Synthesis, Structure−Activity Relationships, and Pharmacological Profile of 9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indoles: Discovery of Potent, Selective Phosphodiesterase Type 4 Inhibitors

Catherine Burnouf,* Eric Auclair, Nadine Avenel, Bernadette Bertin, Christèle Bigot, Alain Calvet,[#] Kam Chan,[#] Corinne Durand, Veronique Fasquelle, Frédéric Féru, Richard Gilbertsen,[#] Henry Jacobelli, Adel Kebsi, Emmanuelle Lallier, Jacquie Maignel, Brigitte Martin, Stéphane Milano, Malika Ouagued, Yves Pascal, Marie-Pierre Pruniaux, Jocelyne Puaud, Marie-Noëlle Rocher, Christophe Terrasse, Roger Wrigglesworth, and Annette M. Doherty

Pfizer Global Research & Development, Fresnes Laboratories, 3 à 9 rue de la Loge, 94265 Fresnes, France, and Ann Arbor Laboratories, 2800 Plymouth Road, Ann Arbor, Michigan 48105

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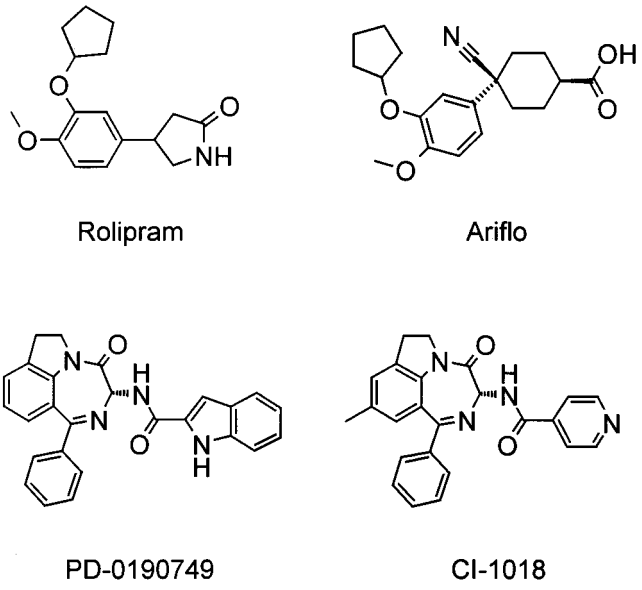
The synthesis, structure−activity relationships, and biological properties of a novel series of potent and selective phosphodiesterase type 4 (PDE4) inhibitors are described. These new aminodiazepinoindoles displayed in vitro PDE4 activity with submicromolar IC_{50} values and PDE4 selectivity vs PDE1, -3, and -5. Specifically, one compound (CI-1044, **10e**) provided efficient in vitro inhibition of TNFα release from hPBMC and hWB with IC_{50} values of 0.34 and 0.84 μM, respectively. This compound was found to exhibit potent in vivo activity in antigen-induced eosinophil recruitment in Brown-Norway rats (ED_{50} = 3.2 mg/kg po) and in production of TNFα in Wistar rats (ED_{50} = 2.8 mg/kg po). No emetic side effects at therapeutic doses were observed in ferrets.

Introduction

Phosphodiesterases (PDEs), responsible for the hydrolysis of intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP), have been classified into 11 major families (PDE1−11) with respect to their substrate sensitivity, Ca^{2+}/calmodulin requirement, and inhibitor selectivity.[1−5] PDE4 is a cAMP-specific enzyme localized in airway smooth muscles in immune and inflammatory cells. Therefore, PDE4 is a potential molecular target for the development of new antiasthmatic and antiinflammatory drugs.[1,3,6−13] Previous efforts have mainly focused on asthma. Several PDE4 inhibitors are still reported in clinical development against asthma such as Ariflo (SB-207499), Roflumilast, and V-112294A.[14−16,7] Ariflo (Chart 1) has also shown efficacy in humans against chronic obstructive pulmonary disease (COPD) leading to a rationale for the efficacy of PDE4 inhibitors in the treatment of COPD.[17−20] Moreover, various PDE4 inhibitors have demonstrated efficacy in models of atopic dermatitis, rheumatoid arthritis, multiple sclerosis, septic shock, gastrointestinal diseases, autoimmune diseases, and various neurological diseases.[6,7]

Four human cDNA isoforms of PDE4 (A, B, C, and D) have been cloned, and the chromosomal localization of the human genes has been defined.[21−23] Each subtype mRNA is expressed in human lung, and PDE4A, -B, and -D are found in eosinophils.[24] To date, the main objective of the research effort in the PDE4 area concerns the identification of novel, selective, and orally active inhibitors with antiinflammatory properties and reduced side effects compared with those reported with the first-generation inhibitors including Rolipram (Chart 1). A high-affinity Rolipram binding site (HARBS) has been reported in rat brain homogenates and has also been found to be a component of PDE4.[25−28] The clinical utility of the pioneer compounds has been limited by their propensity to induce nausea, emesis, and gastric acid secretion.[29−31] To obtain efficient PDE4 inhibitors with reduced side effects, two strategies have been reported. The first approach is based on the design of compounds with reduced affinity for one of the conformations of PDE4 (HPDE4 is inhibited by Rolipram with a K_i value of 1 nM and is argued to be related to side effects) and increased activity for the other conformation (LPDE4 is inhibited by Rolipram with a K_i value of ∼100

Chart 1. Structures of Rolipram, Ariflo, PD-0190749, and CI-1018



* To whom correspondence should be addressed. Tel: 01 40 96 76 00. Fax: 01 40 96 76 87. E-mail: catherine.burnouf@pfizer.com.
[#] Ann Arbor Laboratories.

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nM and is assumed to be linked to the therapeutic actions of PDE4 inhibition).[27,30,32−39] This has led to the identification of a second generation of inhibitors with an improved margin of safety in preclinical and clinical studies.[32−41] The second strategy is related to the relevance of PDE4 subtype-selective inhibitors to distinguish between beneficial actions and side effects or to open the opportunity to increase the tissue or cell selectivity in a next generation of compounds: this remains to be determined. Recently, naphthyridine-related PDE4 inhibitors have been reported to be PDE4D-selective.[42,43]

The primary objective of our research effort has been to identify novel, selective (PDE4 vs other PDEs), and orally active PDE4 inhibitors as antiinflammatory and antiasthmatic drugs with reduced side effects. To achieve this goal, it had been decided in the early 1990s to screen our library against PDE4 enzymes. Focus was placed on a diazepinoindole series derivative which was not structurally related to the PDE4 inhibitors described in the literature. The initial structure−activity relationship (SAR) studies led to the identification of a candidate for development, CI-1018 (**9b**) (Chart 1).[44−47] However, the research program in this field has been continued, and an SAR evolution of CI-1018 (**9b**), leading to the discovery of a new family of aminodiazepinoindoles with improved in vitro PDE4 activity and selectivity (PDE4 vs other PDEs), is described.[48] These derivatives showed increased inhibitory potency against TNFα release in vitro and in vivo. Several analogues displayed good potency and efficacy, after oral administration, in antigen-induced lung eosinophil recruitment in sensitized rats. Finally, the aminodiazepinoindole analogues were devoid of emesis at therapeutic doses in ferrets.

Chemistry

Ariflo and Rolipram were synthesized as described in the literature.[39,49] All the final diazepino derivatives were prepared via elaboration of the key chiral intermediates **8a**−**f** as presented in Scheme 1, starting from commercially available indolines **1a**,**b** or from 5-methoxyindoline **1c** prepared by reduction with sodium cyanoborohydride of the corresponding commercial indole. A procedure for the preparation of the basic benzodiazepine scaffold **7a** (R^1 = H) was reported in the literature (general procedures C−F).[50−52] The synthesis was modified in order to introduce various substituents on position 9 of the multinucleus intermediates **8b**−**f**. 3-Amino-9-methyl-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (**7b**) was obtained starting from the commercially available 5-methyl-2,3-dihydro-1*H*-indole (**1b**) and following general procedures C−F. The elaboration of the methoxy analogue **7c** was performed starting from 5-methoxy-2,3-dihydro-1*H*-indole (**1c**) obtained via reduction of the commercially available corresponding indoline. To avoid treatment with Lewis acids such as BCl_3 and $AlCl_3$ (general procedure C) that led to a mixture of compounds, Boc protection of the amino group of **1c** was carried out (general procedure A) and was followed by reaction using *sec*-butyllithium providing **4c**. The 5-substituted indole phenylmethanone derivatives **4a**−**c** were converted to the related racemates **7a**−**c** by successive treatments from general procedures D−F: seven-membered ring formation using the ethyl glycinate hydrochloride (**5a**−**c**), incorporation of the primary amino group via oxime conversion (**6a**−**c**), and reduction (**7a**−**c**). The chiral intermediates **8a**−**c** were then resolved via crystallization using either *N*-acetyl-L-phenylalanine, *N*-acetyl-D-phenylalanine, or di-*p*-toluyl-D-tartaric acid to afford **8a**−**c**, respectively (general procedure G). The final nitro and amino analogues were obtained by nitration of **8a** on position 9 of the benzodiazepine core leading to the intermediate **8d**.[53] To elaborate a new aminodiazepinoindole series with a variety of final primary analogues (**9e**−**20e**) and substituted aminodiazepinoindole derivatives (**9h, 14f**−**h**), the nitro intermediate **8d** was reduced in the presence of tin chloride providing the key chiral diamino target **8e**.

