Novel Selective PDE4 Inhibitors. 3. *In Vivo* Antiinflammatory Activity of a New Series of N-Substituted *cis*-Tetra- and *cis*-Hexahydrophthalazinones

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The synthesis and biological activities of a series of N-substituted *cis*-4a,5,6,7,8,8a-hexa- and *cis*-4a,5,8,8a-tetrahydro-2*H*-phthalazin-1-ones are described. It was found that compounds bearing a cycloalkyl group at the 2-position exhibit the highest PDE4 inhibitory activities (pIC_{50} = 8.6-9.4). The *N*-cycloheptyl- and *N*-adamantanyltetrahydrophthalazinones (**7h**, **8**, **10**, **11**) show high in vivo antiinflammatory activities after oral application. Additionally, some phthalazinones were found to exhibit potent suppression of LPS-induced TNFα release and show moderate potency against fMLP-stimulated production of ROS.

Introduction

In most of the inflammatory cells, elevated cAMP levels are known to inhibit cellular responses.[1] One effective means to elevate cyclic nucleotide levels is to attenuate cyclic nucleotide breakdown mediated by the action of phosphodiesterase (PDE) enzymes. Therefore, in recent years, much research focused on the discovery and development of potent and selective PDE inhibitors for the treatment of acute and chronic inflammatory diseases such as rheumatoid arthritis (RA) and asthma.[2,3]

In previous papers we have described the discovery of a novel class of *cis*-annealed hexa- and tetrahydrophthalazinones as selective PDE4 inhibitors.[4] Structure-activity relationship studies for the fused hydrocarbon ring and catechol moiety of these phthalazinones have been summarized.[4,5] In the present study the synthesis and structure-activity relationships of *cis*-tetra- and hexahydrophthalazinones with a large variety of substituents at the 2-position of the heterocyclic subunit are studied (Figure 1). The target structures contain the 3,4-dimethoxy moiety, which, among others, was found to be optimal for PDE4 inhibitory activity. Selected members of the series are evaluated for their *in vitro* and *in vivo* antiinflammatory properties to establish their potential benefit in the treatment of chronic inflammatory conditions such as rheumatoid arthritis and asthma.[6-8]

Chemistry

The synthetic strategies used for the preparation of the target phthalazinones (general structure) are summarized in Scheme 1. Introduction of substituents to the 2-position of the heterocycle was carried out by two different methods. The first process involves alkylation

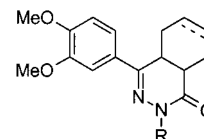
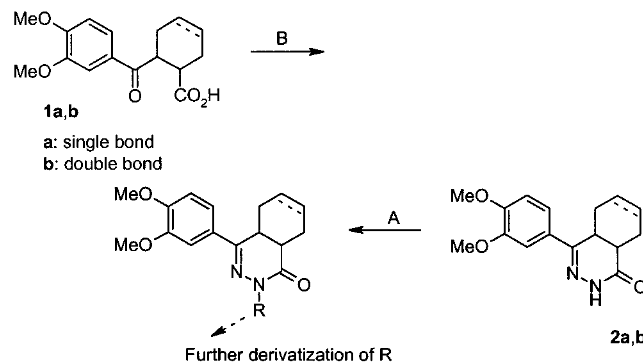


Figure 1. Target *cis*-fused phthalazinones.

Scheme 1[a]



[a] Reagents. Method A: (1) NaH, DMF; (2) RX. Method B: $RNHNH_2$, toluene/1-propanol, TEA, reflux.

of the phthalazinones **2** by successive treatment with sodium hydride and an alkyl halide (method A), while the other method involves condensation of γ-keto acids **1** with substituted hydrazines (method B). These hydrazines were either commercially available or synthesized according to literature procedures.[9]

The structures of the target compounds are listed in Tables 1-3.

