_{18}FN_3O_2$) C, H, N.

B. Biological Tests. IL-4 and IL-5 Release Inhibition. Complete medium was RPMI 1640 containing HEPES (Boehringer Mannheim, Germany) supplemented with 2 mM glutamine (Boehringer Mannheim), 50 μM mercaptoethanol (Sigma Chemie GmbH, Germany), 100 IU/mL penicillin/100 μg/mL streptomycin (Biochrom, Germany), and 10% fetal calf serum (Boehringer Mannheim). Rat T-STIM was purchased from Collaborative Biomedical Products (Beldford, MA), Cyclosporin A (Sandimmun ad Infus.) was obtained from Sandoz AG, Nürnberg, Germany. Dimethylsufoxyde (DMSO), dexamethasone and mitomycin C were obtained from Sigma Chemie GmbH.

The hamster anti-mouse-CD3 mAb (145-2C11) was obtained from Cedar Lane Laboratories Hornby, Ontario, Canada.

Preparation of Syngeneic Splenocytes. Spleens from male AKR/J (H-2k) mice (Charles River, Wiga Laboratories, Germany) were aseptically removed and minced with scissors and gently pressed through a No. 60 sieve. Cells were collected by centrifugation. The cell pellet was resuspended in 5 mL of lysing buffer (8.29 g/L NH_4Cl, 1.0 g/L $KHCO_3$, 0.037 g/L EDTA) and incubated for 2−5 min to lyse the red cells. Medium was added (45 mL) and spun down. The cell pellet was resuspended in phosphate buffered saline (PBS) containing 50 μg/mL mitomycin C with a cell concentration of 2×10^7 cells/mL and incubated for 30 min at 37 °C. Immediately the cells were diluted with PBS and washed exhaustively.

Cell Culture. The Th-2 clone D10.G4.1 (D10) is specific for conalbumin in the context of H-2k and was obtained from American Type Culture Collection (ATCC), Rockville, MD. It was stimulated every 10 days with the antigen conalbumin (100 μg/mL) and mitomycin C treated syngeneic splenocytes as antigen presenting cells (APC). The ratio of D10 to APC was 1:4. As a source of lymphokines, 5% Rat T-STIM was added to the culture medium. After several cycles of restimulation and 3 days after the last stimulation with antigen and APC, several freezes were made, and all experiments reported here were performed with this batch of cells. After thawing, cells were cultured for 3 days in complete medium with Rat T-STIM.

Exposition of Cells and Stimulation by Anti-CD3 mAb. Cells were cultivated in 96-well microtiter plates (4×10^4 cells/well/100 μL) for 3.5 h in complete medium without Rat T-STIM. Stock solutions of the various drugs were made in DMSO (1000 times concentrated vs final concentration) and subsequent dilutions were made in complete medium. Fifty microliters of drug solutions and solvent, respectively (4 times concentrated), were added to the cultures and incubated for 30 min. Cells were stimulated by application of 50 μL of anti-CD3 mAb (final dilution 1:100) and further incubated for 16 h. Supernatants were centrifuged and stored at −70 °C until estimation of their cytokine contents.

Determination of Cytokine Release. The supernatants were assayed for their cytokine contents by using the commercially available ELISA kits for murine IL-4 and IL-5, respectively (Endogen Inc., Boston, MA).

Results. Sixteen hours following stimulation with anti-CD3 mAb D, 10 cells produced 461 ± 54 pg IL-5/mL and 5210 ± 1319 pg IL-4/mL. Without stimulation no cytokine levels were detectable. DMSO, in concentrations up to 0.1%, affects neither IL-4 nor IL-5 production (data not shown).

The effects of the test compounds on anti-CD3 mAb induced IL-4 and IL-5 production were tested at 10 μM and are depicted in Table 3. Inhibition of IL-4 and IL-5 production by the most potent compounds was determined over a range of concentrations and the corresponding estimated IC_{50} values are listed in Table 4.