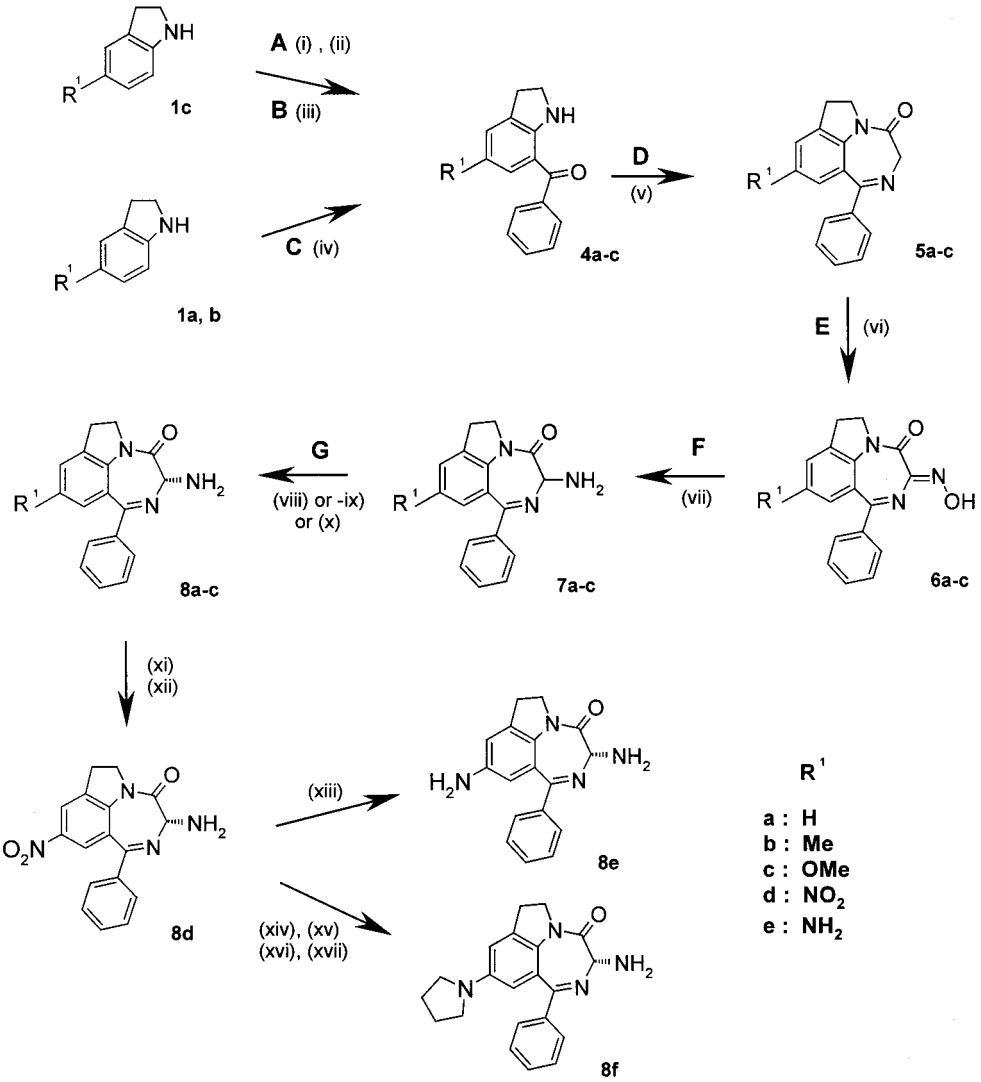
As illustrated in Scheme 2, all the final analogues were obtained via various classical condensation routes: condensation between **8a**−**f** with pentafluorophenyl esters derived from the related carboxylic acids providing compounds **9a**,**c, 16c**, and **17b,c** (general procedure H); condensation between **8a**−**f** with acidic chlorides in pyridine producing compounds **9b**,**f** and **13b** or in dichloromethane/triethylamine for **10d** (general procedure I); or condensation between **8a**−**f** with carboxylic acids in solvent, in the presence of a base and a coupling reagent − dichloromethane/diisopropylethylamine (DIEA)/*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TOTU) (general procedure J) or dimethylformamide/triethylamine (TEA)/bromotris(pyrrolidino)-phosphonium hexafluorophosphate (PyBrop) (general procedure K) − giving respectively compounds **9e**, **10a**,**e**, **11a,b**,**e**, **13e**, **14b**,**e**, **15e**, **16e**, **17d,e**, **18b,c**,**e,f**, and **12a**,**e**. The diamino derivatives **19a,e** and **20e** were produced by Boc deprotection of the respective compounds **12a**,**e** and **15e** (general procedure B). Various substituents on the 9-amino group were incorporated at the last step of the synthesis by classical chemical routes from **9e** and/or **14e**: acetylation provided **9h** and **14h** (general procedure L), pyrrolidine formation with dibromoethane afforded **14f** (general procedure M), and dimethylation by treatment using formaldehyde and sodium borohydride produced **14g** (general procedure N). Alternatively, syntheses of pyrrolidine derivatives **14f**, **9f**, and **18f** were elaborated via formation of the pyrrolidine intermediate **8f** (Scheme 1) obtained by successive steps from **8d** (Boc protection, hydrogenation using Ru/C (5%), dibromobutane substitution, and final Boc deprotection).

Biology

For the high-throughput screening, a PDE4 isozyme mixture from the U937 human cell line was used. The optimization of PDE4 selectivity vs other PDEs was carried out using isolated PDE3 (dog aorta) and a mixture of PDE1/5 (guinea pig trachea). As in vitro PDE4 activity was described as being related to in vitro inhibition of TNFα release for PDE4 inhibitors such as Rolipram and Ariflo, our selected compounds were evaluated for in vitro TNFα-related potency from human peripheral blood monocytes (hPBMC) and from human whole blood (hWB).[54,55]

In vivo activity of selected PDE4 inhibitors was determined in an asthma-related model by measuring the

Scheme 1[a]



[a] Reagents: general procedure A: (i) $O(CO_2tBu)_2$, THF; (ii) $(CH_3)_2NCH_2CH_2N(CH_3)_2$, Et_2O, sBuLi, $C_6H_5CO_2CH_3$; general procedure B: (iii) trifluoroacetic acid, CH_2Cl_2; general procedure C: (iv) BCl_3, CH_2Cl_2, C_6H_5CN, $AlCl_3$; general procedure D: (v) $H_2NCH_2CO_2Et$, Py; general procedure E: (vi) tBuOK, THF, iAmONO; general procedure F: (vii) H_2, 5% Ru/C, MeOH; general procedure G: (viii) *N*-acetyl-L-phenylalanine, EtOAc or (ix) *N*-acetyl-D-phenylalanine, EtOAc or (x) di-*p*-toluoyl-D-tartaric acid, PhCN; (xi) H_2SO_4, KNO_3; (xii) concd NH_4OH; (xiii) $SnCl_2$, EtOH; (xiv) $O(CO_2tBu)_2$, THF; (xv) H_2, 5% Ru/C, MeOH; (xvi) $Br(CH_2)_4Br$, $NaHCO_3$, DMF; (xvii) trifluoroacetic acid, CH_2Cl_2 (general procedure B).

