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# Inhibition of FcεRI-Mediated Activation of Mast Cells by 2,3,4-Trihydropyrimidino[2,1-*a*]isoquinolines

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Assays based on reporter gene technology represent today an important tool in the pharmaceutical industry for discovering novel compound classes interfering with the activation and signaling of target cells after stimulation. Here we describe a reporter gene assay targeting mast cell activation by IgE plus antigen, established in an attempt to identify substances preventing type I allergy (allergic rhinitis, allergic conjunctivitis, allergic asthma, and acute and chronic urticaria). The assay is based on a murine mast cell line designated CPII, stimulation by IgE plus antigen, and a reporter gene construct with the TNFα promoter linked to luciferase as a read-out system. Via screening about 50 000 substances, compound **2** was found to inhibit the reporter gene induction in the submicromolar range in this assay. Analogues of compound **2** of the 2,3,4-trihydropyrimidino[2,1-*a*]isoquinoline type were synthesized starting from 2-alkyl-substituted benzonitriles via aminolysis with 1,3-diaminopropane, dimetalation of 2-substituted 2-phenyl-1,4,5,6-tetrahydropyrimidines with *n*- and *sec*-butyllithium, reaction with carboxylic acid methyl esters, and finally acidic dehydration. From about 50 derivatives, compound **41** was selected as a lead structure with an $IC_{50}$ of 0.2 μM and a $TC_{50}$ of 2.7 μM. In a first profiling in secondary assays, it effectively interfered with the production of mediators such as TNFα, IL-4, IL-6, IL-13, and leukotriene synthesis as measured by the corresponding ELISAs. In addition, a passive cutaneous anaphylaxis in mice (a typical type I reaction) is inhibited to more than 90% by compound **41**, when administered intradermally 90 min before challenge.

## Introduction

Triggering of mast cells via the FcεRI is the final step in a complex chain of events in atopic individuals, resulting in diseases such as allergic rhinitis, conjunctivitis, acute and chronic urticaria, and acute allergic asthma.[1] While antihistaminika of the first and second generation has proven to be effective to relieve most of the characteristic symptoms, the bronchoconstriction in asthma, the nasal blockage in rhinitis, and the axon reflex in the skin are unaffected by these drugs.[1] In addition, late-phase reactions resulting in an enhanced and sustained inflammation are not a direct consequence of histamine, strongly suggesting that other mediators are involved in the pathogenic picture observed in these patients. After FcεRI stimulation mast cells release a vast amount of leukotrienes and cytokines/chemokines into the tissue in a tightly and timely controlled fashion.[2−4] The cytokines and chemokines in particular serve as a clear link to the inflammatory process that is characteristic for these diseases and could also explain how chronic manifestations develop.

In an attempt to prevent mediator release from mast cells in general, two intervention strategies can be followed: First, inhibition of receptor cross-linking by preventing the binding of IgE and polyvalent allergen (or in the case of certain types of chronic urticaria of IgG autoantibodies) to the FcεRI—anti-IgE antibodies, currently in clinical trials, are an example of this type of approach;[5] second, inhibition of the signaling cascades initiating at the FcεRI after such binding—this approach relates to the success of cyclic undecapeptides and macrolides as specific inhibitors of the TCR/CD28 signaling in T-cells and their widespread use in the clinical practice of transplantation surgery.[6,7] To screen for compounds interfering with the signaling in mast cells, a reporter gene assay (RGA) in the murine mast cell line CPII was established.[4] It uses a stably integrated DNA construct of the human TNFα promoter linked to luciferase as a reporter gene and TNFα 3′-nontranslated sequences.[8,9] The validation of this read-out system in CPII cells in transient transfections was demonstrated recently.[9] Upon triggering this recombinant cell line with IgE plus antigen/allergen, a strong induction (10−30-fold) of luciferase activity can be detected which parallels the induction of the endogenous proinflammatory mediators (histamine, leukotrienes, and cytokines). In particular, it is an ideal surrogate parameter for measuring the endogenous TNFα production, a cytokine shown to contribute to the allergic late-phase inflammation in a murine in vivo model by applying neutralizing antibodies.[10]

More than 25 000 pure compounds and 22 000 broths were screened in this assay for their inhibitory potential. Here, we show that 2,3,4-trihydropyrimidino[2,1-*a*]-isoquinolines efficiently inhibit not only the RGA but also endogenous TNFα, IL-4, IL-6, and IL-13 production as well as the degranulation reaction (hexosaminidase release) and arachidonic acid metabolism (leukotrienes). The in vivo effect of this class of compounds is demonstrated in a passive cutaneous anaphylaxis in mice,

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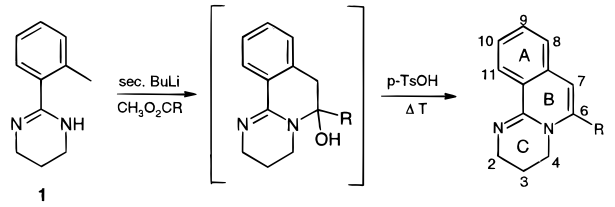
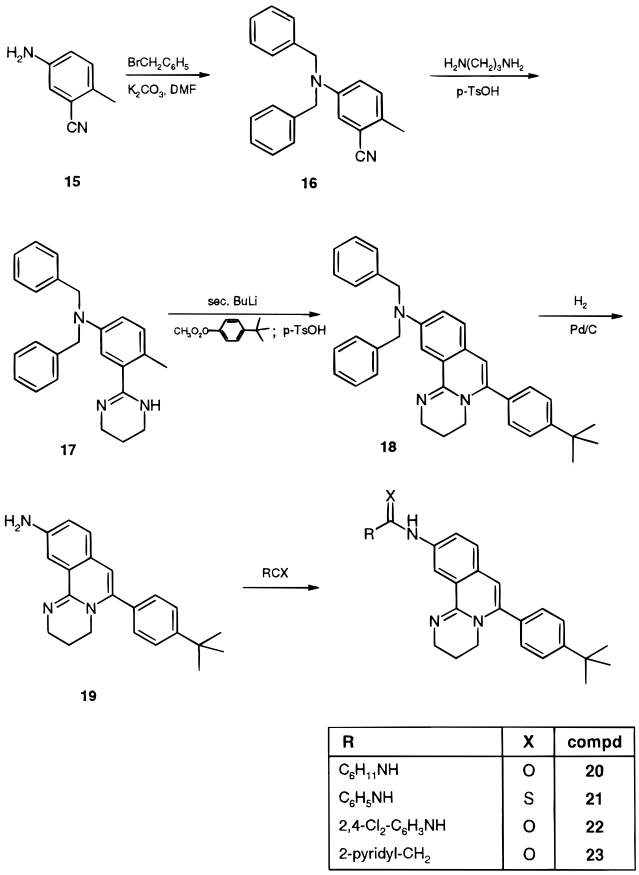
**Scheme 1**




**Scheme 2**




| R | X | compd |
|---|---|---|
| $C_6H_{11}NH$ | O | **20** |
| $C_6H_5NH$ | S | **21** |
| 2,4-$Cl_2$-$C_6H_3NH$ | O | **22** |
| 2-pyridyl-$CH_2$ | O | **23** |

where the parental compound (**2**) inhibits this experimental allergic reaction by 54% while the lead compound (**41**) prevents it by 92%.

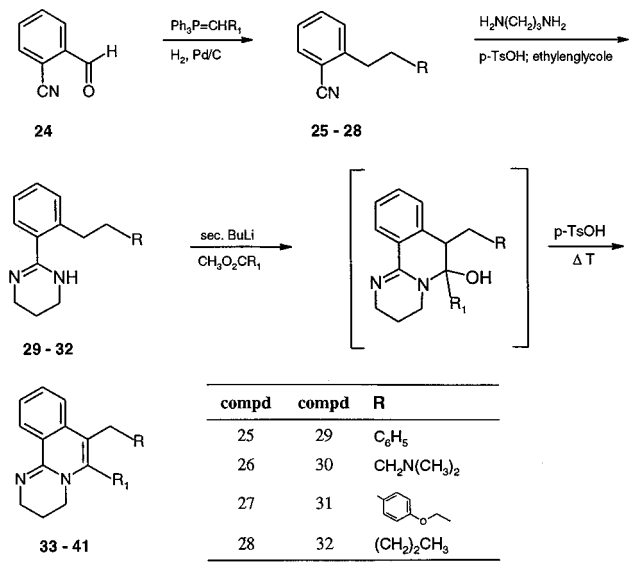
## Chemistry

2,3,4-Trihydropyrimidino[2,1-*a*]isoquinolines substituted in position 6 were synthesized as published[11] (Scheme 1), with the modification of using *sec*-butyllithium instead of *n*-butyllithium as the second basic equivalent. This improved the overall yield and gave consistent results. 10-Anilino derivatives were synthesized according to Scheme 2, starting from commercially available 4-amino-2-methylbenzonitrile. Compounds substituted in positions 6 and 7 were synthesized according to Scheme 3. All amidines and 2,3,4-trihydropyrimidino[2,1-*a*]isoquinolines were purified on silica gel as hydrochlorides due to superiority in terms of yield and purity compared to the free bases.