Inhibition of Histamine Release. In vitro study of inhibition of allergically induced histamine release was carried out with rat peritoneal mast cells by test compounds. Sprague−Dawley rats were sensitized by sc injection of 30 mg of whole egg white (WEW) and killed *Bordetella pertussis* organisms (5×10^{10}); 4 weeks later, peritoneal cells were harvested. The cells were centrifuged and suspended in Tris-Gel CM buffer of the following composition (mM): Tris, 25; NaCl, 120; KCl, 5; $CaCl_2$, 0.6; $MgCl_2$, 1; and 0.01% gelatin; pH 7.6. The reaction mixture containing 2×10^6 peritoneal cells (2−6% mast cells) and phosphatidylserine (10 μg/mL, known to enhance allergic histamine release) was preincubated in the presence of the drug in polypropylene tubes at 37 °C for 15 min. After antigen challenge (WEW, 50 μg/mL), the cell suspensions were incubated for an additional 30 min at 37 °C and were then centrifuged at 2000 rpm for 5 min at 4 °C. Histamine in the supernatant is measured by a fluometric method.[42] Histamine release, corrected for the spontaneous release, is expressed as a percentage of the total cell content. Total histamine from a separate, duplicate cell suspension is released by boiling for 10 min. Histamine release, induced by whole egg white, is assayed in the presence and absence of drug. Test compounds were first screened at the fixed concentration of 10 μM (data not shown) and then at 4−6 increasing concentrations for the calculation of the half-maximal inhibition (IC_{50}).

In the ionomycin-induced histamine release assay, peritoneal mast cells were obtained from naive animals. The final concentration of ionomycin was 0.300 μM.

Inhibition of TEA-Induced Hyperreactivity to Acetylcholine in Rat Tracheal Segments. Male Wistar rats (200−250 g) obtained from Charles River Wiga, Sulzfeld, (Germany) were sacrificed by CO_2 inhalation, and the tracheas were taken out. Tracheal segments (rings, 2−3 cartilages) were prepared and set up in organ baths under isotonic conditions with 1 g of preload in Krebs-Henseleit solution at 37 °C gassed with carbogen. After an equilibration period of 30 min, the cumulative concentration curve (CRC) for acetylcholine (0.01 μM to 1 M) was determined. After the sample was washed and rested, another CRC for acetylcholine in the presence of TEA (10 mM, 30 min) was established. Under these conditions TEA-induced hyperreactivity was about 30−50% at 100 μM acetylcholine. After the sample was washed and rested, another CRC for acetylcholine was estimated in the presence of the test drug (30 min equilibration before TEA) and TEA (30 min equilibration before acetylcholine). Millimeter contraction (mean and standard deviation) for 100 μM acetylcholine from 3 to 5 preparations was calculated and percent inhibition of hyperreactivity calculated by the following formula

$$(A - C) - (B - C)/(A - C) \times 100 = \text{percent inhibition}$$

where A = millimeters of contraction for 100 μM acetylcholine in the presence of TEA alone, B = millimeters of contraction for 100 μM acetylcholine in the presence of TEA and test drug, and C = millimeters of contraction for 100 μM acetylcholine alone.

Usually the screening concentration of the drug is 30 μM. If more than 50% inhibition is achieved, the IC_{50} value is determined from 3 to 4 concentrations by regression analysis.[43]

Relaxation of Carbachol-Precontracted Guinea Pig Trachea. Guinea pigs (250−400 g) obtained from Charles-River Wiga (Sulzfeld, Germany) are stunned by a blow on the neck and exsanguinated. Tracheas were taken out, freed from surrounding tissue, cut into spirals, and set up in organ baths in Tyrode solution, under isotonic conditions with preload of 2 g. The temperature was 37 °C, and the bath was gassed with carbogen. After washing and equilibration period of 30 min, carbachol (0.164 μM) is injected to give contraction. After a

stable contraction is achieved (3−4 equal spasms ± 10% from the mean), isoprenaline is injected cumulatively every 5 min until full relaxation is achieved. After the sample was washed and rested, carbachol is given, and then the cumulative concentration response curve for the test compound is estimated. Percentage relaxation for each concentration is calculated. IC_{50} values for the reference drug salbutamol (0.050 μM) and the test drug were calculated from 3 to 5 preparations in parallel by regression analysis.[43]