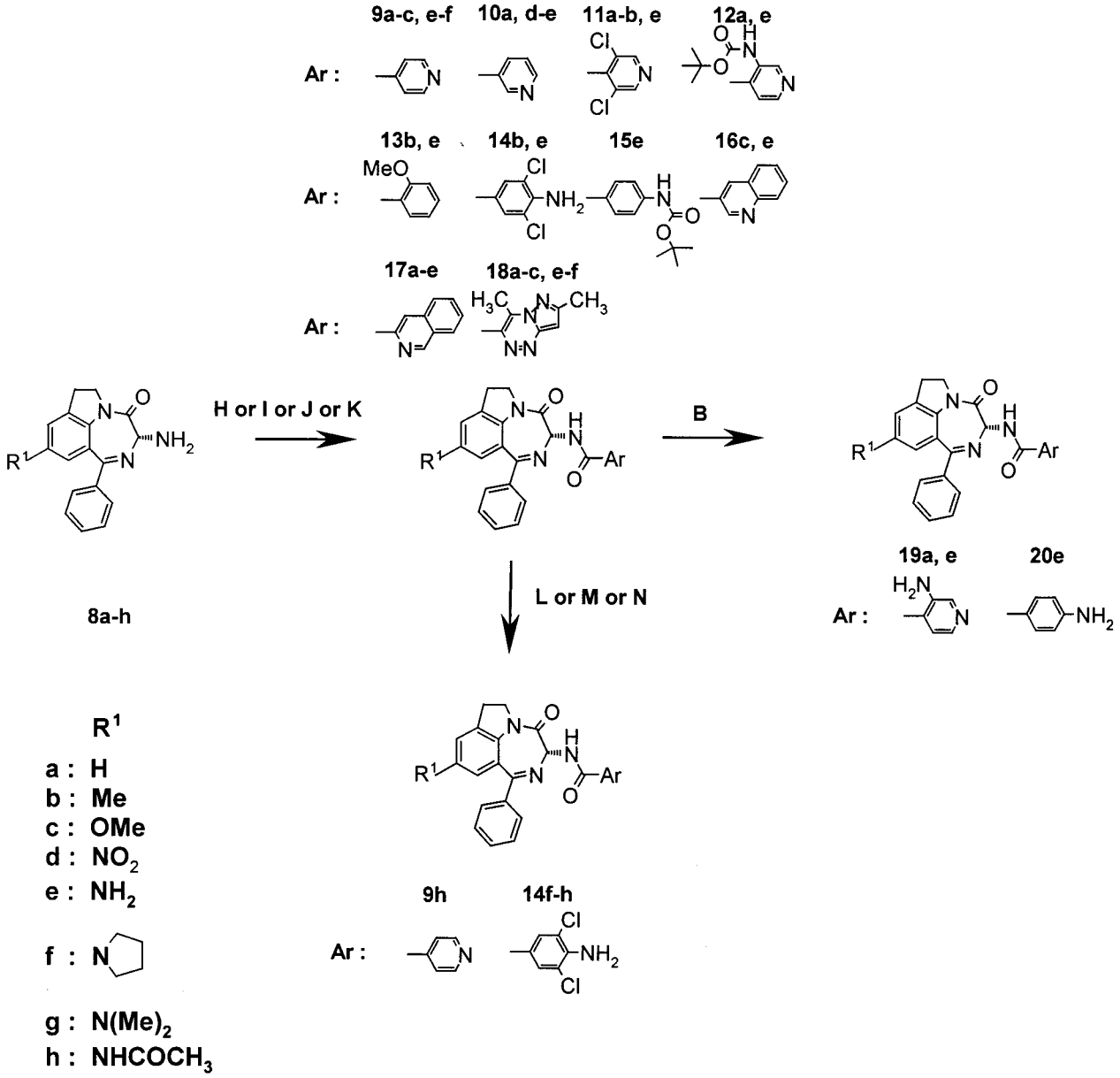
ability to block the eosinophil recruitment in bronchoalveolar lavages of sensitized and ovalbumin-challenged Brown-Norway rats.[56] Compounds were subsequently tested via the same route of administration in a modified model of in vivo inhibition of LPS-stimulated TNFα release in Wistar rats.[57] To measure PDE4-related side effects, emetic activity (vomiting and retching events) was determined in ferrets after iv administration and the ability of compounds to compete for high-affinity [^{3}H]Rolipram binding site (HARBS) was evaluated.[26]

Structure−Activity Relationships

As mentioned in the Introduction, in the early 1990s, our chemical library was screened against PDE4 enzymes. Among various chemical hits identified, PD-0190749 (Chart 1) was determined to be an interesting chemical hit. This compound was not structurally related to PDE4 inhibitors described in the literature and particularly was not a Rolipram-like derivative. Earlier, SAR studies on the new diazepinoindole series were performed leading to pharmacophore determination.[45] The five-membered ring of the multiring diazepinoindole nucleus was found to be strongly implicated in PDE4 inhibitory potency as well as the indicated absolute configuration of the asymmetric carbon. Various substitutions on the different aromatic moieties were undertaken, and the initial SAR studies led to the identification of **9b** (CI-1018) which was declared a candidate for development.[44,45,47] CI-1018 is a PDE4 inhibitor with some properties that were not optimized. Therefore, SAR optimization of CI-1018 was undertaken with the aim of improving in vitro PDE4 activity, PDE4 selectivity vs PDE1, -3, and -5, in vitro inhibition of TNFα release from hWB, in vivo activities in rats after oral administration in both inhibition of antigen-induced airway eosinophil recruitment and inhibition of LPS-stimulated TNFα release, and, finally, keeping a good safety profile with no emesis at therapeutic doses.

With the initial SAR data in hand, a chemistry program was initiated to evaluate the effects of substitutions on both aromatic rings (R^1 and Ar).

Scheme 2[a]



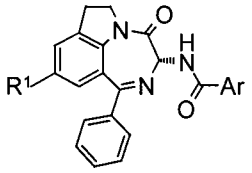
[a] Reagents: general procedure H: $ArCO_2H$, pentafluorophenol, 4-(dimethylamino)pyridine *p*-toluenesulfonate, CH_2Cl_2; general procedure I: ArCOCl, Py; general procedure J: $ArCO_2H$, TOTU, DIEA, CH_2Cl_2; general procedure K: $ArCO_2H$, PyBrop, DMF, TEA; general procedure B: trifluoroacetic acid, CH_2Cl_2; general procedure L: acetic anhydride, Py; general procedure M: $Br(CH_2)_4Br$, PhCN; general procedure N: HCHO, PhCN, $NaNH_3CN$, CH_3CO_2H.

As reported in Table 1, introduction of a methyl, methoxy, or primary amino group at the 9-position of the diazepinoindole derivatives enhanced between 2- and 100-fold in vitro PDE4 activity (**9b,c,e**, **10e**, **11b,e**, **12e**, **17b,c,e**, **19e** in comparison respectively to **9a**, **10a**, **11a**, **12a**, **17a**, **19a**). However, in comparison to the methyl and methoxy derivatives, the primary amino analogues were found the most potent PDE4 inhibitors with submicromolar PDE4 IC_{50}s (**9e**−**14e**, **16e**−**19e**), similar to that of Rolipram. The best primary amino derivative in terms of in vitro PDE4 activity and a high PDE4 selectivity vs other PDEs was **14e** with a PDE4 IC_{50} of 0.04 μM (20-fold more potent than Rolipram (0.93 μM) and similar to Ariflo (0.03 μM)). Moreover, in the cases tested, the NH_2 group, instead of both methyl and methoxy substituents, provided improved PDE4 selectivity vs PDE1, -3, and -5 **(9e**, **11e**−**14e**, **16e**−**17e**). Replacement of the amino moiety with a nitro substituent induced drastic reduction of PDE4 inhibitory activity (**10d**, **17d**).

With the above promising data of the primary amino derivatives, various secondary or tertiary amino analogues were evaluated to determine if they maintained promising in vitro PDE4 activity and selectivity. On the basis of the results of the in vitro PDE4 testing of the mono- and disubstituted amino compounds (**9f**,**h**, **14f**−**h**, **18f**), it was observed that the substitutions of the NH_2 group induced a reduction in PDE4 selectivity vs other PDEs (PDE4 vs PDE3 selectivity was decreased between 2- and 80-fold). Subsequently, the above compounds were not selected for further evaluations.