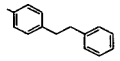
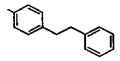
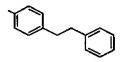
## Biology

The recently established mouse mast cell line CPII conveyed the starting point for the search of signaling inhibitors.[4] A genetically modified subline, designated

**Scheme 3**




| compd | compd | R |
|---|---|---|
| 25 | 29 | $C_6H_5$ |
| 26 | 30 | $CH_2N(CH_3)_2$ |
| 27 | 31 |  |
| 28 | 32 | $(CH_2)_2CH_3$ |

| compd | R | $R_1$ |
|---|---|---|
| 33 | $C_6H_5$ | H |
| 34 | $C_6H_5$ | 4-$(CH_3)_3SiC_6H_4$ |
| 35 | $C_6H_5$ | 4-$(CH_3)_3CC_6H_4$ |
| 36 | $CH_2N(CH_3)_2$ | 4-$(CH_3)_3CC_6H_4$ |
| 37 |  |  |
| 38 |  |  |
| 39 | $C_6H_5$ |  |
| 40 | $(CH_2)_2CH_3$ | 4-$(CH_3)_3CC_6H_4$ |
| 41 | $(CH_2)_2CH_3$ |  |

clone 12, was generated which harbors a TNFα promoter−luciferase−TNFα 3′ construct stably integrated into its genome. Upon triggering with IgE plus antigen (Ag), clone 12 cells do not only induce the pathophysiologically relevant mediators histamine, hexosaminidase, leukotriene C4 (LTC4), and a variety of cytokines/chemokines but also upregulate luciferase activity. The latter generates a cost-effective, nonradioactive, robust primary read-out system for activation of those cells by IgE plus Ag (Figure 1). FK506, a macrolide immunosuppressant, is able to prevent this activation effectively[9] and served as a positive standard in the initial screening. Organic solvents were tolerated without any inhibitory effect up to 1.0% for both DMSO and EtOH. High-throughput screening was done at a single dose of 10 μM for pure compounds and with 50 μg/well for broths and plant extracts. Compounds and broths were considered to be a "hit" if a more than 50% inhibition of induction was found and confirmed at this concentration where less than 20% toxicity was observed. A chemical derivatization program was started if in addition the initial "hits" showed some specificity by failing to inhibit two further RGA at a concentration of 10 μM (the human T-cell line Jurkat after anti-CD3/CD28 stimulation, read-out IL-2, the murine DC18 antigen-presenting cell line after IgG complex stimulation, read-

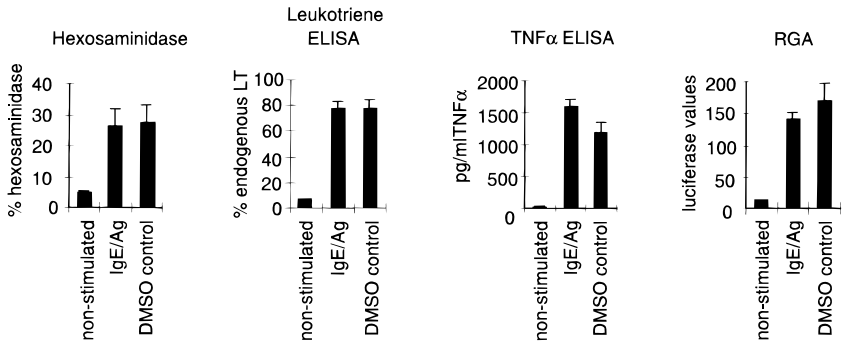



**Figure 1.** Comparison of the reporter gene read-out to pathophysiological parameters of CPII activation. Panels from left to right represent hexosaminidase release (% release in comparison to total hexosaminidase content), leukotriene release (% release in comparison to total leukotriene content), murine TNFα production (pg/mL), and TNFα reporter gene assay (luciferase values). All experiments were done in triplicate, and SD is indicated. Values for the nonstimulated, IgE-plus-antigen-stimulated (IgE/Ag), and solvent (DMSO control; at the highest concentration later used in the compound screening) treated CPII clone 12 cells are shown for all parameters.

out TNFα).[12,13] In total 25 368 pure compounds and 22 603 broths were tested with a hit rate of 0.08% and 0.04%, respectively. Pure compounds fall into eight chemical classes, broths belong to four natural product classes.

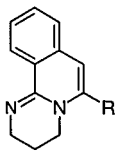
For guiding the chemical derivatization program, a hexosaminidase release assay and an extended cellular toxicity assay (over 3 days) were performed for all the derivatives in addition to the RGA. The best derivative ("lead") was evaluated further by measuring the inhibition of the production of various mast cell cytokines and leukotrienes in ELISAs. The final proof of efficacy for this compound class was done by a passive cutaneous anaphylaxis in mice.

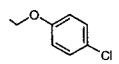
## Results

Compound **2** is one of the initial hits in this mast cell reporter gene assay that fulfilled the outlined criteria of inhibition, toxicity, and selectivity at the test concentration of 10 $\mu$M. Its $IC_{50}$ in the RGA assay was determined to be 0.8 $\mu$M, the $IC_{50}$ in the hexosaminidase release assay to be 0.5 $\mu$M, and the toxicity ($TC_{50}$) as measured by an XTT test over 3 days on clone 12 cells at 3.2 $\mu$M. On the basis of these data, a chemical derivatization program was started to improve the biological activity of this compound and in parallel reduce its toxic potential. As shown in Table 1 aliphatic chains (**3**, **4**) or phenyl rings substituted with polar groups instead of the trimethylsilyl group (**6**, **7**) abolished the inhibitory effect. Also, inserting a C,C double bond (**9**) or $CH_2O$ (**10**) that enlarges the distance of the aromatic substituent to ring B resulted in inactive compounds. Substitution of the trimethylsilyl group by a *tert*-butyl group led to a slightly less active and less toxic derivative (**8**). As a consequence we investigated the influence of lipophilic substituted aromatic rings in position 6 (Table 2). None of the three compounds in this series resulted in a significant improvement; however, activity was not lost by the modification. Compound **14** bearing a 2-phenylethyl substitution in the para position was active in the same range as compound **2**. This determined the importance of the substituent for the inhibitory effect with respect to distance, ring structure, and lipophilicity.

We next investigated the contribution of substitutions of ring A (Table 3) to the activity of this compound class.

**Table 1.** Influence of Substituents in Position 6 on the Inhibition Potency in the TNFα Reporter Gene, Cell Toxicity, and Hexosaminidase Release Assays

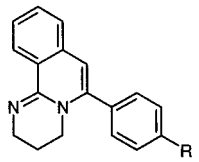



| Compd | R | $IC_{50}$ - CPII [a] μM | $TC_{50}$ - CPII Tox [b] μM | $IC_{50}$ - Hexo [c] μM |
|---|---|---|---|---|
| 2 | 4-$(CH_3)_3SiC_6H_4$ | 0.8 | 3.2 | 0.5 |
| 3 | $CH_3$ | > 10 | > 10 | >25 |
| 4 | n-$(CH_2)_5CH_3$ | > 10 | > 10 | 4.5 |
| 5 | $C_6H_5$ | > 10 | > 10 | 12 |
| 6 | 3,4,5-$(CH_3O)_3C_6H_2$ | > 10 | > 10 | > 25 |
| 7 | 4-$ClC_6H_4$ | > 10 | > 10 | 5.5 |
| 8 | 4-$(CH_3)_3CC_6H_4$ | 0.9 | 5.7 | 0.7 |
| 9 |  | > 10 | > 10 | 4.1 |
| 10 |  | 9 | > 10 | > 25 |
| 11 |  | > 10 | > 25 | > 25 |

[a] CPII mouse mast cells stable transfected by a TNFα promoter-driven luciferase reporter construct were incubated for 1 h with the substance and then triggered by a (dinitrophenyl)albumin/anti-dinitrophenyl-IgE complex; incubation time: 6 h. [b] CPII cells were incubated for 3 days with substance in growth medium (RPMI 1640 + 10% FCS). Cell growth is measured by formazane formation (XTT staining). [c] CPII mouse mast cells were treated overnight with substance and anti-dinitrophenyl-IgE. Degranulation was then induced by the addition of (dinitrophenyl)albumin for 2 h and quantified by colorimetric measurement of released hexosaminidase.

No improvement was seen within a series of six derivatives, with compound **18** (the best out of this series) being in the same range as compound **2**. This indicates the importance of a certain overall lipophilicity of the molecule, a fact often observed in a cell-based assay (see Discussion). We therefore decided to study the influence of simultaneous substitutions in positions 6 and 7 (Table 4). Compound **33**, which is unsubstituted in position 6 and has a benzyl substitution in position 7 was inactive. If compound **2** is benzyl-substituted in position 7 (**34**), reduced inhibition of hexosaminidase release is observed. Exchange of the trimethylsilyl group to a *tert*-

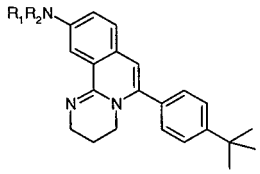
**Table 2.** Influence of Substituted Aromatic Rings in Position 6 on the Inhibition Potency in the TNFα Reporter Gene, Cell Toxicity, and Hexosaminidase Release Assays



| Compd | R | $IC_{50}$ - CPII [a] μM | $TC_{50}$ - CPII Tox [b] μM | $IC_{50}$ - Hexo [c] μM |
|---|---|---|---|---|
| 2 | $(CH_3)_3Si$ | 0.8 | 3.8 | 0.5 |
| 12 | $C_6H_5$ | 1.5 | 3.9 | 1.7 |
| 13 | $OCH_2C_6H_5$ | 0.8 | 3.5 | 0.9 |
| 14 | $CH_2CH_2C_6H_5$ | 0.7 | 2.1 | 0.5 |

[a–c] See corresponding footnotes in Table 1.