Hexahydrophthalazinone Series (Table 1). The preparation of phthalazinones **2a**, **4**, and **5** has been described previously.[4] Phthalazinones **3e** and **3k** were prepared from γ-keto acid **1a**[4] (Scheme 1) and the corresponding hydrazines (method B). Phthalazinone **6r** was synthesized from **3k** in two steps. First, **3k** was brominated using bromotriphenylphosphonium bromide, which was formed in situ. Subsequently, the resulting alkyl bromide was reacted with imidazole to yield the desired compound. The remainder of the

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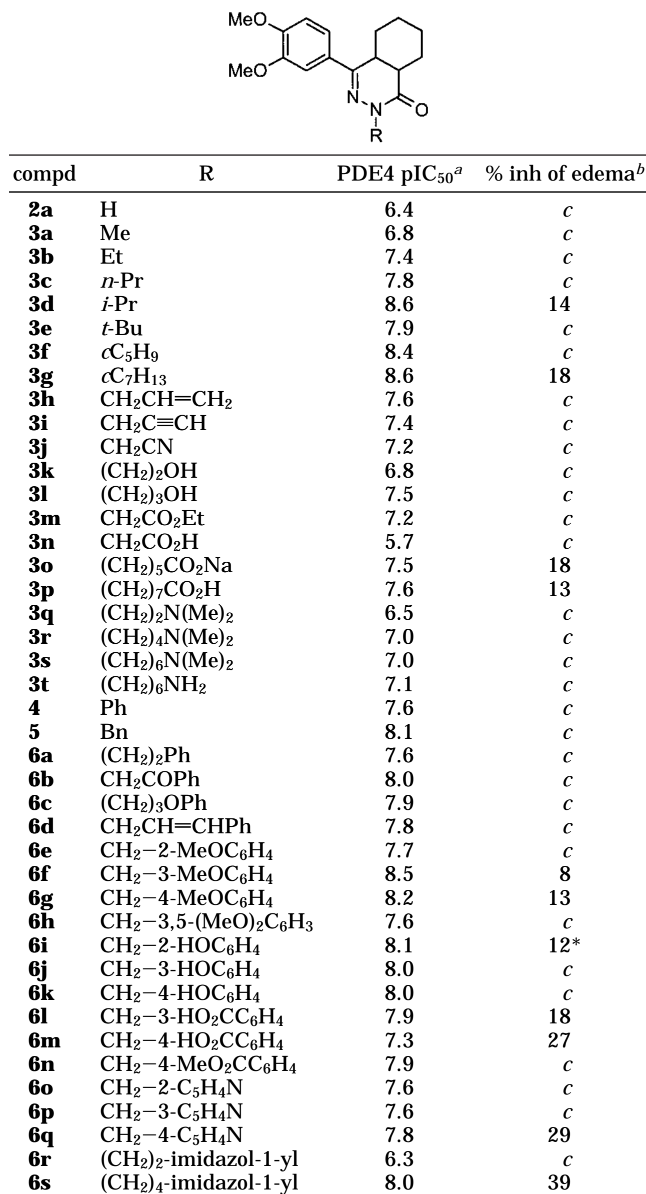
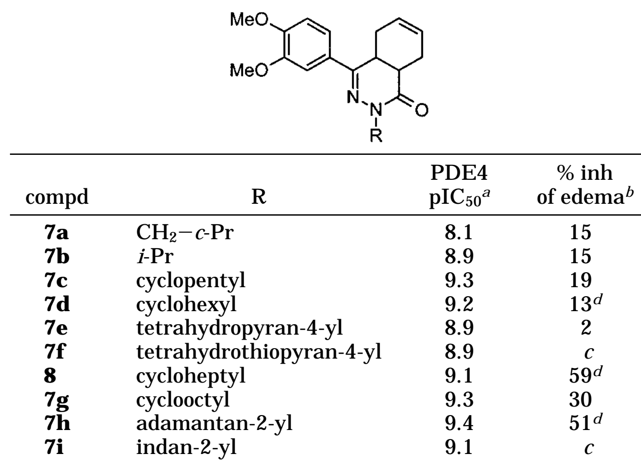
Table 1. PDE4 Inhibitory and *in Vivo* Antiinflammatory Activities of N-Substituted Hexahydrophthalazinones