As mentioned above, previous SAR studies were carried out leading to pharmacophore determination. However, in the aromatic related area of the main chain,

Table 1. SARs of Aminodiazepinoindoles and PDE Inhibition



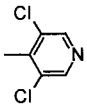
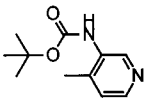
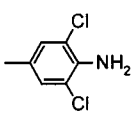
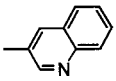
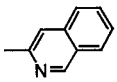
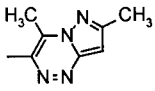
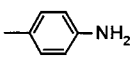
compd	Ar	R^1	PDE4 U937 human cells IC_{50}[a] (μM) ±SEM (n)	PDE3 dog aorta IC_{50}[a] (μ) ±SEM (n)	PDE1/5 guinea pig trachea IC_{50}[a] (μM) ±SEM (n)
Rolipram			0.93 ± 0.17 (3)	>100 (6)	94.9 ± 17.1 (8)
Ariflo			0.03 ± 0.01 (3)	nt[b]	nt[b]
9a		H	20.5 ± 4.2 (3)	>100	>100 (3)
9b		CH_3	1.1 ± 0.1 (30)	35.8 ± 11.4 (12)	73.4 ± 19.5 (4)
9c		OCH_3	4.4 ± 4.4 (3)	40.8 ± 10.2 (3)	16.1 ± 2.5 (3)
9e		NH_2	0.5 ± 0.2 (4)	>100 (3)	>100 (3)
9f		Pyrrolidin	1.4 ± 0.6 (3)	18.0 ± 1.4 (3)	34 ± 4.9 (3)
9h		$NHCOCH_3$	0.7 ± 0.1 (5)	70.0 ± 30.7 (3)	30.1 ± 18.3 (3)
10a		H	10.5 ± 1.4 (7)	>100 (3)	>100 (3)
10d		NO_2	10.8 ± 1.92 (3)	nt[b]	nt[b]
10e		NH_2	0.27 ± 0.02 (3)	>100 (3)	>100 (3)
11a		H	4.7 ± 0.9 (3)	>100 (3)	>100 (3)
11b		CH_3	1.0 ± 0.02 (4)	19.5 ± 2.2 (3)	38.0 ± 9.5 (3)
11e		NH_2	0.20 ± 0.08 (4)	80.0 ± 21.0 (3)	>100 (3)
12a		H	6.2 ± 1.7 (4)	54.7 ± 23.7 (3)	53.3 ± 23.3 (3)
12e		NH_2	0.30 ± 0.09 (3)	58.2 ± 15.5 (3)	67.7 ± 19.4 (4)
13b		CH_3	1.3 ± 0.4 (4)	28.8 ± 2.9 (3)	50.6 ± 13.7 (3)
13e		NH_2	0.5 ± 0.1 (3)	>100 (3)	93.2 ± 7.8 (4)
14b		CH_3	0.30 ± 0.04 (4)	10.3 ± 4.0 (3)	>100 (3)
14e		NH_2	0.040 ± 0.004 (6)	51.6 ± 20.3 (3)	>100 (3)
14f		Pyrrolidin	0.080 ± 0.017 (5)	10.6 ± 2.4 (3)	39.2 ± 31.0 (3)

Table 1 (Continued)

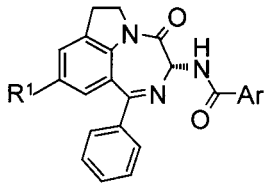
compd	Ar	R^1	PDE4 U937 human cells IC_{50}[a] (μM) ±SEM (n)	PDE3 dog aorta IC_{50}[a] (μ) ±SEM (n)	PDE1/5 guinea pig trachea IC_{50}[a] (μM) ±SEM (n)
14g		$N(CH_3)_2$	0.20 ± 0.04 (4)	3.2 ± 1.9 (3)	5.3 ± 2.5 (3)
14h		$NHCOCH_3$	0.090 ± 0.019 (4)	4.5 ± 2.7 (3)	3.5 ± 2.5 (3)
16c		OCH_3	1.7 ± 0.4 (3)	19.4 ± 6.7 (3)	16.1 ± 7.7 (3)
16e		NH_2	0.20 ± 0.03 (4)	53.9 ± 23.7 (3)	90.7 ± 10.2 (4)
17a		H	5.5 ± 0.4 (17)	>100 (3)	>100 (3)
17b		CH_3	2.6 ± 0.4 (17)	>100 (3)	>100 (3)
17c		OCH_3	1.6 ± 0.8 (4)	82.3 ± 18.7 (3)	65.3 ± 13.6 (3)
17d		NO_2	>100 (1)	nt[b]	nt[b]
17e		NH_2	0.10 ± 0.03 (5)	48.3 ± 18.3 (3)	>100 (3)
18b		CH_3	0.7 ± 0.2 (5)	>100 (3)	>100 (3)
18c		OCH_3	2.7 ± 0.5 (5)	46.0 ± 5.0 (3)	14.1 ± 1.5 (4)
18e		NH_2	0.20 ± 0.04 (4)	48.6 ± 18.0 (4)	72.0 ± 17.1 (4)
18f		Pyrrolidin	0.20±0.1 (4)	22.2±10.2 (3)	>100(3)
19a		H	16.2 ± 7.7 (4)	90.3 ± 10.2 (3)	>100 (3)
19e		NH_2	0.7 ± 0.2 (4)	>100 (3)	>100 (3)
20e		NH_2	0.69 ± 0.10 (4)	86.8 ± 14.2 (3)	93.2 ± 7.7 (4)

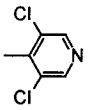
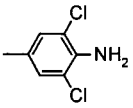
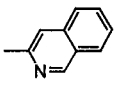
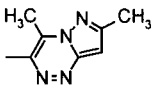
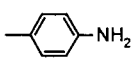
[a] IC_{50} values are the average of at least 3 independent determinations. [b]nt, not tested.

conclusions were ambiguous. Presented here are the most effective compounds with various heteroaromatic rings (**9a**−**e**, **10a**,**d**,**e**, **11a**,**b**,**e**, **12a**,**e**, **16c**,**e**, **17a**−**e**, **18b**,**c**,**e**, **19a**,**e**) or substituted phenyl moieties (**13b**,**e**, **14b**,**e**, **20e**) that were recognized as being the most positive features for in vitro PDE4 activity.

Optimization of activity of the new aminodiazepinoindole series in the hPBMC and hWB in vitro TNFα release assays has been a primary focus (Table 2). Compared to CI-1018 (**9b**), the most potent PDE4 inhibitors were found to be 2−100-fold more active in blocking TNFα liberation from hPBMC with an IC_{50} of 0.011 μM for the best compound (**14e**), which was comparable to that for Rolipram. Looking at in vitro TNFα inhibition assays run in more physiological conditions, more significant was the inhibitory potency of the new amino series in hWB. Replacement of CH_3 by NH_2 led to analogues that were by far more effective than the methyl-substituted compounds: **9e** and **18e** displaying IC_{50}s of 1.09 and 0.750 μM were 55- and 40-fold more active than their corresponding methyl compounds, **9b** and **18b**. The most potent PDE4 inhibitors provided

Table 2. Inhibition of TNFα Release from Human Peripheral Blood Monocytes and Human Whole Blood



compd	Ar	R^1	hPBMC IC_{50}[a] (μM) ±SEM (*n*)	hWB IC_{50}[a] (μM)) ±SEM (*n*)
Rolipram			0.083 ± 0.035 (3)	0.940 ± 0.110 (3)
Ariflo			nt[b]	7.30 ± 0.19 (2)
9b		CH_3	0.990 ± 0.089 (22)	61 (14)
9e		NH_2	0.480 ± 0.230 (4)	1.090 ± 0.340 (4)
10e		NH_2	0.340 ± 0.180 (3)	1.240 ± 0.390 (3)
11e		NH_2	0.038 ± 0.009 (3)	0.213 ± 0.066 (3)
13e		NH_2	0.123 ± 0.014 (4)	1.061 ± 0.281 (3)
14e		NH_2	0.011 ± 0.001 (2)	0.438 ± 0.081 (2)
17e		NH_2	0.048 ± 0.007 (2)	3.366 ± 0.590 (2)
18b		CH_3	5.350 ± 2.670 (3)	>30 (2)
18e		NH_2	0.260 ± 0.072 (3)	0.750 ± 0.120 (3)
19e		NH_2	0.080 ± 0.016 (3)	0.398 ± 0.133 (4)
20e		NH_2	0.443 ± 0.164 (3)	1.52 ± 0.49 (2)

[a] IC_{50} values are generated from the plot of percent inhibition vs concentration. [b] nt, not tested.

submicromolar IC_{50}s in hWB, similar to the Rolipram value (0.94 μM) but lower than the Ariflo value (7.30 μM), thus showing an important improvement within the new aminodiazepinoindole family.