**Table 3.** Influence of Anilides in Position 2 on the Inhibition Potency in the TNFα Reporter Gene, Cell Toxicity, and Hexosaminidase Release Assays



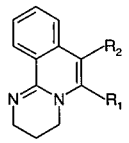
| Compd | $R_1$ | $R_2$ | $IC_{50}$ - CPII [a] μM | $TC_{50}$ - CPII Tox [b] μM | $IC_{50}$ - Hexo [c] μM |
|---|---|---|---|---|---|
| 18 | $C_6H_5CH_2$ | $C_6H_5CH_2$ | 0.6 | 2.6 | 0.6 |
| 19 | H | H | 1.7 | 7.8 | 3 |
| 20 | $C_6H_{11}NHCO$ | H | 1.7 | 4.8 | 2.1 |
| 21 | $C_6H_{11}NHCS$ | H | 2.6 | 9.9 | 3.3 |
| 22 | $2,4\text{-}Cl_2C_6H_3NHCO$ | H | 3.3 | 5.8 | 17 |
| 23 |  | H | 4.4 | 8.8 | 4.8 |

[a–c] See corresponding footnotes in Table 1.

butyl group (**35**) led to reduced activity, although this exchange from C to Si had nearly no influence on the activity in earlier cases (e.g., **2**, **8**). Polar substitution in position 7 (**36**) again abolished the activity. 4-Ethoxybenzyl substitution in position 7 reduced the inhibition of hexosaminidase release to some extent (**37**, **38**), whereas a 4-(2-phenylethyl)phenyl substituent in position 6 and in addition a benzyl group in position 7 (**39**) was again as active as **34** but showed enhanced cellular toxicity. An *n*-butyl group in position 7 in combination with the 4-*tert*-butylphenyl group in position 6 (**40**) showed clearly improved activity compared to compound **34**. Finally, switching to the 4-(2-phenylethyl)phenyl side chain in position 6 in addition to the *n*-butyl group in position 7 (**41**) enhanced the activity compared to compound **2** by a factor of 4, with a cell toxicity in the same range as that of compound **2**.

We therefore studied the biological activity of compound **41** in more detail. The compound did not inhibit the IgE interaction with the FcεRI, as measured in a cell-free ELISA system (data not shown). A dose-dependent inhibition, however, at slightly higher concentrations as in the RGA was observed for the cytokines TNFα, IL-4, IL-6, and IL-13 (Figure 2). This difference in effective concentration can be explained in part by a certain amount of preformed mediators in mast cells. The differing $IC_{50}$ values for inhibition of the various cytokines (from 0.25 to 0.45 μM) parallel

**Table 4.** Influence of Substituents in Positions 5 and 6 on the Inhibition Potency in the TNFα Reporter Gene, Cell Toxicity, and Hexosaminidase Release Assays



| Compd | $R_1$ | $R_2$ | $IC_{50}$ - CPII [a] μM | $TC_{50}$ - CPII Tox [b] μM | $IC_{50}$ - Hexo [c] μM |
|---|---|---|---|---|---|
| 2 | $4\text{-}(CH_3)_3SiC_6H_4$ | H | 0.8 | 3.2 | 0.5 |
| 33 | H | $CH_2C_6H_5$ | > 10 | > 10 | > 10 |
| 34 | $4\text{-}(CH_3)_3SiC_6H_4$ | $CH_2C_6H_5$ | 0.8 | 5.5 | 1.2 |
| 35 | $4\text{-}(CH_3)_3CC_6H_4$ | $CH_2C_6H_5$ | 2 | 5 | 2.9 |
| 36 | $4\text{-}(CH_3)_3CC_6H_4$ | $n\text{-}(CH_2)_2N(CH_3)_2$ | > 10 | > 10 | > 10 |
| 37 |  |  | 1.5 | 5.5 | 3.5 |
| 38 |  |  | 0.9 | 9 | 2.6 |
| 39 |  | $CH_2C_6H_5$ | 0.6 | 2.2 | 0.9 |
| 40 | $4\text{-}(CH_3)_3CC_6H_4$ | $n\text{-}(CH_2)_3CH_3$ | 0.3 | 2.1 | 0.6 |
| 41 |  | $n\text{-}(CH_2)_3CH_3$ | 0.2 | 2.7 | 0.3 |

[a–c] See corresponding footnotes in Table 1.

recent findings of the effect of FK506 on these mediators in mast cells. Here proinflammatory cytokines (like TNFα) are inhibited at 10 times lower concentrations than growth factors (like IL-13).[14] Also the production of leukotrienes was inhibited in the same range, while the phosphorylation (activation) of the MAP kinase erk 1/2, a key kinase in this pathway, was unaffected (Figure 3) (see Discussion). All these findings characterize compound **41** as being of interest for interfering with these important mediators in allergic diseases.

To finally prove the efficacy of this compound class in type I allergic reactions, a passive cutaneous anaphylaxis in mice was performed. This (exclusively) IgE-driven in vivo skin reaction was done to exclude the possibility that substituted isoquinolines are specific inhibitors of our selected screening system (CPII and clone 12 mast cells). Both the parental compound (**2**) as well as the selected lead compound (**41**) were inhibiting the extravasation, if applied intradermally 90 min before an allergic challenge (Figure 4). The observed inhibition ranged from 54% (for **2**) to 92% (for **41**), further demonstrating the improved activity of our lead compound. The direct effect of the compounds on mast cell activation is indicated by their inability to inhibit an extravasation provoked by the administration of histamine. It shows that substituted isoquinolines comprise novel and suitable potential drugs for the treatment of type I allergic diseases.

## Discussion

Signaling/activation inhibitors at various levels have provided valuable pharmaca for the treatment of immunological disorders and include such diverse drugs as FK506 and glucocorticoids.[1] Depending on their target specificity, they are either broadly antiinflammatory (glucocorticoids) or more or less restricted to T-cell-mediated phenomena (FK506). Mast cell-specific signaling inhibitors which would prevent all symptoms related to type I allergy in the indications allergic

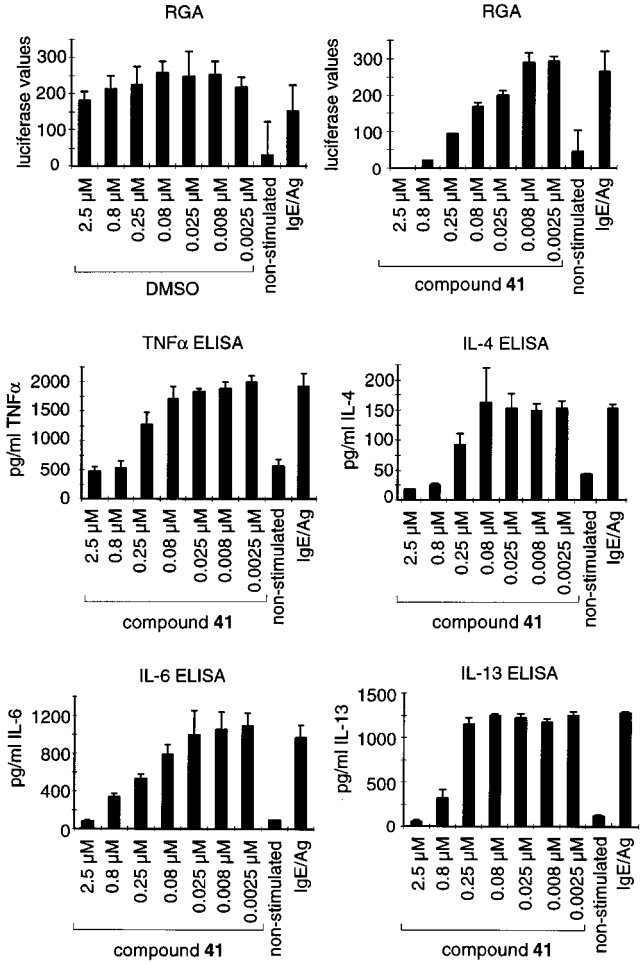



**Figure 2.** Inhibition of mast cell cytokine release by compound **41**. Dose titration of the solvent (DMSO) and compound **41** on the reporter gene assay is shown in the top left and right panels. Middle and bottom panels show dose titration of compound **41** on the inhibition of TNFα ($IC_{50} = 0.3\ \mu M$), IL-4 ($IC_{50} = 0.25\ \mu M$), IL-6 ($IC_{50} = 0.25\ \mu M$), and IL-13 ($IC_{50} = 0.45\ \mu M$) in CPII clone 12 cells. Release is shown on the *y*-axis in pg; concentration of compound and stimulation conditions are given on the *x*-axis. All experiments were done in triplicate, and SD is indicated.

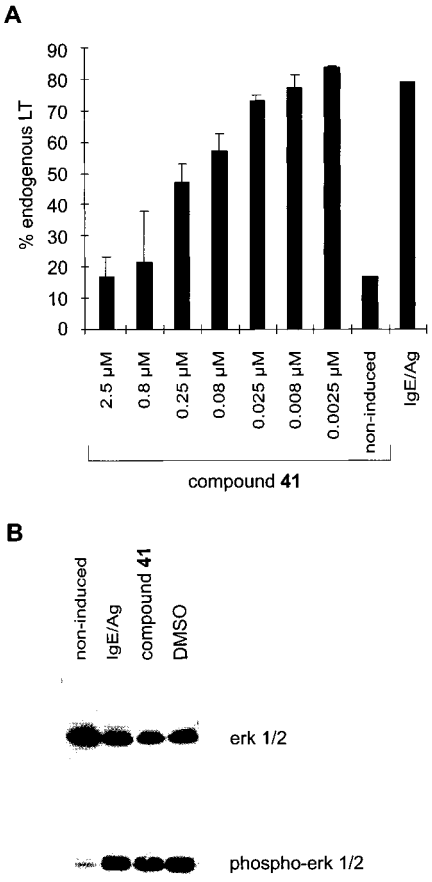



**Figure 3.** Leukotriene release, but not erk 1/2 activation, affected by compound **41**. (A) Dose titration of compound **41** on leukotriene synthesis. Release is shown on the *y*-axis in % endogenous LT; concentration of compound and stimulation conditions are given on the *x*-axis. All experiments were done in triplicate, and SD is indicated. (B) Western blot detecting erk 1/2 and phospho-erk 1/2 after 15 min of activation of CPII clone 12 cells in untreated, stimulated, and stimulated and compound **41**-treated cells. Stimulations, inhibitor (compound **41**), and solvent are indicated on the *x*-axis.

rhinitis, allergic conjunctivitis, allergic asthma, and acute and chronic urticaria still need to be developed. Such potential drugs require a relatively high specificity due to the fact that allergic diseases are highly uncomfortable and disturbing but usually not life-threatening (fatal asthma and anaphylactic reactions excluded). Therefore, besides toxicity, effects of compounds especially on T-cells and other antigen-presenting cells must be excluded to prevent general immunosuppression in those patients. This was the basis for choosing an IL-2 RGA in the Jurkat T-cell line and a TNFα RGA in the antigen-presenting DC18 cell line as initial counterscreening assays for specificity.[12,13]