Inhibition of Allergically Induced Late Phase Eosinophilia. Male Dunkin-Hartley guinea pigs (200−250 g) obtained from Charles-River Wiga (Sulzfeld, Germany) were actively sensitized with two ip injections of a suspension of 20 μg of ovalbumin (OVA) (Sigma, Germany) + 20 mg of $Al(OH)_3$ in 0.5 mL of saline, on two consecutive days. Fourteen days after the second injection, animals were preteated with mepyramine (10 mg/kg, ip) to protect against lethal anaphylaxis. Thirty minutes later, guinea pigs were placed in a chamber and exposed to an OVA aerosol for 30 s. The OVA aerosol was generated from a 5 mg/mL solution via nebulizer (Bird micronebulizer, USA) driven by compressed air at 19.6 kPa and operating at 3 L/s. Test compounds (amide **45** and zardaverine) were given intraperitoneally or orally by gavage either 2 h prior to or 4 h after OVA challenge. Animals were sacrificed 24 h later by urethane overdose (1.5 g/kg, ip). Bronchoalveolar lavage (BAL) was performed with 2 × 5 mL of PBS (37 °C). Cells from pooled BAL samples were recovered by centrifugation (400*g* for 10 min at 4 °C) and resuspended in 1 mL of saline. Eosinophils were counted under microscope in a Neubauer chamber, after staining with a Becton-Dickinson test (No. 5877) containing Phloxin B (a specific coloring of eosinophils) by persons who were not involved in the experimental part. Percentage of inhibtion was calculated by the following formula:

$$(A - C) - (B - C)/(A - C) \times 100 = \text{percent inhibition}$$

where A = number of eosinophils in challenged and placebo-treated animals, B = eosinophils in OVA-challenged and test-compound-treated guinea pigs, C = number of eosinophils in naive (nonsensitized) animals.

Inhibition of Experimentally Induced Rhinitis in Anesthetized Rats. Male Brown Norway rats (280−320 g) obtained from Charles-River Wiga (Sulzfeld, Germany) were used. Animals were sensitized by an ip injection of a mixture containing 0.5 mg/kg ovalbumin (OVA) and 7.5 mg/kg $Al(OH)_3$ in 0.5 mL. The sensitization was optimal between day 18 and day 24 after this treatment. Usually, the experiments were carried out on day 20 or 21. Animals were anesthetized by sodium thiopental (50 mg/kg, ip). For perfusion of the nasal cavity, a polyethylene tube was inserted retrogradly into the trachea in a way that its tip laid above the epiglottis, just inside of the inner opening of the choanes. A short tube was introduced orthogradly into the trachea to keep respiration patent. The rats were then placed in a supine position. Thus, the perfusion fluid, driven by a roller pump (Ismatec SA 8031, Switzerland) dripping from the nostrils, was collected by a fraction sampler (Circular II fraction collector, Germany). As a plasma marker, Evans blue (Sigma, Germany) was used and injected through a catheter placed in the jugular vein. After intitial perfusion with phosphate buffered saline (PBS) (0.5 mL/min) in order to remove nasal mucus, test compounds were administered either topically or systemically (po or ip). Antazoline and mepyramine were used as reference compounds. Test compounds for topical administration were dissolved in the PBS solution, and the nasal cavity was perfused for 30 min prior to challenge. The po and iv administration of **45** took place 60 and 30 min, respectively, before challenge. Evans blue was injected 15 min prior to allergic provocation. Following OVA-induced challenge, samples were collected for 60 min. The concentration of Evans blue was measured at 620 nm by a photometer (Digiscan 340, Austria) and calculated. The effect of compounds on the allergically induced increase in the nasal vascular permeability was compared to placebo-treated control animals and expressed in % inhibition.

Inhibition of Capsaicin-Induced Rhinitis in Anesthetized Rats. Male Sprague Dawley rats (280−320 g) obtained from Charles-River Wiga (Sulzfeld, Germany) were used. The experimental protocol was identical to that described previously. The only difference was that the increase in the nasal vascular permeability was induced by a PBS solution containing 0.5 mg/mL capsaicin (Sigma, Germany). Morphine was used as the reference compound.

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