To determine whether the improved potency could be maintained upon oral administration, we evaluated the ability of the most promising compounds to inhibit LPS-stimulated TNFα release and antigen-induced

eosinophil recruitment in rats (Table 3). In vivo, CI-1018 (**9b**) was not active (25% inhibition at 10 mg/kg po) in the TNFα-related assay, but its amino analogue (**9e**) inhibited TNFα release by 71% at 10 mg/kg po. In vivo CI-1018 inactivity in the TNFα-related model might be related to the lack of in vitro TNFα release inhibition in hWB. The most potent candidates provided efficient in vivo TNFα inhibition of more than 70% at 10 mg/kg po (**9e**−**10e**, **12e**, **19e**, **20e**). These activities were in the range of activity of Ariflo with an ED_{50} value of 3 mg/kg po. The inactivity of **17e** correlated with its poor in vitro TNFα inhibition in whole blood (IC_{50} = 3.37 μM) and its weak bioavailability in the rat as well (<10%, data not shown). In the antigen-induced lung eosinophilia model, CI-1018 (**9b**) inhibited eosinophil recruitment with an ED_{50} value of 5.06 mg/kg po. As illustrated in Table 3, CI-1018 amino analogue (**9e**) was found ∼2-fold more active than CI-1018. Moreover, various other examples showed that CH_3 substitution with a NH_2 group led to more potent compounds than Rolipram, Ariflo (not active in this model), and CI-1018 with ED_{50}s in a range between 1.8 and 11 mg/kg po (**9e**, **10e**, **12e**, **18e**, **20e**).

Finally, selected compounds failed to show emetic liability in the ferret upon iv administration (Table 4). While Rolipram and Ariflo induced several retching and emetic events at 0.1 mg/kg (37.0 in 90 min) and 10 mg/kg (59.5 in 90 min), respectively, most of our selected compounds displayed no or very few retching and emetic events (3.5 in 90 min) at 4 or 10 mg/kg, similar to the CI-1018 safety profile. The lack of emetic side effects within this series was observed for compounds showing HARBS IC_{50}s between 300 and 3000 nM which are low affinities compared to Rolipram and Ariflo IC_{50}s at 5.3 and 49.0 nM, respectively.

In addition, no PDE4 subtype selectivities with respect to PDE4A−D were observed (data not shown) with this new series including the compounds evaluated for emesis (Table 4). These results would suggest that the strategy based on the selective PDE4 subtype compound identification is not the path forward to nonemetic candidates.

Conclusions

In conclusion, a new structural series of PDE4 inhibitors was discovered leading to compounds with submicromolar PDE4 activity and PDE4 selectivity vs PDE1, -3, and -5. These derivatives showed an improvement in therapeutic potential as they inhibited TNFα release from hWB in the same concentration range obtained from purified hPBMC. In vivo, the new candidates showed improved potency and efficacy in inhibiting eosinophil recruitment and TNFα release in rats and displayed no emetic propensity at therapeutic doses. Optimization provided a back-up compound to CI-1018 (CI-1044, **10e**) showing potent inhibition of PDE4 activity both in vitro and in vivo and maintaining the good safety profile of our previous compound in development.[58] In addition, compared to Ariflo − the current standard in development against COPD and asthma − our most potent candidates displayed improvements in terms of in vitro and in vivo activities and increased therapeutic index between efficacy and emesis.

Experimental Section

Biology. 1. High-Throughput PDE Activity. The PDE assay was adapted to a 96-well microtiter plate format with modified method.[59] Reaction components were added to 96-well microtiter plates in sequence: 4 μL of compound or DMSO, 50 μL of 4X assay buffer (40 mM Tris, 5 mM $MgCl_2$, 4 mM β-mercaptoethanol, pH 8), 150 μL of [^{3}H]cAMP/cAMP (0.1 μCi/1 μM), 20 μL of PDE enzyme (for blank, 1 mg/mL BSA was added). Components were mixed in microtiter plates. Plates were incubated at 37 °C for 1 h. The reaction was stopped by adding 25 μL of 0.4% TCA. From each well of the reaction plate, 200 μL of the reaction mix was applied to 50 mg of alumina hydroxide/well, equilibrated with washing buffer (0.1 M TES, pH 8) using the Millipore multiscreen plates in conjunction with a Pall Industries silent monitor system (Loprodyne microtiter plates). The unbound [^{3}H]cAMP was washed from alumina with 3 mL/well wash buffer. [^{3}H]AMP was subsequently eluted with 4 × 0.25 mL of NaOH. Each fraction was collected in picovials and 4.5 mL of scintillant was added. After mixing, radioactivity measurement was carried out with a scintillation counter.

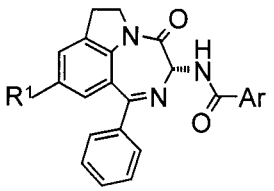
2. PDE Purification. U937 cells (PDE4), obtained from European Collection of Animal Cell Cultures, were stored in liquid nitrogen. Each experiment was carried out between the 5 and 20 generations of cells. The pellet of cells was diluted by extraction buffer (10 mM Tris HCl, 2 mM $MgCl_2$, 1 mM dithiotreitol (DTT), pH 7.5, 2 U/mL aprotinin) at 2.5 × 10^7 cells/mL. Enzymatic homogenate was obtained by sonication with microwaves (Labsonic U sonicator, Braun, 3 × 15 s + 30 s between each sonication) at 4 °C. Homogenate was centrifuged (40000*g* for 30 min; L5-50B Beckman, rotor TI50 Beckman) at 4 °C and supernatant, containing the soluble material, was stored at −80 °C.

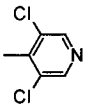
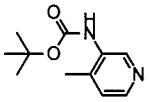
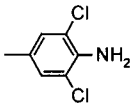
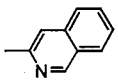
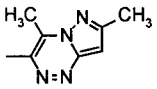
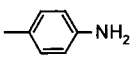
Guinea pig tracheas (PDE1,5) or dog aorta (PDE3) were weighed on Teflon small dishes put on dry ice. Tissues were cut in 200-μm and 50-μm small cubes with a chopper (chopper tissue, Mickle Lab) in the presence of extracting buffer (10 mM Tris, 1 mM $MgCl_2$, 1 mM DTT, 2 U/mL aprotinin, pH 7.5). The preparation was homogenized (polytron: sonde PT20, 3 × 20 s + 30 s between each homogenization, speed 10) at 4 °C in 4 volumes of extracting buffer. The homogenate was sonicated with microwaves (Labsonic U sonicator Braun, 3 × 15 s + 30 s between each sonication) at a similar temperature. The homogenate was centrifuged (40000*g* for 30 min, L5-50B Beckman, rotor TI50 Beckman) at 4 °C and supernatant, containing the soluble material, was frozen at −80 °C.

PDE4 isoenzymes and PDE3 enzymes were separated using an anion-exchange column (Mono-Q HR 5/5, volume = 1 mL, ref 17 0546.01, Pharmacia Biotech) according to the Lavan technical procedure.[60] This column was coupled with fast protein liquid chromatography (FPLC; Pharmacia). The flow was 1 mL/min and the column was preequilibrated with 10 column volumes of elution buffer (20 mM Bis Tris, 10 mM EDTA, 2.5 mM DTT, adjusted to pH 6.5 with 10 N HCl, 2 mM benzamidine, 2 mg/mL soybean trypsin inhibitor, filtered (0.22 μm), 10 mg/mL bacitracin added extemporaneously). The enzymatic sample was filtered (0.22 μM) and put on the top of column. Column was washed with 10 volumes of elution buffer. The bound proteins were obtained using NaCl gradient (0−1 M) and samples (1.5 mL) were collected at 4 °C. BSA (0.1 mg) was added to protect enzymatic activity. From each sample, 100 μL was conserved at 4 °C for analysis and characterization of enzymatic content using Rolipram and Siguazodan, selective PDE4 and PDE3 inhibitors, respectively. The cAMP-dependent Rolipram-sensitive enzymatic fractions were pooled and aliquots (500 μL) were stored at −80 °C as PDE4 enzymes (U937). The cAMP-dependent and Siguazodan cGMP-sensitive enzymatic fractions were pooled and aliquots (500 μL) were stored at −80 °C as PDE3 enzymes (dog aorta).