compd	R	PDE4 pIC_{50}[a]	% inh of edema[b]
2a	H	6.4	*c*
3a	Me	6.8	*c*
3b	Et	7.4	*c*
3c	*n*-Pr	7.8	*c*
3d	*i*-Pr	8.6	14
3e	*t*-Bu	7.9	*c*
3f	cC_5H_9	8.4	*c*
3g	cC_7H_{13}	8.6	18
3h	$CH_2CH{=}CH_2$	7.6	*c*
3i	$CH_2C{\equiv}CH$	7.4	*c*
3j	CH_2CN	7.2	*c*
3k	$(CH_2)_2OH$	6.8	*c*
3l	$(CH_2)_3OH$	7.5	*c*
3m	CH_2CO_2Et	7.2	*c*
3n	CH_2CO_2H	5.7	*c*
3o	$(CH_2)_5CO_2Na$	7.5	18
3p	$(CH_2)_7CO_2H$	7.6	13
3q	$(CH_2)_2N(Me)_2$	6.5	*c*
3r	$(CH_2)_4N(Me)_2$	7.0	*c*
3s	$(CH_2)_6N(Me)_2$	7.0	*c*
3t	$(CH_2)_6NH_2$	7.1	*c*
4	Ph	7.6	*c*
5	Bn	8.1	*c*
6a	$(CH_2)_2Ph$	7.6	*c*
6b	CH_2COPh	8.0	*c*
6c	$(CH_2)_3OPh$	7.9	*c*
6d	$CH_2CH{=}CHPh$	7.8	*c*
6e	$CH_2{-}2{-}MeOC_6H_4$	7.7	*c*
6f	$CH_2{-}3{-}MeOC_6H_4$	8.5	8
6g	$CH_2{-}4{-}MeOC_6H_4$	8.2	13
6h	$CH_2{-}3,5{-}(MeO)_2C_6H_3$	7.6	*c*
6i	$CH_2{-}2{-}HOC_6H_4$	8.1	12*
6j	$CH_2{-}3{-}HOC_6H_4$	8.0	*c*
6k	$CH_2{-}4{-}HOC_6H_4$	8.0	*c*
6l	$CH_2{-}3{-}HO_2CC_6H_4$	7.9	18
6m	$CH_2{-}4{-}HO_2CC_6H_4$	7.3	27
6n	$CH_2{-}4{-}MeO_2CC_6H_4$	7.9	*c*
6o	$CH_2{-}2{-}C_5H_4N$	7.6	*c*
6p	$CH_2{-}3{-}C_5H_4N$	7.6	*c*
6q	$CH_2{-}4{-}C_5H_4N$	7.8	29
6r	$(CH_2)_2$-imidazol-1-yl	6.3	*c*
6s	$(CH_2)_4$-imidazol-1-yl	8.0	39

[a] $pIC_{50} = -\log IC_{50}$. Inhibition of PDE4 was investigated in the cytosol of human neutrophils. The data are means of two independent determinations in triplicate. [b] Percent inhibition of the formation of AA-induced mouse ear edema after pretreatment with the target compound (1 h before AA) at a drug concentration of 30 μmol/kg po, except where indicated by *, which is at 100 μmol/kg po. $n = 1$. [c] Not determined.

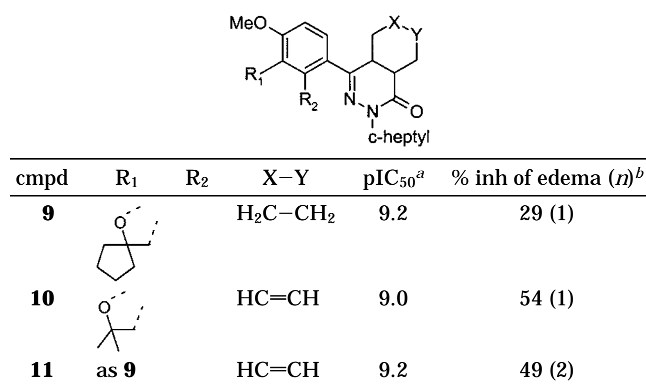
Table 2. PDE4 Inhibitory and *in Vivo* Antiinflammatory Activities of N-Substituted 4a,5,8,8a-Tetrahydrophthalazinones



compd	R	PDE4 pIC_{50}[a]	% inh of edema[b]
7a	$CH_2{-}c{-}Pr$	8.1	15
7b	*i*-Pr	8.9	15
7c	cyclopentyl	9.3	19
7d	cyclohexyl	9.2	13[d]
7e	tetrahydropyran-4-yl	8.9	2
7f	tetrahydrothiopyran-4-yl	8.9	*c*
8	cycloheptyl	9.1	59[d]
7g	cyclooctyl	9.3	30
7h	adamantan-2-yl	9.4	51[d]
7i	indan-2-yl	9.1	*c*

[a–c] See corresponding footnotes from Table 1. [d] $N = 2$.