Several signaling pathways in mast cells were recently revealed; however, their interplay and cross-talk still need to be elucidated. PI-3 kinase catalytic activity was shown to be important for the degranulation reaction (hexosaminidase release) and LT synthesis but is dispensable for cytokine/chemokine induction.[15] On the contrary, the MAP kinase pathway (erk 1/2 serine/threonine kinase) and the phosphatase calcineurin (the target for FK506, the positive standard control in the high-throughput screening) were linked to the transcriptional activation of cytokines/chemokines.[16,17] All these late-signaling molecules are triggered by common, mostly tyrosine-based phosphorylation events initiated at the $\beta$ and $\gamma$ chain of the tetrameric Fc$\epsilon$RI after cross-linking.[18−20] This primarily includes the lyn and syk kinases, a number of adaptor proteins such as Shc, SOS, and Grb2, and $Ca^{2+}$ influx.[21−26] The inhibitory effect of 2,3,4-trihydropyrimidino[2,1-*a*]isoquinolines on all pathophysiological mediators would strongly argue for an interference with one of these central steps relatively proximal to the receptor, thereby inhibiting all further downstream pathways. From the structure it is not unlikely that the strongly basic 2,3,4-trihydropyrimidino[2,1-*a*]isoquinolines interfere by forming salt bridges to the phosphorus acids, thereby preventing the binding of the important SH2 domains in the tyrosine kinases and adaptor proteins. Interference with the inositol triphosphate-mediated $Ca^{2+}$ release from internal stores mechanistically based on the same salt bridge-building ability is also plausible.

The finding that erk 1/2 phosphorylation (activation) is not inhibited by this compound is interesting and also allows a different interpretation. The erk 1/2 MAP kinase directly activates PLC$\gamma$1, the enzyme releasing

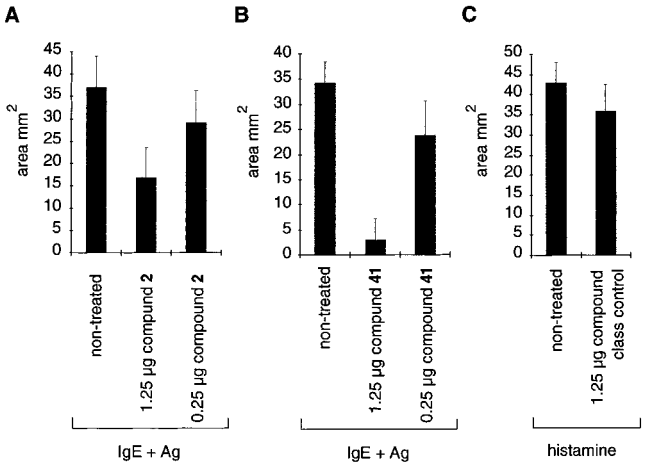



**Figure 4.** Passive cutaneous anaphylaxis (PCA) is inhibited by 2,3,4-trihydropyrimidino[2,1-*a*]isoquinolines: (A) dose-dependent inhibition of an IgE-plus-antigen-provoked PCA by compound **2**, (B) dose-dependent inhibition of an IgE-plus-antigen-provoked PCA by compound **41**, (C) control reaction that substituted isoquinolines do not inhibit a PCA provoked by histamine. The score of the PCA in $mm^2$ of dye extravasation is given on the *y*-axis; stimulations, compounds, and concentrations are given on the *x*-axis, and SD is indicated.

arachidonic acid from the membrane as the first step in the leukotriene pathway. That leukotriene production is inhibited but not this kinase suggests that further signaling pathways are required for the overall induction/activation. We have recently described such an additional signaling pathway in CPII cells which is blocked by inhibitors of atypical PKCs, primarily PKC$\mu$.[17] This pathway is required in addition to the MAPK pathway and $Ca^{2+}$ influx for the activation of all so far tested mast cell functions and seems to be equally important as the other two for the overall activation. In this respect it is interesting that PKC$\mu$ is membrane-bound and dependent on lipids for its activity.[27] This could be an explanation for the requirement of a certain amount of lipophilicity of the inhibitors. Currently in vitro kinase assays after immunoprecipitation of PKC$\mu$ are being performed to address this question.

## Experimental Section

**Chemistry: General.** $^1$H NMR spectra were recorded with a Bruker WC-250 or AMX-500 spectrometer; chemical shifts are reported in ppm ($\delta$) relative to internal $Me_4Si$. Elemental analyses were performed by the Analytical Department, Novartis, Basel, Switzerland, and are within ±0.4% of the theoretical values. Analytical thin-layer chromatography was performed on silica gel 60 $F_{254}$ glass plates (HPTLC, Merck). Preparative column chromatography was performed on silica gel (40−63 $\mu$m) under pressure (≈0.2 mPa). Solvents were AR grade and were used without further purification. All reagents were obtained from commercial suppliers and were used without further purification. Evaporations were carried out in vacuo with a rotary evaporator. Melting points were determined with a thermovar apparatus (Reichert-Jung) and are not corrected.

**General Procedures: A. Ring Closure of 2-(2-Methylphenyl)-4,5-dihydro-6*H*-pyrimidine to 2,3,4-Trihydropyrimidino[2,1-*a*]isoquinolines.** (Slightly modified procedure of ref 11; 2,3-dihydroimidazo[2,1-*a*]isoquinolines have been synthesized analogously[28].) All yields relate to purified products (via chromatography), in general they are between 22 and 50%. The exceptions, **12** (9%) and **40** (7%), are due to purification problems. **1** (840 mg, 4 mmol) (2-(2-methylphenyl)-4,5-dihydro-6*H*-pyrimidine hydrochloride),[11] carefully predried over $P_2O_5$ (this is essential to obtain good yields), was dissolved in 30 mL of absolute tetrahydrofuran (THF), and 1.31 mL (8.8 mmol) of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, cooled to −10 °C and lithiated[29,30] with 6.6 mL (8.6 mmol) of a 1.3 M solution of *n*-butyllithium in hexane, was added followed by addition of 3.38 mL (4.4 mmol) of a 1.3 M solution of *sec*-butyllithium in hexane. The reaction mixture was kept for 6 h at −50 °C; then 4 mmol of the chosen methyl ester dissolved in 3 mL of absolute THF was added. The whole procedure was run under the protecting atmosphere of argon. The reaction mixture was allowed to warm to room temperature overnight and then was poured into 100 mL of saturated ammonium chloride solution, and the aqueous solution was extracted with methylene chloride. The organic phase was dried and evaporated, and the residue was dissolved in 30 mL of toluene; 270 mg of *p*-toluenesulfonic acid was added, and the solution was refluxed for 3 h. Then toluene was evaporated in vacuo, the residue dissolved in methylene chloride, and the resulting solution extracted with a mixture of NaOH (20%) and saturated NaCl solution. The organic phase was dried and the solvent evaporated in vacuo. Adding methanolic HCl to the residue, evaporation of excess solvent, and chromatography of the resulting hydrochloride with silica gel (methylene chloride/methanol) gave the product.

**B. Synthesis of Starting Materials for Compounds Carrying a Substituent in Position 7 (Wittig Reaction and Hydrogenation).** 2-Cyanobenzaldehyde **24** (3.57 g, 27.2 mmol) was added at 0 °C in 10 mL of absolute THF to a solution of the Wittig ylid, prepared from 42 mmol of a triphenylphosphonium bromide, predried over $P_2O_5$, and 40 mmol of potassium *tert*-butylate in THF. The solution was stirred for 30 min at room temperature, then diluted with methylene chloride, and washed with 1 M HCl. The organic layer was separated, dried, and evaporated to dryness. Chromatography of the residue over silica gel (toluene/ethyl acetate) gave the product in varying amounts of cis/trans isomers. This mixture was dissolved in 250 mL of ethanol (in case of poor solubility methylene chloride was added) and, after addition of 1.4 g of Pd (5%) on charcoal, was hydrogenated at room temperature and atmospheric pressure. After filtration and evaporation of the solvent, the residue was chromatographed on silica gel or used crude for the synthesis of the 2-(2-substituted phenyl)-4,5-dihydro-6*H*-pyrimidines as described in ref 11. Ring closures to 2,3,4-trihydropyrimidino[2,1-*a*]-isoquinolines were done as described above.

**6-[4-(Trimethylsilyl)phenyl]-3,4-dihydro-2*H*-pyrimido-[2,1-*a*]isoquinoline Hydrochloride (2·HCl).** Procedure A, **1**, and 4-(trimethylsilyl)benzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3$-OH: 8/1), yield 50% of **2·HCl** (amorphous): $^1$H NMR ($CDCl_3$) $\delta$ 0.60 (s, 9H, $(CH_3)_3$), 2.17 (quin, 2H, $CH_2$), 4.01 (t, 2H, $NCH_2$), 4.18 (t, 2H, $NCH_2$), 6.79 (s, 1H, =CH), 7.43−7.51 (m, 3H, ArH), 7.60−7.75 (m, 4H, ArH), 9.38 (dd, 1H, ArH), 11.78 (bs, 1H, $H^+$); MS 333 ($MH^+$). Anal. ($C_{21}H_{24}N_2Si$·HCl) C, H, N.

**6-Methyl-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (3·HCl).** Procedure A, **1**, and acetic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 8/1), yield 41% of **3·HCl**: mp 210 °C subl; $^1$H NMR ($CDCl_3$/$CD_3OD$) $\delta$ 2.24 (quin, 2H, $CH_2$), 2.60 (s, 3H, $CH_3$), 3.74 (t, 2H, $NCH_2$), 4.39 (t, 2H, $NCH_2$), 7.00 (s, 1H, =CH), 7.63−7.72 (m, 2H, ArH), 7.82−7.90 (m, 1H, ArH), 8.40 (d, 1H, ArH); MS 199.2 ($MH^+$). Anal. ($C_{13}H_{14}N_2$·HCl) C, H, N.