PDE1,5 enzymes were separated using an anion-exchange column (DE52 = column of diethyl aminocellulose (Whatman, ref 4057 050) contained in Polyprep (Biorad, ref 731-1550) according to the technical procedure used by Thompson et al.[61,62] The DE52 column was preequilibrated under agitation

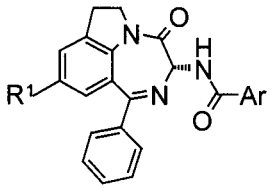
Table 3. In Vivo Inhibition on Antigen-Induced Eosinophil Recruitment in Brown-Norway Rats and in Vivo Inhibition on LPS-Induced TNFα Release in Wistar Rats

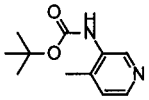
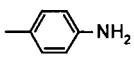


compd	Ar	R^1	Inh. eosinophils ED_{50}[a] (mg/kg po) [min;max] (*n*)[b] or % inh.[c] at 10 mg/kg po	Inh. TNFα ED_{50}[d] (mg/kg po) [min;max] (*n*) % inh.[e] at 10 mg/kg po [min;max]
Rolipram			4.66[f] [2.32;9.36] (7-8)	-94% [-89%;-99%]
Ariflo			-25.0%[g] ns[h] (?)	3.0
9b		CH_3	5.06[g] [3.24;6.88] (8)	-25% [0%;-52%]
9e		NH_2	1.89[g] [0;3.80] (8)	-71% [-32%;-87%]
10e		NH_2	3.2[g] [0;10.63] (8)	2.8 ± 1.9[i] -75% [-65%;-89%]
11b		CH_3	+63.7%[f] ns[g] (7)	nt[j]
12e		NH_2	5.03[f] [3.08;7.97] (9)	-87% [-62%;-100%]
14b		CH_3	-21%[g] ns[h] (8)	nt[j]
14e		NH_2	-61.9%[f] (9)	nt[j]
17b		CH_3	10.7[g] [3.44;33.26] (6)	nt[j]
17e		NH_2	-62.9%[g] (9)	-16% ns[h]
18b		CH_3	+33.9%[f] ns[h](7)	nt[j]
18e		NH_2	1.8[g] [0.86;3.72] (7)	nt[j]
19e		NH_2	-82.5%[f] (7)	-89% [-76%;-100%]
20e		NH_2	10.84[f] [1.49;17.63] (8)	-71% [-65%;-100%]

[a] ED_{50} values generated by linear regression of inhibition curve on at least 3 doses and expressed in mg/kg after oral administration 24 and 3 h before the antigen challenge. [b] Number of animals per group. [c] Percentage inhibition of eosinophil recruitment in bronchoalveolar lavages of animals treated orally at 10 mg/kg. Statistical analysis of inhibition percentage was performed using the nonparametric Mann and Whitney U test: $p < 0.001$ was considered when ns is not indicated. [d] ED_{50} values generated by linear regression of inhibition curve. [e] Percentage inhibition of LPS-induced TNFα release of animals treated orally at 10 mg/kg. [f] Methylcellulose (1%) as vehicle. [g] Methylcellulose (1%) + polyethylene glycol 400 (95/5) as vehicle. [h] ns, not significant. [i] Mean ED_{50} value from 2 separate experiments. [j] nt, not tested.

Table 4. Emesis in Ferrets and [^{3}H]Rolipram Binding Assay in Wistar Rats



compd	Ar	R^1	Emesis R+V[a] (90 min) ± SEM after iv route	HARBS Wistar rats IC_{50} (nM) ± SEM (*n*)
Rolipram			36.5[b] ± 0.6 (4)	5.3 ± 0.3 (7)
Ariflo			59.5[c] ± 21.0 (6)	49.0 ± 2.4 (4)
9b		CH_3	0[c]	>3000 (3)
9e		NH_2	0[c]	654 ± 72 (3)
10e		NH_2	0[d]	1700 ± 300 (4)
12e		NH_2	0[c]	309 ± 36 (2)
19e		NH_2	3.5[c] ± 3.8 (2)	340± 38 (2)
20e		NH_2	0[c]	>1000 (3)

[a] R + V indicates the number of retching and vomiting events per 90 min. The data is a mean value obtained on a minimum of 5 ferrets. [b] At 0.6 mg/kg. [c] At 10 mg/kg. [d] At 40 mg/kg. IC_{50} refers to the concentration of tested compounds inducing an inhibition of 50% of [^{3}H]Rolipram binding on rat brain membranes. IC_{50} values are the average of 3 independent determinations, with variation in individual values of <10%.

with buffer I (70 mM sodium acetate buffer, 5 mM β-mercaptoethanol, pH 6.5). 2 mL DE52 suspension was distributed in each Polyprep column and washed with 5 mL of buffer I.

Enzymatic sample (2 mL) was applied on the column and washed with 3 × 5 mL of buffer I. The bound proteins were obtained with successive elutions (1 × 4 mL and 2 × 1 mL) of buffer II (220 mM sodium acetate buffer, 5 mM β-mercaptoethanol, pH 6.5). Only elution samples provided from buffer II and displaying an efficient PDE1,5 activity characterized by the selective inhibitory effect of Zaprinast (mixed PDE1,5 inhibitor) were stored at −80 °C as PDE1,5 enzymes (guinea pig trachea).

3. In Vitro Inhibition of TNFα from hPBMC and hWB. Heparinized normal human blood was centrifuged at 500*g* for 10 min at room temperature to sediment erythrocytes. The leukocyte-rich layer was removed, diluted with phosphate-buffered saline (PBS) to 40 mL, and layered over Histopaque (1.077 sp gr; Sigma, St. Louis, MO), after which the cells were centrifuged at 1100*g* for 20 min at room temperature. The mononuclear cell interface was removed and washed × 3 with PBS. The resulting mononuclear cells (PBMC) were resuspended at 2.0 × 10^6 cells/mL in RPMI-1640 containing 10% heat-inactivated fetal bovine serum (FBS). For the whole blood assay, human whole blood (hWB) was diluted with an equal volume of RPMI-1640 without FBS prior to use.

Compounds were dissolved and diluted in DMSO, followed by a 1:100 dilution in RPMI-1640 not containing FBS. The final in-well concentration of DMSO was 0.25% for all conditions. Compound dilutions (50 μL) were preincubated with either PBMC or hWB (100 μL/well) in a 96-well flat bottom plate for 15 min at 37 °C in a 5% CO_2 incubator. 50 μL of lipopolysaccharide (LPS; *E. coli* 055:B5, Difco Laboratories, Detroit, MI), diluted in RPMI-1640, was added to give a final concentration of 1 μg LPS/mL. Plates were incubated for 18−20 h as described above, then centrifuged at 500*g* for 5 min at 4 °C to pellet cells, after which supernatants (120 μL) were collected and stored at −20 °C. Supernatants were evaluated for TNFα by ELISA (R&D Systems, Minneapolis, MN).

Positive control wells were treated with LPS without addition of test compound. Medium control wells were treated

with neither test compound nor LPS. Percent inhibition was calculated as: {1 − [(drug-treated) − (medium control)] ÷ [(positive control) − (medium control)]} × 100. IC_{50}s are the concentrations of compounds (in μM) that reduced the maximum release by about 50%. IC_{50}s were generated from the plot of percent inhibition vs concentration, using Table Curve 2D (SPSS Inc., Chicago, IL).

4. In Vivo Inhibition of Aerosolized Antigen-Induced Airway Eosinophil Recruitment in Rats. Inbred male Brown-Norway rats (weight 175−200 g) were sensitized twice at 14-day interval by ip and sc injection of 0.5 mL of a suspension containing ovalbumin (OA; 2 mg/mL) and aluminum hydroxide ($Al(OH)_3$; 40 mg/mL) in 0.9% saline. From 22−30 days after the initial sensitization, rats were exposed to an aerosol of either OA (10 and 50 mg/mL in 0.9% saline for 30 min each concentration) or 0.9% saline. Aerosol exposure was accomplished by placing the rats in a Plexiglass chamber connected to a Devilbiss ultrasonic nebulizer (model UltraNEB 99); 48 h later, rats were anesthetized with sodium pentobarbitone (6%, 2.5 mg/kg ip; Sanofi Santé Animale) and the lungs were lavaged with 3 × 5 mL of 0.9% sterile saline containing 1 mg/mL EDTA (Sigma) via polyethylene tube through an upper tracheostomy site. Lavage fluid was centrifuged (350*g* for 15 min at 4 °C) and the cell pellet was resuspended in 5 mL of PBS without calcium and magnesium. Total cell count per milliliter of bronchoalveolar lavage (BAL) fluid was determined by diluting 200 μL of sample in 20 mL of Isoton II (Coultronics), adding 4 drops of Zapoglobin II (Coultronics) to lyse erythrocytes, and reading the sample using a Coulter Counter Z2 (Coultronics). To determine the percent composition of each leukocyte type, slides from each rat BAL sample were obtained by centrifuging 50 μL of the lavage fluid for 10 min at 700 rpm in a Cytospin 2 centrifuge (Shandon). The slides were stained with May Grünwald-Giemsa for differential cell count. At least 200 leukocytes were counted on each slide and differentiated into macrophages, eosinophils, and neutrophils following morphological criteria. Sensitized animals were treated orally 24 and 3 h before the aerosol challenge either with vehicle (control group) or with test compound (test group) (n = 6−9 animals/group). The vehicles used were 1% methylcellulose or 1% methylcellulose + polyethylene glycol 400 (95/5).