Table 3. PDE4 Inhibitory and *in Vivo* Antiinflammatory Activities of 4-Benzofuran-Substituted *N*-Cycloheptylphthalazinones



cmpd	R_1	R_2	X−Y	pIC_{50}[a]	% inh of edema (n)[b]
9			$H_2C{-}CH_2$	9.2	29 (1)
10			$HC{=}CH$	9.0	54 (1)
11	as **9**		$HC{=}CH$	9.2	49 (2)

[a,b] See corresponding footnotes from Table 1.

hexahydrophthalazinone series was obtained via method A using phthalazinone **2a**. In some cases additional reaction steps were needed to provide the preferred target compounds. Hydrolysis of ethyl ester **3m** gave access to phthalazinone **3n**. Phenols **6i**−**k** were synthesized by catalytic hydrogenation of the respective *N*-(benzyloxybenzyl)phthalazinone intermediates. Esterification of benzoic acid **6m** by treatment with thionyl chloride in methanol afforded **6n**. To achieve amines **3r**−**t** and imidazole **6s**, phthalazinone **2a** was alkylated with the suitable α,ω-dibromoalkanes to provide the corresponding *N*-(ω-bromo-*n*-alkyl)phthalazinones (n = 4 or 6), which were then reacted with the selected amines.

Tetrahydrophthalazinone Series (Tables 2 and 3). Phthalazinones **7a**−**c** and benzofuran **9** were prepared via method A. Method B was used to synthesize **7d**−**i**. The synthesis of γ-keto acid **1b** and tetrahydrophthalazinones **2b**, **8**, **10**, and **11** has been described in a previous paper.[4,5]

Pharmacology

The N-substituted hexa- and tetrahydrophthalazinones were screened for their activity against PDE3 and PDE4. The inhibitory activities (pIC_{50} values) were determined as described previously[4] and are listed in Tables 1−3. Structure−activity relationships for the present series of phthalazinones are discussed below.

1. PDE3 Inhibition. None of the target compounds display substantial PDE3 inhibitory activity ($pIC_{50} < 5.5$, data not shown).

2. PDE4 Structure−Activity Relationships. To study the influence of the steric, electronic, and lipophilic properties of the substituents attached to N2 on the PDE4 inhibitory activity, structurally diverse hexa- and tetrahydrophthalazinones were examined (Tables 1 and 2). Comparison of phthalazinone **2a** with compounds **3**−**6** reveals that in general N-substitution is beneficial for PDE4 inhibitory activity. An increase in the length of linear alkyl substituents (compare **3a**−**c**) enhances PDE4 inhibition. The introduction of branched alkyl chains initially leads to a 6-fold increase in activity for isopropyl analogue **3d** over *n*-propyl derivative **3c**;

however, further branching (**3e**) is not favorable. These results indicate that lipophilicity and steric interactions in this region play an important role in PDE4 inhibition. Changing the isopropyl group to cyclic alkyl structures does not affect the activity (compare **3d**, **3f**, and **3g**). From analysis of the analogues **2a**−**3g**, it seems that a trisubstituted carbon atom attached to N2 (*N*-CHR_2) is preferable for PDE4 inhibitory activity. Derivatives **3h**−**j**, having unsaturated (hetero)carbon chains connected to N2, possess similar or slightly decreased activities in comparison to **3c**. The introduction of relatively small but polar hydroxy-, carboxy-, or dimethylaminoalkylene moieties to the phthalazinone skeleton negatively influences the PDE4 inhibitory potential (**3k**, **3n**, and **3q** vs **3b**). Enlargement of the alkyl chain lengths (**3k** vs **3l**, **3n** vs **3o**, and **3q** vs **3r**) or esterification (**3m** vs **3n**) leads to substantial increases in activity, suggesting that the lipophilicity of the N-substituents and/or the distance of the polar group to N2 plays a major role in PDE4 inhibition. Further elongation of the alkyl chains, from $n = 4$ to $n = 7$, does not alter the inhibitory activities (**3o** vs **3p**; **3r** vs **3s**).