**6-Hexyl-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (4·HCl).** Procedure A, **1**, and hexoic acid methyl ester were used; product crystallized from toluene, yield 35% of **4·HCl** (amorphous, hygroscopic): $^1$H NMR ($CDCl_3$) $\delta$ 0.96 (t, 3H, $CH_3$), 1.20−1.58 (m, 6H, $(CH_2)_3$), 1.62−1.77 (m, 2H, $CH_2$), 2.25 (quin, 2H, $CH_2$), 2.76 (t, 2H, $CH_2$), 3.85 (t, 2H, $NCH_2$), 4.22 (t, 2H, $NCH_2$), 6.65 (s, 1H, =CH), 7.45 (d, 1H, ArH), 7.60−7.79 (m, 2H, ArH), 9.25 (d, 1H, ArH), 11.42 (bs, 1H, $H^+$); MS 269.0 ($MH^+$). Anal. ($C_{18}H_{24}N_2$·HCl) C, H, N.

**6-Phenyl-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (5·HCl).** Procedure A, **1**, and benzoic

acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 8/1), yield 40% of **5·HCl**: mp 200 °C subl; $^1H$ NMR ($CDCl_3$) $\delta$ 2.10 (quin, 2H, $CH_2$), 3.96 (t, 2H, $NCH_2$), 4.05 (t, 2H, $NCH_2$), 6.77 (s, 1H, =$CH_2$), 7.10−7.18 (m, 2H, ArH), 27.22 (t, 1H, ArH), 7.42−7.44 (m, 2H, ArH), 7.50−7.55 (m, 1H, ArH), 7.60 (dt, 1H, ArH), 7.73 (dt, 1H, ArH), 8.95 (d, 1H, ArH), 11.42 (bs, 1H, $H^+$); MS 261.2 ($MH^+$). Anal. ($C_{18}H_{16}N_2$·HCl·0.6$H_2O$) C, H, N.

**6-(3,4,5-Trimethoxyphenyl)-3,4-dihydro-2*H*-pyrimido-[2,1-*a*]isoquinoline Hydrochloride (6·HCl).** Procedure A, **1**, and 3,4,5-trimethoxybenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3$-OH: 8/1), yield 35% of **6·HCl**: mp 225−230 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 2.12 (quin, 2H, $CH_2$), 3.86−4.07 (m, 11H, 3 $OCH_3$, $NCH_2$), 4.21 (t, 2H, $NCH_2$), 6.74 (s, 1H, =$CH_2$), 6.85 (s, 2H, ArH), 7.42−7.55 (m, 2H, ArH), 7.63 (t, 1H, ArH), 9.16 (d, 1H, ArH), 11,43 (bs, 1H, $H^+$); MS 351 ($MH^+$). Anal. ($C_{21}H_{22}N_2O_3$·-HCl) C, H, N.

**6-(4-Chlorophenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]iso-quinoline Hydrochloride (7·HCl).** Procedure A, **1**, and 4-chlorobenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 7/1), yield 26% of **7·HCl** (amorphous): $^1H$ NMR ($CDCl_3$) $\delta$ 2.20 (quin, 2H, $CH_2$), 3.90 (t, 2H, $NCH_2$), 4.03 (t, 2H, $NCH_2$), 6.82 (s, 1H, =CH), 7.40−7.51 (ABX, 4H, ArH), 7.58 (dd, 1H, ArH), 7.78−7.82 (m, 2H, ArH), 9.00 (dd, 1H, ArH), 11,40 (bs, 1H, $H^+$); MS 294 ($MH^+$). Anal. ($C_{18}H_{15}N_2Cl$·HCl) C, H, N.

**6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]-isoquinoline Hydrochloride (8·HCl).** Procedure A, **1**, and 4-*tert*-butylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 8/1), yield 45% of **8·HCl**: mp 242−250 °C (amorphous); $^1H$ NMR ($CDCl_3$) $\delta$ 1.41 (s, 9H, $(CH_3)_3$), 2.17 (quin, 2H, $CH_2$), 4.01 (t, 2H, $NCH_2$), 4.18 (t, 2H, $NCH_2$), 6.78 (s, 1H, =CH), 7.42−7.79 (m, 7H, ArH), 9.38 (dd, 1H, ArH), 11.73 (bs, 1H, $H^+$); MS 317.2 ($MH^+$). Anal. ($C_{22}H_{24}N_2$·HCl·0.8$H_2O$) C, H, N.

**6-Styryl-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (9·HCl).** Procedure A, **1**, and cinnamic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 8/1), yield 36% of **9·HCl**: mp 231−240 °C (amorphous); $^1H$ NMR ($CDCl_3/CD_3OD$) $\delta$ 2.33 (quin, 2H, $CH_2$), 3.78 (t, 2H, $NCH_2$), 4.44 (t, 2H, $NCH_2$), 7.23 (dd, 1H, =CH), 7.28 (d, 1H, =CH), 7.32 (s, 1H, =CH), 7.38−7.47 (m, 4H, ArH), 7.63 (td, 2H, ArH), 7.74 (dt, 1H, ArH), 7.84 (d, 1H, ArH), 7.91 (dt, 1H, ArH), 8.41 (td, 1H, ArH); MS 287.2 ($MH^+$). Anal. ($C_{20}H_{18}N_2$·HCl) C, H, N.

**6-[(4-Chlorophenoxy)methyl]-3,4-dihydro-2*H*-pyrimido-[2,1-*a*]isoquinoline Hydrochloride (10·HCl).** Procedure A, **1**, and (4-chlorophenoxy)acetic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3$-OH: 8/1), yield 23% of **10·HCl**: mp 182−190 °C; $^1H$ NMR ($CDCl_3/CD_3OD$) $\delta$ 2.23 (quin, 2H, $CH_2$), 3.80 (t, 2H, $NCH_2$), 4.40 (t, 2H, $NCH_2$), 5.18 (s, 2H, $OCH_2$), 6.92 (td, 2H, ArH), 7.08 (s, 1H, =CH), 7.26 (td, 2H, ArH), 7.58−7.82 (m, 3H, ArH), 8.87 (d, 1H, ArH); MS 325.1 ($MH^+$). Anal. ($C_{19}H_{17}N_2OCl$·-HCl) C, H, N.

**1-(3,4-Dihydro-2*H*-pyrimido[2,1-*a*]isoquinolin-6-yl)-2-phenylethylamine Dihydrochloride (11·2HCl).** Procedure A, **1**, and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine methyl ester were used (reaction leads to cleavage of the BOC protecting group); the product was chromatographed on silica gel ($CH_2$-$Cl_2/CH_3OH$: 4/1), yield 22% of **11·2HCl**: mp 138−145 °C; $[\alpha]^{25}_D$ −0.7° ($c$ = 1, $C_2H_5OH$); $^1H$ NMR ($CDCl_3/CD_3OD$) $\delta$ 2.01−2.25 (m, 2H, $CH_2$), 3.06−3.21 (m, 2H, $CH_2$), 3.49−3.78 (m, 2H, $NCH_2$), 4.01−4.18 (m, 1H, $CHNH_2$), 4.46−4.62 (m, 2H, $NCH_2$), 7.20−7.28 (m, 5H, ArH), 7.40 (s, 1H, =CH), 7.74 (dt, 1H, ArH), 7.84−7.95 (m, 2H, ArH), 8.32 (dd, 1H, ArH); MS 304 ($MH^+$). Anal. ($C_{20}H_{21}N_3$·1.4HCl·2$H_2O$) C, H, N.

**6-Biphenylyl-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquino-line Hydrochloride (12·HCl).** Procedure A, **1**, and 4-phenylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 7/1), yield 9% of **12·HCl**: mp 272−280 °C ($CH_2Cl_2$/ethyl acetate); $^1H$ NMR ($CDCl_3$) $\delta$ 2.19 (quin, 2H, $CH_2$), 4.01 (t, 2H, $NCH_2$), 4.22 (t, 2H, $NCH_2$), 6.82 (s, 1H, =CH), 7.42−7.95 (m, 12H, ArH), 9.38 (dd, 1H, ArH), 11.69 (bs, 1H, $H^+$); MS 337.2 ($MH^+$). Anal. ($C_{24}H_{20}N_2$·HCl) C, H, N.

**6-[4-(Benzyloxy)phenyl]-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (13·HCl).** Procedure A, **1**, and 4-(benzyloxy)benzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 9/1), yield 29% of **13·HCl**: mp 252−260 °C (amorphous); $^1H$ NMR ($CDCl_3$) $\delta$ 2.10 (quin, 2H, $CH_2$), 3.96 (t, 2H, $NCH_2$), 4.12 (t, 2H, $NCH_2$), 5.18 (s, 2H, $OCH_2$), 6.76 (s, 1H, =CH), 7.18 (td, 2H, ArH), 7.38−7.76 (m, 10H, ArH), 9.32 (dd, 1H, ArH), 11.55 (bs, 1H, $H^+$); MS 366.0 ($MH^+$). Anal. ($C_{25}H_{22}N_2O$·HCl) C, H, N.

**6-(4-Phenethylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]-isoquinoline Hydrochloride (14·HCl).** Procedure A, **1**, and 4-phenethylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 8/1), yield 49% of **14·HCl**: mp 200 °C subl (amorphous); $^1H$ NMR ($CDCl_3$) $\delta$ 2.17 (quin, 2H, $CH_2$), 3.01 (s, 4H, $CH_2CH_2$), 3.96 (t, 2H, $NCH_2$), 4.08 (t, 2H, $NCH_2$), 6.78 (s, 1H, =CH), 7.20−7.59 (m, 10H, ArH), 7.62−7.79 (m, 2H, ArH), 9.38 (dd, 1H, ArH), 11.55 (bs, 1H, $H^+$); MS 364.0 ($M^+$). Anal. ($C_{26}H_{24}N_2$·HCl) C, H, N.