5. In Vivo Inhibition of TNFα Release. This assay was used to evaluate the ability of a compound to inhibit in vivo the TNFα release after LPS stimulation. Groups of animals were constituted at random with 7 Wistar rats/group. The LPS concentration, the timing of drug administration, and the time of sacrifice were previously determined using Rolipram as a selective PDE4 inhibitor. LPS was suspended in sterile saline and injected ip at a dose of 0.5 mg/kg in a volume of 3 mL/kg of bodyweight. The compound (10 mg/kg) or the vehicle as a suspension prepared with 1% methylcellulose + 5% polyethylene glycol 400 was orally administered in a volume of 2 mL/kg, 30 min before LPS. Blood (5 mL) was collected in EDTA tubes 90 min after LPS stimulation, plasma was separated after centrifugation (1400*g* for 15 min at 4 °C), and aliquots (400 μL) were stored at −80 °C.

Plasma TNFα levels were estimated by ELISA assay from diluted samples according to the manufacturer's instructions (kit for rat TNFα, Genzyme Corp., Cambridge, MA). Appropriate dilutions were performed in order to obtain absorbance readings within the range of standard curve. For each compound, the data were expressed as a percent inhibition of plasma TNFα in treated vs nontreated LPS-stimulated rats.

6. Ferret Emesis Assay. Male ferrets (1−1.4 kg; Marshall Farm) that were to be administered compound (or vehicle) received chronic left jugular vein cannulation 72 h prior to the start of the experiments. Compounds were dissolved into hydroxy-β-cyclodextrin (20% v/w) and administered iv at 1 mL/kg. Then, animals were transferred to individual observation cages and observed continuously over 90 min. Their behavior was recorded by video camera and tapes were subsequently read at the end of the experiment to score emesis. Emesis was defined as rhythmic abdominal contractions that were either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting) or were not associated with the passage of material (i.e. retching movements). Episodes of retching were considered separate, i.e. "bouts" when the animal changed it location in the observation cage or when the interval between retches exceeded 5 s. Data are expressed as number of emetic events (vomiting + bouts of retches) scored throughout the 90-min observation period.

7. [^{3}H]Rolipram Binding Assay in Rats. Affinity of tested compounds for high-affinity Rolipram binding site (HARBS) was evaluated on Wistar rat brain membranes. Brain material was washed with PBS and homogenized in 10 parts (v/w) of buffer A (20 mM Tris HCl pH 7.5, 2 mM $MgCl_2$, 0.1 mM DTT). The pellet fraction was then separated by centrifugation (30 min at 30000*g*), resuspended in the Tris buffer volume, aliquoted by 1-mL fractions (8−10 mg/mL) and stored at −80 °C. Competitive experiments for HARBS radiolabeled with [^{3}H]-Rolipram was carried out according to a modification of Schneider's protocol, i.e. by adding successively in 96-well microplates increasing concentrations of tested compounds, DMSO for total binding, or Rolipram (1 μM final concentration) for nonspecific binding.[26] In each well, 80 μL of buffer A, 50 μL of AMP, 0.05% BSA, 20 μL of [^{3}H]Rolipram (2 nM final concentration) and 100 μL of rat brain membranes (0.8 mg protein/mL) were added. Microplates were incubated for 60 min at 25 °C; 2 h before this incubation was complete, Packard Unifilters gF/B (96 wells) were soaked in buffer A and 0.3% polyethylenimine and stored at 4 °C. After incubation completion, rapid vacuum filtration on a Brandell cell harvester (96 wells) was performed through Unifilters gF/B and filters were washed 4-fold with cold buffer. Filters were dried overnight and 25 μL of scintillation cocktail was added before the count in a Packard Topcount.

Chemistry. General Methods. Commercial chemicals were of reagent grade and used as supplied by the manufacturer, except for solvents which were purified and dried by standard methods before use. All reactions, except those performed in aqueous solution, were carried out with the use of standard techniques for the exclusion of moisture and under a N_2 atmosphere. Organic extracts were routinely dried over anhydrous Na_2SO_4. Concentration refers to rotory evaporation under reduced pressure. Flash chromatography was conducted using silica gel (0.004−0.063 μm; E. Merck). Thin-layer chromatography (TLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25 mM) and components were usually visualized using UV light, iodine vapor, or Dragendorff preparation. Melting points were determined on a Büchi (B-530 or B-540 models) capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at ambient temperature on Brüker ACM-400 and DPX-400 spectrometers with an internal lock on the deuterium of the indicated solvent. Chemical shifts were reported as apparent centers of multiplets in ppm (δ) downfield from the internal lock and apparent first-order coupling constants reported in hertz. Mass spectra were recorded on a Platform LC micromass spectrometer, the ionization being done by electrospray in positive mode. HPLC was performed on a Merck LaChrom instrument, using a Pirckle covalent phenylglycine column and a diode array detector. All optical active compounds were pure (ee >95% by comparison with the related racemate). Optical rotations were measured on a Polartronic D polarimeter (Schmidt & Haensch) in a 1-dm cell. Elemental analyses were determined using a chromatography separation-based model with detection mode performed by measuring the thermic conductibility; the amorphous and/or hygroscopic nature of certain compounds resulted in incorporation of fractions of water molecules into their elemental analyses.

5-Methoxy-2,3-dihydro-1*H*-indole (1c). After being stirred for 15 min at room temperature, a homogeneous solution of 5-methoxyindole (25.1 g, 170.5 mmol) in glacial acetic acid (250 mL) was obtained. The reaction mixture was cooled to 0 °C and sodium cyanoborohydride (16 g, 255 mmol) was added stepwise maintaining temperature under control. Stirring was continued overnight at room temperature. Water (250 mL) was

introduced and keeping the reaction at 0 °C, concentrated NaOH (500 mL) was added up to pH 12. The aqueous layer was extracted with CH_2Cl_2 (3 × 1.5 L). The combined organic extracts were washed with saturated NaCl, dried (Na_2SO_4) and filtered. Concentration and chromatography (98:2 CH_2Cl_2/MeOH) afforded **1c**: oil; 16.3 g, 91%; R_f 0.4 (98:2 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, DMSO) δ 2.85 (t, J = 8.6 Hz, 2H), 3.33 (m, 2H), 3.62 (s, 3H), 5.02 (bs, 1H), 6.42 (d, J = 10 Hz, 1H), 6.50 (d, J = 10 Hz, 1H), 6.70 (s, 1H).

General Procedure A: Boc Protection. 5-Methoxy-2,3-dihydroindole-1-carboxylic Acid *tert*-Butyl Ester (2c). To a stirred solution of **1c** (24.1 g, 161 mmol) in anhydrous THF (250 mL), cooled to 0 °C, was introduced a solution of di-*tert*-butyl dicarbonate (77.6 g, 355.4 mmol) in anhydrous THF (400 mL). The reaction was allowed to warm to room temperature and stirred overnight. Concentration provided 92.6 g of a dark brown oil. Chromatography eluting with 50:50 cyclohexane/CH_2Cl_2 followed with recrystallization from petroleum ether provided **2c**: 36 g, 89%; white powder; R_f 0.55 (CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 1.5 (bs, 9H), 3.00 (t, J = 8.6 Hz, 2H), 3.70 (s, 3H), 3.89 (t, J = 8.6 Hz, 2H), 6.70 (d, J = 10 Hz, 1H), 6.82 (s, 1H), 7.15−7.70 (m, 1H).

5-Methoxy-7-(1-phenylmethanoyl)-2,3-dihydroindole-1-carboxylic Acid *tert*-Butyl Ester (3c). To a stirred solution of **2c** (36 g, 144 mmol) in anhydrous Et_2O (800 mL) was introduced *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (28.9 mL, 191 mmol). The reaction mixture was cooled to −60 °C and *sec*-butyllithium (1.3 M in cyclohexane) (133 mL, 173 mmol) was added dropwise over a 30-min period. After being stirred for a further 90 min, at −60 °C, a solution of methyl benzoate (59 g, 433 mmol) in anhydrous Et_2O (50 mL) was dropped in 15 min. The reaction was allowed to warm to room temperature and stirring was maintained overnight. The reaction was quenched adding dropwise over 15 min 10% NH_4Cl (230 mL, 433 mmol) then water (750 mL). The separated aqueous layer was extracted with Et_2O (3 × 1 L). The combined organic extracts were washed with saturated NaCl (1.5 L), dried and filtered. Concentration and chromatography (70:30 CH_2Cl_2/cyclohexane) afforded **3c**: 32.2 g, 63%; oil; R_f 0.29 (99:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, DMSO) δ 1.12 (bs, 9H), 3.08 (t, J = 8.6 Hz, 2H), 3.77 (s, 3H), 3.95 (t, J = 8.6 Hz, 2H), 6.72 (s, 1H), 7.09 (s, 1H), 7.40−7.48 (m, 5H).