Most phthalazinones (**4**−**6s**), substituted with different aromatic substituents, show similar activities. Comparison of analogues **4**, **5**, and **6a**−**d** shows that the linker for 0−3 carbon atoms connecting the aromatic ring to N2 does not largely affect the activity. Next, the influence of methoxy, hydroxy, and carboxy moieties at the benzyl aromatic ring on PDE4 inhibition was studied. Although minor variations in activity are observed (**6e**, **6f**, and **6h**), phthalazinones **6g**, **6i**−**l**, and **6n** have activities comparable to that of the unsubstituted benzyl analogue **5**. In contrast, benzoic acid **6m** is approximately 1 log unit less potent than compound **5**. Substitution of the benzyl group for an *o*-, *m*-, or *p*-pyridinemethylene moiety leads to a small decrease in activity (compare **5** with **6o**−**q**). Again, the introduction of a relatively small but polar substituent, an imidazolylethylene moiety, to the phthalazinone skeleton negatively influences the PDE4 inhibitory potential (**6r** vs **6a**). Elongation of the alkyl chain length from $n = 2$ to $n = 4$ in imidazole **6r** leads to a 50-fold increase in potency for **6s**, which may be due to increased lipophilicity and/or enlarged distance between the imidazol ring and N2, as suggested above for phthalazinones with polar N-substituents. Comparison of the PDE4 inhibitory activities of the entire hexahydrophthalazinone series (Table 1) reveals that in general *N*-(cyclo)alkyl and selected branched alkyl substitution provides the most potent compounds.

To find the optimal *N*-cycloalkyl substituent(s), a series of tetrahydrophthalazinones was tested (Table 2). All compounds exhibit nanomolar activities except for cyclopropylmethyl analogue **7a**, which is about 1 log unit less active. Comparison of **7a** with **7b**−**i** and **8** again indicates that a *N*-CHR_2 motif is optimal for activity. Changes in the steric bulk and lipophilicity (compare **7b**−**i** and **8**) of the *N*-CHR_2 group does not have a substantial effect on the PDE4 inhibitory activity. In general, the tetrahydrophthalazinones are more active than the corresponding hexahydro derivatives (**3d** vs **7b**, **3f** vs **7c**, and **3g** vs **8**).

Other 4-catechol-substituted phthalazinones have also been substituted with a cycloheptyl ring as shown in Table 3. Similar to the 3,4-dimethoxyphenyl subunit, the benzofuran moieties are optimal for PDE4 inhibition. All three compounds (**9**−**11**) exhibit nanomolar activity; however, the PDE4 inhibitory activity of 4-(dimethoxyphenyl)tetrahydrophthalazinone **8** is not exceeded.

Table 4. Further Pharmacological Data for Selected Phthalazinones

compd	PDE4 pIC_{50}[a]	% inh of edema[b]	TNFα pIC_{30}[c]	ROS pIC_{35}[d]	ratio[e] ROS/PDE4
3g	8.6	18	7.30	[f]	[f]
7h	9.4	51	7.22	6.30	1250
8	9.1	59	7.05	6.82	190
10	9.0	54	7.05	[f]	[f]
(−)-Rolipram	7.3	55	5.70	7.22	1
Ariflo	7.0	33	4.89	7.04	1
CDP840	7.7	48	4.82	7.20	3

[a,b] See corresponding footnotes from Table 1. [c] Log of concentration of test compound, which inhibited LPS-stimulated TNFα release in human whole blood by 30% of control. [d] Log of concentration of test compound, which inhibited fMLP-stimulated production of ROS in human PMNL by 35% of control. [e] Ratio ROS IC_{35}/PDE4 IC_{50}. [f] Not determined.

3. Further Pharmacological Examination of Selected Phthalazinones. Besides the measurement of the PDE4 inhibitory potencies of the ligands, additional pharmacological testing is required to determine their *in vitro* and *in vivo* antiinflammatory efficacy to establish their benefit in the treatment of chronic inflammatory conditions such as rheumatoid arthritis and asthma. The phthalazinones with the highest PDE4 inhibitory activity were selected for further pharmacological evaluation. For comparison, the PDE4-selective inhibitors (−)-rolipram,[10] ariflo,[11] and CDP840[12] were employed as well.