**5-(Dibenzylamino)-2-methylbenzonitrile (16). 15** (396 mg, 3 mmol) (5-amino-2-methylbenzonitrile) was dissolved in 3 mL of dimethylformamide, and 1.07 mL (9 mmol) of benzyl bromide and 1.243 g (9 mmol) of $K_2CO_3$ were added at room temperature. After the suspension stirred for 5 h at room temperature, 552 mg (4 mmol) of $K_2CO_3$ and 357 $\mu$L (3 mmol) of benzyl bromide were added in addition. After 24 h at room temperature, the mixture was poured into water and extracted with methylene chloride, the organic phase was washed twice with water and dried, the solvent was evaporated under reduced pressure, and the residue was chromatographed over silica gel (toluene/ethyl acetate: 98/2), yield 899 mg (96%) of **16**, clear syrup: $^1H$ NMR ($CDCl_3$) $\delta$ 2.40 (s, 3H, $CH_3$), 4.61 (s, 6H, $N(CH_2)_2$), 6.81 (dd, 1H, ArH), 6.90−7.08 (ABX, 2H, ArH), 7.12−7.40 (m, 10H, ArH); MS 313.2 ($MH^+$). Anal. ($C_{22}H_{20}N_2$) C, H, N.

**Dibenzyl[4-methyl-3-(1,4,5,6-tetrahydropyrimidin-2-yl)phenyl]amine Hydrochloride (17·HCl). 16** (876 mg, 2.8 mmol) was dissolved in 5 mL of ethylene glycol and 280 $\mu$L (3.36 mmol) of 1,3-diaminopropane; 319 mg (1.68 mmol) of *p*-toluenesulfonic acid was added, the solution was kept for 4 days at 200 °C, and then after cooling the reaction mixture was diluted with $CH_2Cl_2$ and extracted with a solution of NaOH (20%) and saturated sodium chloride. The organic phase was separated, dried, evaporated, and transformed to the hydrochloride with methanolic HCl. The residue was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 7/1), yield 363 mg (35%) of **17·HCl**: mp 215−222 °C (acetic acid isopropyl ester); $^1H$ NMR ($CDCl_3$) $\delta$ 1.70 (quin, 2H, $CH_2$), 2.22 (s, 3H, $CH_3$), 3.34 (t, 4H, $(NCH_2)_2$), 4.59 (s, 4H, $(CH_2)_2$), 6.63 (dd, 1H, ArH), 6.82−6.90 (m, 2H, ArH), 7.16−7.38 (m, 10H, ArH), 11.55 (bs, 1H, $H^+$); MS 370.0 ($M^+$). Anal. ($C_{25}H_{27}N_2$·HCl) C, H, N.

**Dibenzyl[6-(4-*tert*-butylphenyl)-3,4-dihydro-2*H*-pyrim-ido[2,1-*a*]isoquinolin-10-yl]amine Hydrochloride (18·HCl).** Procedure A, **17·HCl**, and 4-*tert*-butylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 9/1), yield 56% of **18·HCl**, amorphous: $^1H$ NMR ($CDCl_3$) $\delta$ 1.38 (s, 9H, $C(CH_3)_3$), 2.04 (quin, 2H, $CH_2$), 3.81 (t, 2H, $NCH_2$), 3.89 (t, 2H, $NCH_2$), 5.00 (s, 4H, $N(CH_2)_2$), 6.64 (s, 1H, =CH), 7.08−7.76 (m, 16H, ArH), 8.37 (d, 1H), 11.24 (bs, 1H, $H^+$); MS 512 ($MH^+$). Anal. ($C_{36}H_{37}N_3$·HCl) C, H, N.

**[6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]-isoquinolin-10-yl]amine Hydrochloride (19·HCl). 18·HCl** (110 mg, 0.2 mmol) was dissolved in 10 mL of ethanol and acidified with ethanol/HCl to pH 3; 50 mg of Pd (5%) on charcoal was added and the reaction mixture hydrogenated at room temperature and atmospheric pressure for 3 h. After filtration the organic solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel ($CH_2Cl_2/CH_3OH$: 9/1), yield 61 mg (92%) of **19·HCl**: mp 250 °C dec (methanol/diethyl ether); $^1H$ NMR ($CDCl_3$) $\delta$ 1.35 (s, 9H, $C(CH_3)_3$), 2.04 (quin, 2H, $CH_2$), 3.70 (t, 2H, $NCH_2$), 3.92

(t, 2H, $NCH_2$), 6.62 (s, 1H, =CH), 7.09 (dd, 1H, ArH), 7.24 (td, 2H, ArH), 7.33 (d, 1H, ArH), 7.46 (td, 2H, ArH), 7.78 (d, 1H, ArH), 10.04 (bs, 1H, $H^+$); MS 332 ($MH^+$). Anal. ($C_{22}H_{25}N_3$·HCl) C, H, N.

**1-[6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinolin-10-yl]-3-cyclohexylurea Hydrochloride (20·HCl). 19·HCl** (100 mg, 0.27 mmol) was dissolved in 5 mL of dry THF, and 38 μL (0.3 mmol) of cyclohexylisocyanate was added. The reaction mixture was kept at 70 °C for 24 h, the solvent evaporated, and the residue chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 96/4), yield 37 mg (30%) of **20·HCl**: mp 235−240 °C; $^1H$ NMR ($CDCl_3$/$CD_3OD$) δ 1.18−1.61 (m, 5H, $(CH_2)_3$), 1.42 (s, 9H, $C(CH_3)_3$), 1.63−1.75 (m, 1H, CH), 1.80−2.02 (m, 4H, 2 $CH_2$), 2.24 (quin, 2H, $CH_2$), 3.64−3.78 (m, 1H, NCH), 3.80 (t, 2H, $NCH_2$), 4.18 (t, 2H, $NCH_2$), 7.08 (s, 1H, =CH), 7.48 (dt, 2H, ArH), 7.62 (td, 2H, ArH), 7.75−7.78 (m, 2H, ArH), 8.43 (d, 1H, ArH); MS 457.6 ($MH^+$). Anal. ($C_{29}H_{36}N_4O$·HCl) C, H, N.

**1-[6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinolin-10-yl]-3-phenylthiourea Hydrochloride (21·HCl). 19·HCl** (100 mg, 0.27 mmol) was dissolved in 5 mL of THF and 0.5 mL of DMF, 72 μL (0.6 mmol) of phenyl isothiocyanate was added ,and the reaction mixture was kept at 60 °C for 20 h. After evaporation of the solvent, the residue was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 96/4), yield 99 mg (79%) of **21·HCl**: mp 200 °C dec; $^1H$ NMR ($CDCl_3$) δ 1.47 (s, 9H, $C(CH_3)_3$), 2.17 (quin, 2H, $CH_2$), 3.84 (t, 2H, $NCH_2$), 4.10 (t, 2H, $NCH_2$), 6.84 (s, 1H, =CH), 7.17 (t, 1H, ArH), 7.25−7.36 (m, 3H, ArH), 7.43 (td, 2H, ArH), 7.75 (d, 1H, ArH), 7.80 (td, 2H, ArH), 7.76−7.80 (m, 2H, ArH), 8.52 (s, 1H, NH), 8.97 (d, 1H, ArH), 10.08 (s, 1H, NH), 10.85 (bs, 1H, $H^+$); MS 467 ($MH^+$). Anal. ($C_{29}H_{30}N_4S$·HCl) C, H, N.

**1-[6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinolin-10-yl]-3-(2,4-dichlorophenyl)urea Hydrochloride (22·HCl). 19·HCl** (100 mg, 0.27 mmol) was dissolved in 5 mL of THF and cooled to −15 °C. Then 56 mg (0.3 mmol) of 2,4-dichlorophenyl isocyanate was added, and the reaction mixture was allowed to come to room temperature slowly overnight. The solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 96/4), yield 106.5 mg (76%) of **22·HCl**, amorphous powder: $^1H$ NMR ($CDCl_3$/$CD_3OD$/DMSO-$d_6$) δ 1.37 (s, 9H, $C(CH_3)_3$), 2.20 (quin, 2H, $CH_2$), 3.82 (t, 2H, $NCH_2$), 4.10 (t, 2H, $NCH_2$), 6.85 (s, 1H, =CH), 7.18 (dd, 1H, ArH), 7.234−7.36 (m, 1H, ArH), 7.38 (td, 2H, ArH), 7.50 (td, 2H, ArH), 7.61 (d, 1H, ArH), 8.11 (d, 1H, ArH), 8.25 (d, 1H, ArH), 8.42 (dd, 1H, ArH); MS 519 ($MH^+$). Anal. ($C_{29}H_{28}Cl_2N_4O$·HCl) C, H, N.

***N*-[6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinolin-10-yl]-2-pyridin-2-ylacetamide Dihydrochloride (23·2HCl).** 2-Pyridylacetic acid (45 mg, 0.27 mmol) was dissolved in 5 mL of DMF, and the reaction mixture was cooled to −10 °C. Then 259 μL (1.5 mmol) of diisopropylamine, 211 mg (0.405 mmol) of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, and 100 mg (0.27 mmol) of **19·HCl** were added, and the solution was allowed to come to room temperature overnight. After diluting with $CH_2Cl_2$ the reaction mixture was extracted with 0.1 N NaOH and $H_2O$ and dried, and the organic solvent was evaporated under reduced pressure. The residue was dissolved in methanol and acidified to pH 5 with methanolic HCl and the solvent evaporated. The resulting dihydrochloride was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 96/4), yield 59.6 mg (49%) of **23·2HCl**: mp 212−220 °C; $^1H$ NMR ($CDCl_3$) δ 1.38 (s, 9H, $C(CH_3)_3$), 2.08 (quin, 2H, $CH_2$), 3.81−4.01 (m, 4H, $CH_2$-CO, $NCH_2$), 4.17 (t, 2H, $NCH_2$), 6.78 (s, 1H, =CH), 7.14−7.76 (m, 8H, ArH), 8.62−8.90 (m, 3H, ArH), 11.04 (bs, 1H, $H^+$), 11.43 (s, 1H, NH); MS 450 ($M^+$). Anal. ($C_{29}H_{30}N_4O$·2HCl) C, H, N.