3.1. Tumor Necrosis Factor-α. Among the growing list of (pro-)inflammatory mediators assumed to be involved in pathologies such as arthritis,[13] AIDS, and asthma, the tumor necrosis factor-α (TNFα) may be of particular importance in initiating chronic inflammation by activating the secretion of cytokines from a variety of cells.[14,15] TNFα is mainly produced by cells of the monocyte/macrophage lineage. Repeatedly, PDE4 inhibitors have been shown to influence lipopolysaccharide (LPS)-induced TNFα synthesis in monocytes.[16] In addition, the archetypal selective PDE4 inhibitor rolipram and other PDE4 inhibitors display inhibitory effects of LPS-induced TNFα release in human whole blood.[17]

Accordingly, a small number of phthalazinones (compounds **3g**, **7h**, **8**, and **10** with pIC_{50} values of 8.6−9.4) have been examined for their inhibition of LPS-induced TNFα release in human whole blood. Preincubation of blood with the selected compounds (and other known PDE4 inhibitors; unpublished results) at different concentrations before LPS stimulation led to a concentration-dependent inhibition of TNFα release with a maximum inhibition of about 60%. Therefore, for quantitative analysis, mean half-maximal inhibitory drug concentrations have been determined, and these are displayed (as pIC_{30} values) in Table 4. The phthalazinones **3g**, **7h**, **8**, and **10** showed similar inhibitory activities with pIC_{30} values ranging from 7.05 to 7.30. Under the same conditions, the reference compounds (−)-rolipram, ariflo, and CDP840 were 28- to 214-fold less potent than the phthalazinones. It should be mentioned, however, that

the overall potency of the compounds tested in this whole blood assay system results from the cellular activity (monocytes), cell permeability (monocytes), and the plasma protein binding of the compounds.

3.2. Reactive Oxygen Species. Among the cell types discussed to be involved in RA pathobiology as well as in airway inflammation is the polymorphonuclear leukocyte (PMNL).[18] An important cell response occurring after receptor-agonist stimulation of PMNL is the formation of reactive oxygen species (ROS) along the so-called respiratory burst.[19] In a number of studies, selective inhibitors of PDE4, which is the predominant PDE isoenzyme of these cells,[20] have been reported to suppress PMNL responses.[1,21]

Compounds **7h** and **8** were selected for the determination of their inhibitory effects on the *N*-formylmethionylleucylphenylalanine (fMLP)-stimulated formation of ROS in human PMNL by measuring luminol-enhanced chemiluminescence[7] (CL). In several independent experiments, **7h**, **8**, (−)-rolipram, ariflo, and CDP840 concentration-dependently inhibited the fMLP-stimulated CL response. Under the experimental conditions employed, the maximum inhibition of these compounds was around 70%, which also holds true for other PDE(3)/4 inhibitors. Therefore, pIC_{35} values were calculated for quantitative analysis and are listed in Table 4. These results demonstrate that phthalazinones **7h** and **8** are cellularly active and have similar potency ($pIC_{35} = 6.30$ and 6.82, respectively). The inhibition of human PMNL function(s) by the phthalazinones **7h** and **8** may contribute to the antiinflammatory action of these compounds *in vivo*.

In general, the inhibition of fMLP-stimulated ROS production in PMNL, measured as luminol-enhanced CL, by PDE4 inhibitors has been found to correlate well with elevation of the cAMP concentration.[22] For our limited dataset, no correlation is apparent between the suppression of the formation of ROS and inhibition of the catalytic activity of PDE4 according to the different ROS/PDE4 ratios (Table 4) of **7h**, **8**, (−)-rolipram, ariflo, and CDP840.

3.3. Mouse-Ear Edema. Direct evidence for the potential antiinflammatory activity of PDE4 inhibitors by inhibition of mediator release is derived from animal studies; a much used model is the formation of arachidonic acid (AA) induced edema in the ears of mice.[23] In this in vivo assay, the suppression of the formation of arachidonic acid (AA) induced mouse ear edema was measured after oral administration of the drug. At 2 h after oral addition of the target compound and 1 h after provocation, which is application of a solution of AA to the mouse ear, the percentage of inhibition of edema formation was determined with respect to the provoked nontreated control animals. The maximal possible inhibition of edema in this model is 80%.

Relatively large numbers of phthalazinones were examined for their *in vivo* antiinflammatory effects (Tables 1 and 2). The hexahydrophthalic compounds **3d**,**g**,**o**,**p**,**f**,**g**,**i**,**l**,**m**,**q** and **3s** (Table 1) are all poor inhibitors of AA-induced mouse ear edema when administered at a dose of 30 μmol/kg po. In this series, analogue **3s** shows the highest inhibitory activity of 39% (30 μmol/kg po). Only the *N*-adamantanyl- and *N*-cycloheptyl-substituted analogues (**7h** and **8**, respectively) of the tetrahydrophthalazinone series (**7a**−**e**,**g**,**h** and **8**; Table 2) display good antiinflammatory activity in this assay; the formation of mouse ear edema is inhibited by 50−60% (30 μmol/kg po). As described above, the *N*-cycloheptylhexahydrophthalazinone **3g** is inactive whereas the corresponding tetrahydro compound is highly potent. For this reason, we suggest that the double bond present in the tetrahydro series is essential for potent inhibition of the formation of AA-induced mouse ear edema. Additional benzofurans **9**−**11** (Table 3) were screened to see if this effect was consistent. Indeed, benzofurans **10** and **11** exhibit antiinflammatory activities similar to that of **8** and the analogous hexahydro analogue **9** is considerably less potent. The difference in antiinflammatory activity between the *N*-cycloheptylhexahydro and *N*-cycloheptyltetrahydro series suggests a different pharmacokinetic profile for both series.

Conclusion

We have described the development of a new series of N-substituted hexa- and tetrahydrophthalazinones as potent (nanomolar range) PDE4 inhibitors. A large variety of substituents are allowed at the 2-position of the phthalazinone nucleus; however, the *N*-(cyclo)alkyl-substituted compounds possess the highest PDE4 inhibitory activities in the current series of hexahydrophthalazinones. In general, an *N*-CHR_2 motif is optimal for activity in both the hexa- and tetrahydro series probably because of steric reasons.

Inhibition of both the formation of ROS and release of TNFα is of importance in the treatment of chronic inflammatory diseases such as arthritis and asthma. In this study, selected compounds have been found to potently block the release of TNFα (**3g**, **7h**, **8**, and **10**) and some also moderately inhibit the production of ROS (**7h** and **8**). Furthermore, the *N*-cycloheptyl-substituted tetrahydrophthalazinones **8** and **11** show high *in vivo* antiinflammatory activities in the mouse ear edema assay. In contrast, the corresponding hexahydrophthalazinones **3g** and **9** are inactive or display no substantial activity under these conditions, suggesting a distinct pharmacokinetic profile for both series. Because of their high *in vitro* and *in vivo* antiinflammatory activity, analogues **7h**, **8**, **10**, and **11** are considered to be promising agents for the treatment of rheumatoid arthritis, asthma, and other inflammatory diseases.

Experimental Section

1. Inhibition of TNFα Release in Human Whole Blood. 1.1. Chemicals and Solutions. DMSO, hydroxylamine, and LPS (*S. abortus* equi) were purchased from Sigma Chemie (Deisenhofen, Germany), and heparin (Liquemin N 25000) was from Hoffmann-La Roche AG (Grenzach-Wyhlen, Germany). All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany). A stock solution of LPS (1 mg/mL, w/v) was prepared in 0.1% (v/v) hydroxylamine in PBS (Dulbecco's phosphate-buffered saline, pH 7.4). After sonication for 5 min, 1 mL aliquots were stored at −20 °C. Before the start of the experiment, this solution was diluted 1:100 (v/v) in 0.1% (v/v) hydroxylamine/PBS to get the "LPS working solution".

1.2. Human Blood. Venous blood (~40 mL) was drawn from healthy volunteers and anticoagulated with heparin (8 U/mL).

1.3. TNFα Assay. The final assay volume was 0.5 mL. In 96-deep well plates from Beckman (München, Germany),

aliquots of blood (0.4 mL) were preincubated for 15 min at 37 °C in the absence or presence of the compounds (0.05 mL). Stock solutions of the compounds (10 mM) were prepared in DMSO and diluted 1:100 (v/v) in PBS. Subsequent dilutions were made in 1% (v/v) DMSO/PBS to achieve the final drug concentrations in the assays (0.001−10 μM) at a DMSO concentration of 0.1% (v/v), which by itself did not affect TNFα synthesis. After preincubation, the assays were stimulated by the addition of 0.05 mL of "LPS working solution" (LPS 1 μg/mL, final concentration) for 4 h at 37 °C. Afterward, about 150 μL of supernatant (plasma) was removed and diluted 1:30 (v/v) in PBS containing 3% (w/v) BSA. The samples were stored at −20 °C before TNFα measurement by a commercially available nonisotopic immunoassay from Immunotech (Hamburg, Germany) performed essentially according to the manufacturer's instructions.

1.4. Statistics. IC_{30} values were calculated from concentration−inhibition curves by nonlinear regression analysis using GraphPad Prism (version 2.01, 1998). The data are means of two independent determinations in triplicate, and the variance is less than 0.4 log units.