**2-Phenethylbenzonitrile (25).** Procedure B, **24**, and benzylidene triphenylphosphorane were used; the product was chromatographed on silica gel (toluene/ethyl acetate: 9/1), yield 65% of **25**, syrup: $^1H$ NMR ($CDCl_3$) δ 2.96−3.04 (m, 2H, $CH_2$), 3.07−3.18 (m, 2H, $CH_2$), 7.18−7.36 (m, 7H, ArH), 7.49 (dt, 1H, ArH), 7.60 (dd, 1H, ArH); MS 208.2 ($MH^+$). Anal. ($C_{15}H_{13}N$) C, H, N.

**2-[3-(Dimethylamino)propyl]benzonitrile (26).** Procedure B, **24**, and [2-(dimethylamino)ethylidene]triphenylphosphorane were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 6/1), yield 52% of **26**, syrup: $^1H$ NMR ($CD_3OD$) δ 1.86 (quin, 2H, $CH_2$), 2.27 (s, 6H, $N(CH_3)_2$), 2.40 (dd, 2H, $CH_2$), 2.88 (dd, 2H, $CH_2$), 7.25−7.38 (m, 2H, ArH), 7.51 (dt, 1H, ArH), 7.62 (dd, 1H, ArH); MS 189.1 ($MH^+$). Anal. ($C_{12}H_{16}N_2$·$H_2O$) C, H, N.

**2-[2-(4-Ethoxyphenyl)ethyl]benzonitrile (27).** Procedure B, **24**, and (4-ethoxybenzylidene)triphenylphosphorane were used; the product was chromatographed on silica gel (toluene/ethyl acetate: 9/1), yield 94% of **27**, syrup: $^1H$ NMR ($CDCl_3$) δ 1.18 (t, 3H, $CH_3$), 2.80−2.96 (m, 2H, $CH_2$), 3.08−3.20 (m, 2H, $CH_2$), 3.98 (q, 2H, $OCH_2$), 6.80 (td, 2H, ArH), 7.10 (td, 2H, ArH), 7.22 (m, 2H, ArH), 7.47 (dt, 1H, ArH), 7.62 (dd, 1H, ArH); MS 252.1 ($MH^+$). Anal. ($C_{17}H_{17}NO$) C, H, N.

**2-Pentylbenzonitrile (28).** Procedure B, **24**, and butylenetriphenylphosphorane were used; the product was chromatographed on silica gel (toluene/ethyl acetate: 9/1), yield 76% of **28**, syrup: $^1H$ NMR ($CDCl_3$) δ 0.82−0.94 (m, 3H, $CH_3$), 1.29−1.41 (m, 4H, $CH_2CH_2$), 1.62−1.68 (m, 2H, $CH_2$), 2.83 (AB, 2H, $CH_2$), 7.22−7.34 (m, 2H, ArH), 7.49 (dt, 1H, ArH), 7.60 (dd, 1H, ArH); MS 173 ($M^+$). Anal. ($C_{12}H_{15}N$) C, H, N.

**2-(2-Phenethylphenyl)-1,4,5,6-tetrahydropyrimidine (29). 25** reacted as described in ref 11. The product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 6/1), yield 70% of **29**: mp 208−212 °C; $^1H$ NMR ($CD_3OD$) δ 2.09 (quin, 2H, $CH_2$), 2.81−3.06 (m, 4H, $CH_2CH_2$), 3.40−3.51 (m 4H, $(NCH_2)_2$), 7.03−7.59 (m, 9H, ArH); MS 265.2 ($MH^+$). Anal. ($C_{18}H_{20}N_2$·HCl) C, H, N.

**2-[2-[3-(Dimethylamino)propyl]phenyl]-1,4,5,6-tetrahydropyrimidine (30). 26** was transformed as described in ref 11. The product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 9/1 → 2/1), yield 73% of **30**, very hygroscopic: $^1H$ NMR ($CDCl_3$) δ 1.89−2.20 (m, 4H, 2 $CH_2$), 2.64−2.81 (m, 2H, $CH_2$), 2.75 (s, 6H, $N(CH_3)_2$), 2.08 (t, 2H, $NCH_3$), 3.64 (t, 2H, $NCH_2$), 7.38−7.61 (m, 3H, ArH); MS 246 ($MH^+$). Due to hygroscopic problems, no further characterization; **30** was reacted immediately after drying to **36**.

**2-[2-[2-(4-Ethoxyphenyl)ethyl]phenyl]-1,4,5,6-tetrahydropyrimidine Hydrochloride (31·HCl). 27** was transformed as described in ref 11. The product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 9/1 → 2/1), yield 79% of **31·HCl**, precipitated from $CH_3OH$ with diethyl ether: mp 189−196 °C; $^1H$ NMR ($CDCl_3$) δ 1.36 (t, 3H, $CH_3$), 1.90 (quin, 2H, $CH_2$), 2.80−2.87 (m, 2H, $CH_2$), 2.88−2.97 (m, 2H, $CH_2$), 3.28 (t, 4H, 2 $NCH_2$), 3.98 (q, 2H, $OCH_2$), 6.78 (td, 2H, ArH), 6.90 (td, 2H, ArH), 7.11−7.28 (m, 3H, ArH), 7.34 (dt, 1H, ArH), 9.42 (bs, 1H, $H^+$); MS 309.2 ($MH^+$). Anal. ($C_{20}H_{24}N_2O$·HCl) C, H, N.

**2-(2-Pentylphenyl)-1,4,5,6-tetrahydropyrimidine (32). 28** was transformed as described in ref 11. The product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 9/1), yield 82% of **32**, free base recrystallized from toluene/*n*-hexane: mp 98−105 °C; $^1H$ NMR ($CDCl_3$) δ 0.87 (t, 3H, $CH_3$), 1.22−1.40 (m, 4H, 2 $CH_2$), 1.60 (quin, 2H, $CH_2$), 1.83 (quin, 2H, $CH_2$), 2.78 (AB, 2H, $CH_2$), 3.63 (t, 4H, 2 $NCH_2$), 7.18−7.25 (m, 4H, ArH); MS 231.2 ($MH^+$). Anal. ($C_{15}H_{22}N_2$) C, H, N.

**7-Benzyl-3,4-dihyro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (33·HCl).** Procedure A, **29**, and formic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 4/1), yield 48% of **33·HCl**: mp 228−240 °C; $^1H$ NMR ($CDCl_3$) δ 2.20 (quin, 2H, $CH_2$), 3.82 (t, 2H, $NCH_2$), 4.11 (s, 2H, $CH_2$), 4.23 (t, 2H, $NCH_2$), 6.80 (s, 1H, =CH), 7.16−7.38 (m, 5H, ArH), 7.62−7.78 (m, 3H, ArH), 9.17 (d, 1H, ArH), 11.40 (bs, 1H, $H^+$); MS 274.1 ($MH^+$). Anal. ($C_{19}H_{18}N_2$·HCl) C, H, N.

**7-Benzyl-6-[4-(trimethylsilanyl)phenyl]-3,4-dihyro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (34·HCl).** Procedure A, **29**, and 4-(trimethylsilyl)benzoic acid methyl ester were used; the product was chromatographed on silica gel

($CH_2Cl_2$/$CH_3OH$: 9/1), yield 23% of **34·HCl**: mp 232−240 °C ($CH_3OH$, $(C_2H_5)_2O$); $^1H$ NMR ($CDCl_3$) $\delta$ 0.53 (s, 9H, $Si(CH_3)_3$), 2.18 (quin, 2H, $CH_2$), 3.82−3.98 (m, 4H, 2 $NCH_2$), 3.99 (s, 2H, $CH_2$), 7.00 (d, 2H, ArH), 7.18−7.39 (m, 5H, ArH), 7.58−7.63 (m, 5H, ArH), 9.22 (d, 1H, ArH), 11.60 (bs, 1H, $H^+$); MS 422 ($M^+$). Anal. ($C_{28}H_{30}N_2Si$·HCl) C, H, N.

**7-Benzyl-6-(4-*tert*-butylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (35·HCl).** Procedure A, **29**, and 4-*tert*-butylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 9/1), yield 31% of **35·HCl**, amorphous: $^1H$ NMR ($CDCl_3$) $\delta$ 1.38 (s, 9H, $C(CH_3)_3$), 2.15 (quin, 2H, $CH_2$), 3.78−3.84 (m, 4H, 2 $NCH_2$), 3.92 (s, 2H, $CH_2$), 6.97−7.03 (m, 2H, ArH), 7.14−7.30 (m, 5H, ArH), 7.50 (td, 2H, ArH), 7.63−7.74 (m, 3H, ArH), 8.97−9.01 (m, 1H, ArH); MS 407.2 ($MH^+$). Anal. ($C_{29}H_{30}N_2$·HCl) C, H, N.

**[2-[6-(4-*tert*-Butylphenyl)-3,4-dihydro-2*H*-pyrimido-[2,1-*a*]isoquinolin-7-yl]ethyl]dimethylamine Hydrochloride (36·HCl).** Procedure A, **29**, and 4-*tert*-butylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 7/1 → 4/1), yield 17% of **36·HCl**, precipitated from $CH_3OH$ with diethyl ether: mp 175−190 °C (hygroscopic); $^1H$ NMR ($CDCl_3$) $\delta$ 1.41 (s, 9H, $C(CH_3)_3$), 2.18 (quin, 2H, $CH_2$), 2.42 (s, 6H, $N(CH_3)_2$), 2.87−2.96 (m, 4H, 2 $CH_2$), 2.67 (t, 2H, $NCH_2$), 3.87 (t, 2H, $NHC_2$), 7.48 (td, 2H, ArH), 7.75 (td, 2H, ArH), 7.86 (dt, 1H, ArH), 8.09 (dt, 1H, ArH), 8.18 (d, 1H, ArH), 8.48 (d, 1H, ArH); MS 388 ($MH^+$). Due to hygroscopy, no microanalysis was possible.