2. Inhibition of the Formation of ROS in Human PMNL. 2.1. Chemicals. BSA (bovine serum albumin), DMSO, fMLP, glucose, HEPES, and microperoxidase were purchased from Sigma Chemie (Deisenhofen, Germany), ficoll paque was from Pharmacia Biotech (Uppsala, Sweden), and luminol was from Boehringer (Mannheim, Germany). All other chemicals were of analytical grade and were obtained from Merck (Darmstadt, Germany).

2.2. Isolation of Human PMNL. The isolation of PMNL from human blood (anticoagulated with sodium citrate) by dextran sedimentation, centrifugation on ficoll paque, and hypotonic lysis of remaining red blood cells has been performed essentially as described.[24]

2.3. CL Assay. The formation of reactive oxygen species has been determined with the aid of luminol-potentated chemiluminescence and was carried out essentially as described in the literature.[7,24] CL measurements were performed in a buffered solution containing NaCl (140 mM), KCl (5 mM), HEPES (10 mM), $MgCl_2$ and $CaCl_2$ (1 mM), glucose (1 mg/mL), BSA (0.05% (w/v)), luminol (10 μM), and microperoxidase (4 μM) (all values correspond to final concentrations in the assay). Aliquots (0.4 mL) of the cell suspension (1.25×10^7 cells/mL) were preincubated for 5 min at 37 °C in the absence or presence of inhibitors (0.05 mL). Stock solutions of inhibitors (10 mM) prepared in DMSO were diluted 1:100 (v/v) in buffer. Subsequent dilutions were made in 1% (v/v) DMSO/buffer to achieve the final drug concentrations in the assays (0.001−10 μM) at a DMSO concentration of 0.1% (v/v), which by itself only weakly affected the CL response. After preincubation, the test samples were transferred into the measuring apparatus ("Multi-Biolumnat" LB 9505C from Berthold (Wildbad, Germany)) and stimulated by the addition of 0.05 mL of the receptor agonist fMLP (100 nM, final concentration). The CL was recorded continuously for 3 min. From this, the AUC's (*a*rea *u*nder the *c*urve) values were calculated.

2.4. Statistics. IC_{35} values were calculated from concentration−inhibition curves by nonlinear regression analysis using GraphPad Prism (version 2.01, 1998). The data are means of two independent determinations in triplicate, and the variance is less than 0.4 log units.

***In Vivo* Mouse-Ear Inflammation Assay. 1.1. Animals.** Female NMRI mice (breeder, Charles River, Sulzfeld, 25−32 g of body weight, $n = 8$ per group). The animals were housed in groups under standard environmental conditions (6.30 a.m to 6.30 p.m. light cycle, 24−26 °C, relative humidity 60%). Animals were fed with Altromin diet no. 1324 (Altromin, D-32791 Lage) ad libitum with free access to water except during the experimental period.

3.2. Method. The method was performed according to literature procedures.[23] Following the application of 3 mg of arachidonic acid (AA (Sigma), dissolved in 10 μL of acetone) to the right ear of all animals, the thickness of the ear was measured with calipers. In the control group of animals, additional application of 10 μL of acetone was done to the left ear (as a control so that the acetone itself is not responsible for the inflammation). Measurement of the ear thickness (in mm^{-2}) was performed 1 h after the administration of AA. Administration of the PDE drug was done 1 h before AA-induced injury (to detect inhibition of inflammation).

3.3. Evaluation. Inhibition of the AA-induced edema by the substance was calculated as follows: [(increase in ear thickness in the control group − increase in ear thickness in the treated group)/increase in ear thickness in the control group] × 100 = % inhibition. The deviation in the percentage inhibition is 5−10%. The maximal possible inhibition of edema in this model is 80% (indomethacine; 300 μmol/kg po). Significances (drug-treated group versus control group) were calculated with the Kruskal−Wallis test; $p < 0.05$ was assumed to be significant.

3.4. Drugs and Dosages. The compounds were dissolved in water. Dosages used were (1 h before AA) 30 μmol/kg, pH 6.5−7. Administration (volume was 10 mL/kg) was done by gavage.

Supporting Information Available: Experimental procedures for the preparation of compounds **3a**−**t** (Table 1), **6a**−**s** (Table 1), **7a**−**i** (Table 2), and **9** (Table 3) and their melting points and ^{1}H NMR spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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