**7-(4-Ethoxybenzyl)-6-(3-naphthalen-1-ylpropyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (37·HCl).** Procedure A, **31·HCl**, and 4-(1-naphthyl)butyric acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 95/5), yield 48% of **37·HCl**, precipitated from $CH_3OH$ with diethyl ether: mp 192−200 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 1.38 (t, 3H, $CH_3$), 1.82−2.04 (m, 2H, $CH_2$), 2.06−2.20 (m, 2H, $CH_2$), 2.73−2.86 (m, 2H, $CH_2$), 2.90 (t, 2H, $CH_2$), 3.81 (t, 2H, $CH_2$), 3.93 (q, 2H, $CH_2$), 3.93 (s, 2H, $CH_2$), 4.10 (t, 2H, $CH_2$), 6.61−6.70 (m, 4H, ArH), 7.25 (dd, 1H, ArH), 7.41−7.58 (m, 2H, ArH), 7.58−7.71 (m, 4H, ArH), 7.71−7.90 (m, 3H, ArH), 9.01 (dd, 1H, ArH), 11.15 (bs, 1H, $H^+$); MS 487.3 ($MH^+$). Anal. ($C_{34}H_{34}N_2O$·HCl) C, H, N.

**7-(4-Ethoxybenzyl)-6-(4-phenethylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (38·HCl).** Procedure A, **31·HCl**, and 4-phenethylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 95/5), yield 38% of **38·HCl**, precipitated from $CH_3OH$ with diethyl ether: mp 235−240 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 1.37 (t, 3H, $CH_3$), 2.12 (quin, 2H, $CH_2$), 2.92−3.04 (m, 4H, $CH_2CH_2$), 3.81−8.84 (m, 4H, $NCH_2$, $CH_2$), 3.89 (t, 2H, $NCH_2$), 3.96 (q, 2H, $OCH_2$), 6.74 (td, 2H, ArH), 6.87 (td, 2H, ArH), 7.13−7.29 (m, 9H, ArH), 7.56−7.61 (m, 3H, ArH), 9.27 (d, 1H, ArH), 11.57 (bs, 1H, $H^+$); MS 498.1 ($MH^+$). Anal. ($C_{35}H_{34}N_2O$·HCl) C, H, N.

**7-Benzyl-6-(4-phenethylphenyl)-3,4-dihydro-2*H*-pyrimido[2,1-*a*]isoquinoline Hydrochloride (39·HCl).** Procedure A, **29**, and 4-phenethylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 98/2 → 9/1); yield 52% of **39·HCl**, precipitated from $CH_3OH$ with diethyl ether: mp 182−190 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 2.12 (quin, 2H, $CH_2$), 2.97 (s, 4H, $CH_2CH_2$), 3.79 (t, 2H, $NCH_2$), 3.90−4.01 (m, 4H, $CH_2$, $NCH_2$), 6.97 (dd, 2H, ArH), 7.11−7.38 (m, 12H, ArH), 7.61−7.70 (m, 3H, ArH), 9.24−9.32 (m, 1H, ArH), 11.50 (bs, 1H, $H^+$); MS 454.2 ($MH^+$). Anal. ($C_{33}H_{30}N_2$·HCl) C, H, N.

**7-Butyl-6-(4-*tert*-butylphenyl)-3,4-dihydro-2*H*-[2,1-*a*]isoquinoline Hydrochloride (40·HCl).** Procedure A, **32**, and 4-*tert*-butylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 9/1), yield 7% of **40·HCl**, amorphous: $^1H$ NMR ($CDCl_3$) $\delta$ 0.77 (t, 3H, $CH_3$), 1.22 (quin, 2H, $CH_2$), 1.38−1.45 (m, 11H, $CH_2$, $C(CH_3)_3$), 2.14 (quin, 2H, $CH_2$), 2.43 (AB, 2H, $CH_2$), 3.78 (t, 2H, $NCH_2$), 3.92 (t, 2H, $NCH_2$), 7.21 (td, 2H, ArH), 7.58 (td, 2H, ArH), 7.74−7.83 (m, 3H, ArH), 9.37 (d, 1H, ArH), 11.42 (bs, 1H, $H^+$); MS 372.2 ($MH^+$). Anal. ($C_{26}H_{32}N_2$·HCl) C, H, N.

**7-Butyl-6-(4-phenethylphenyl)-3,4-dihydro-2*H*-[2,1-*a*]-isoquinoline Hydrochloride (41).** Procedure A, **32**, and 4-phenethylbenzoic acid methyl ester were used; the product was chromatographed on silica gel ($CH_2Cl_2$/$CH_3OH$: 9/1), yield 38% of **41**: mp 194−202 °C (amorphous); $^1H$ NMR ($CDCl_3$) $\delta$ 0.77 (t, 3H, $CH_3$), 1.22 (quin, 2H, $CH_2$), 1.41 (quin, 2H, $CH_2$), 2.06 (quin, 2H, $CH_2$), 2.43 (AB, 2H, $CH_2$), 2.95−3.08 (m, 4H, $CH_2CH_2$), 3.71 (t, 2H, $NCH_2$), 3.83 (t, 2H, $NCH_2$), 7.16−7.38 (m, 9H, ArH), 7.71−7.86 (m, 3H, ArH), 9.32 (d, 1H, ArH), 11.42 (bs, 1H, $H^+$). Anal. ($C_{30}H_{32}N_2$·HCl) C, H, N.

**Biology: Stable Reporter Gene Cell Line (Clone 12).** CPII cells ($5 \times 10^7$) were transfected by electroporation using a TNFα promoter−luciferase−TNFα 3′ reporter gene plasmid as recently described.[8,9] After 24 h in culture, cells were selected for viability via lymphoprep (Nycomed Pharma AS, Oslo, Norway) and seeded at a density of $1 \times 10^4$ cells/well in 100 μL of medium in a 96-well plate. Selection pressure for G418 (Gibco BRL Life Technologies Ltd., Paisley, U.K.) resistance encoded in cis on the reporter gene plasmid was applied 24 h later (500 μg/mL G418). Eighteen G418-resistant cell lines were obtained out of 550 wells, from which eight expressed luciferase upon IgE plus Ag stimulation. Cell culturing, stimulation, and luciferase read-outs were performed as described.[4,8]

**ELISA for Cytokines.** ELISAs for TNFα (Genzyme, Cambridge, MA), IL-4 (Endogen, Woburn, MA), IL-6 (R&D, Minneapolis, MN), and IL-13 (R&D) were performed according to the protocol provided by the manufacturer; $1 \times 10^5$ cells were pretreated with the indicated compounds 1 h prior to stimulation. Supernatants were analyzed for cytokine production 4 h later.

**ELISA for Leukotrienes.** The ELISA for leukotriene synthesis (Amersham, Little Chalfont, U.K.) was performed according to the protocol provided by the manufacturer; $1 \times 10^4$ cells were pretreated with the indicated compounds 1 h prior to stimulation. Supernatants were analyzed for leukotriene production 4 h later.

**Western Blot.** Cells were lysed in sample buffer (Novex, San Diego, CA) containing 2% β-mercaptoethanol, and 20 μL was loaded on a precast Tris-glycine 4−20% gradient gel (Novex). Proteins were transferred to PVDF membranes (Novex) by electroblotting. Membranes were blocked with 5% skim milk in PBS containing 0.1% Tween-20 (Biorad Laboratories, Hercules, CA) and incubated with the primary antibody overnight. The antibodies for erk 1/2 and p-erk 1/2 were purchased from New England Biolabs (New England Biolabs, Beverly, MA). After being washed three times with PBS/Tween, membranes were incubated at room temperature for 1 h with the secondary antibody (anti-rabbit IgG/alkaline phosphatase; New England Biolabs) and washed four times with PBS/Tween. Detection by chemiluminescence (CDP Star, New England Biolabs) was perfomed as described by the manufacturer.

**Hexosaminidase Assay.** CPII cells ($5 \times 10^4$) were incubated in 200 μL of Tyrode/BSA buffer (1.5 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, 130 mM NaCl, 10 mM Hepes, 0.1% (w/v) D-glucose, 0.1% (w/v) BSA) with or without compounds and 75 ng of murine IgE (BioMakor, Rehovot, Israel) for 18 h. Cells were stimulated by adding 20 ng of DNP-BSA (Calbiochem Corp., La Jolla, CA) for 100 min. After centrifugation 100 μL of supernatants was mixed with 100 μL of substrate solution (3.8 mM *p*-nitrophenyl-*m*-acetylglucosamine; Sigma, St. Louis, MO) and 0.1 M NaOAc, pH 4.5 (Sigma), and incubated for 90 min at 37 °C. The reaction was terminated by the addition of 100 μL of 2 M NaOH and measured using a microplate reader at a wavelength of 405 nm.

**XTT Test.** Clone 12 cells ($1 \times 10^4$) were subjected to an XTT Test (Boehringer Mannheim, Mannheim, Germany) according to the protocol provided by the manufacturer.

**Passive Cutaneous Anaphylaxis (PCA).** Female mice (Charles River) were sensitized after anesthesia (ip) with 225 μg of Ketalar (Parke Davis, Berlin, Germany) and 30 μg of Rompun (Bayer, Leverkusen, Germany) by intracutaneous injection of 5 μg in 10 μL of mouse IgE anti-DNP (BioMakor)

with and without the drug into each of four sites in the back skin. After 90 min the animals were challenged iv with 50 μg of DNP-BSA (Calbiochem−Behring, San Diego, CA) in 100 μL of 1% Evans blue. The PCA response was scored by measuring the diameter of dye extravasation 15 min after the challenge. For each value, eight mice at two different sites were investigated.

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