General Procedure B: Deprotection of 5-Methoxy-7-(1-phenylmethanoyl)-2,3-dihydroindole-1-carboxylic Acid *tert*-Butyl Ester (3c) Using Trifluoroacetic Acid. 1-(5-Methoxy-2,3-dihydro-1*H*-indol-7-yl)-1-phenylmethanone (4c). To a stirred solution of **3c** (32.2 g, 91 mmol) in CH_2Cl_2 (1.1 L), cooled at 10 °C, was added dropwise over 15 min trifluoroacetic acid (280 mL, 319 mmol). The reaction mixture was allowed to warm to room temperature, stirring was maintained for 1 h and the solvent was removed in vacuo providing a brown oil. This residue was diluted in saturated $NaHCO_3$ (600 mL) and the aqueous phase was extracted with CH_2Cl_2 (3 × 500 mL). The joined organics were washed with brine, dried and filtered. The solvent was removed in vacuo affording **4c**: 22 g, 95%); oil; R_f 0.5 (99:1 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, DMSO) δ 3.00 (t, J = 8.6 Hz, 2H), 3.58 (s, 3H), 3.65 (t, J = 8.6 Hz, 2H), 6.58 (s, 1H), 6.98 (s, 2H), 7.48−7.65 (m, 5H).

3-Amino-*N*-((*R*)-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-yl-isonicotinamide (19a). Starting from **12a**, **19a** was finally purified by chromatography (98:2 CH_2Cl_2/MeOH): yellow powder; 68% yield; mp > 240 °C; ^{1}H NMR (400 MHz, DMSO) δ 3.15 (m, 1H), 3.40 (m, 1H), 3.95 (m, 1H), 4.50 (m, 1H), 5.45 (d, J = 7.9 Hz, 1H), 6.43 (bs, 2H), 7.22 (m, 2H), 7.40−7.57 (m, 5H), 7.63 (m, 1H), 7.70 (d, J = 5.2 Hz, 1H), 7.80 (d, J = 5.2 Hz, 1H), 8.18 (s, 1H), 9.62 (d, J = 7.9 Hz, 1H); MS *m*/*z* 397.

3-Amino-*N*-((*R*)-9-amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (19e). Starting from **12e**, **19e** was finally purified by successive chromatography: the first chromatography eluting with a gradient of 0−10% MeOH/CH_2Cl_2, then with a gradient of 0−7% MeOH/CH_2Cl_2, and the last one using a gradient of 0−5% MeOH/CH_2Cl_2: yellow powder; 23% yield; mp 215−220 °C; $[\alpha]^{20}_D$ −4° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.01 (dd, J = 16 Hz, 8.6 Hz, 1H), 3.30 (m,1H), 3.85 (m, 1H), 4.43 (m, 1H), 5.43 (d, J = 7.9 Hz, 1H), 5.45−5.75 (bs, 2H), 6.35 (s, 1H), 6.35−6.60 (bs, 2H), 6.89 (s, 1H), 7.40−7.60 (m, 5H), 7.70 (d, J = 5.1 Hz, 1H), 7.81 (d, J = 5.1 Hz, 1H), 8.17 (s, 1H), 9.54 (d, J = 7.9 Hz, 1H). Anal. ($C_{23}H_{20}N_6O_2 \cdot {}^3/_{10}CH_2Cl_2 \cdot {}^4/_5H_2O$) C, H, N, O, Cl.

4-Amino-*N*-((*R*)-9-amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)benzamide (20e). Starting from **15e**, **20e** was finally obtained after treatment using 1 N NaOH instead of $NaHCO_3$ and was purified by recrystallization from 2-propanol/hexane: yellow powder; 65% yield; mp 276 °C; $[\alpha]^{20}_D$ +63° (c = 1, MeOH); ^{1}H NMR (400 MHz, DMSO) δ 3.00 (dd, J = 15.9 Hz, J = 9.2 Hz, 1H), 3.25 (m, 1H), 3.82 (m, 1H), 4.42 (m, 1H), 5.25 (bs, 2H), 5.45 (d, J = 8.0 Hz, 1H), 5.68 (bs, 2H), 6.35 (s, 1H), 6.58 (d, J = 8.3 Hz, 2H), 6.87 (s, 1H), 7.40−7.58 (m, 5H), 7.75 (d, J = 8.3 Hz, 2H), 8.79 (d, J = 8.0 Hz, 1H). Anal. ($C_{24}H_{21}N_5O_2$) H, N; C: calcd, 70.06; found, 69.11; O: calcd, 7.78; found, 8.48.

General Procedure C: Houben−Hoech Reaction. 1-(2,3-Dihydro-1*H*-indol-7-yl)-1-phenylmethanone (4a). Commercial indoline **1a** (2 mL, 1.12 mol) was dissolved in CH_2Cl_2 (560 mL). The solution was mechanically stirred and cooled under 5 °C (ice bath) and a solution of BCl_3 (112.5 mL, 1.2 mol) in CH_2Cl_2 (560 mL) was added keeping the temperature ∼5 °C. A solution of benzonitrile (222.7 mL, 2.16 mol) in CH_2Cl_2 (500 mL) and finally aluminum chloride (151.8 g, 1.12 mol) were successively poured. The reaction mixture was then heated to 84 °C over a 16-h period and the volatile material was removed. The mixture was allowed to cool to room temperature, HCl (4 N, 1.12 L) was introduced (very cautiously at first) keeping the temperature between 20 and 60 °C. The resulting mixture was maintained at 80 °C for 20 min and finally cooled to room temperature. The precipitate was filtered, washed with 4 N HCl and then with CH_2Cl_2, diluted with CH_2Cl_2 and treated with NaOH. The organic phase was washed with water, dried and concentrated to afford **4a**: yellow powder; 178 g, 71%; mp 119−121 °C; R_f 0.5 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10 (t, J = 8.6 Hz, 2H), 3.80 (t, J = 8.6 Hz, 2H), 6.47 (dd, J = 8.0 Hz, 1 Hz, 1H), 6.55 (m, 1H), 7.07 (bs, 1H), 7.17 (d, J = 7.0 Hz, 1H), 7.25 (m, 1H), 7.40−7.55 (m, 3H), 7.64 (m, 2H).

1-(5-Methyl-2,3-dihydro-1*H*-indol-7-yl)-1-phenylmethanone (4b). Starting from **1b, 4b** was finally purified by chromatography (CH_2Cl_2) followed by recrystallization from heptane: yellow powder; 91% yield; mp 83−84 °C; R_f 0.2 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.20 (s, 3H), 3.08 (t, J = 8.5 Hz, 2H), 3.80 (t, J = 8.5 Hz, 2H), 6.90 (bs, 1H), 7.06 (s, 2H), 7.42−7.70 (m, 5H).

General Procedure D: Seven-Membered Ring Formation. 1-Phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (5a). To a stirred solution of **4a** (116.6 g, 522 mol) in pyridine (815 mL) was added ethyl glycinate hydrochloride (255 g, 1.83 mol). The reaction mixture was heated to reflux (110 °C) for 16 h, water and alcohol were distilled from the mixture and pyridine was regularly introduced maintaining a constant volume. 200 mL were distilled during the first 3 h and 100 mL overnight. The mixture, concentrated by removing pyridine (∼200 mL) under reduced pressure, was allowed to cool room temperature. CH_2Cl_2 (1 L) was added and a sodium carbonate solution (2.5%) was cautiously dropped maintaining stirring. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 500 mL). The combined organic layers were washed with water, dried and filtered. Concentration, chromatography using 90:10 CH_2Cl_2/acetone, and crystallization from 2-propanol afforded **5a**: 114 g, 84%; mp 137−138 °C; R_f 0.5 (90:10 CH_2Cl_2/acetone); ^{1}H NMR (400 MHz, DMSO) δ 3.20 (t, J = 8 Hz, 2H), 4.15 (t, J = 8 Hz, 2H), 4.23 (s, 2H), 7.10 (m, 2H), 7.40−7.55 (m, 6H).

9-Methyl-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (5b). Starting from **4b**, **5b** was finally obtained by a recrystallization from diethyl ether: white powder; 80% yield; mp 132−34 °C; R_f 0.5 (ethyl acetate); ^{1}H NMR (400 MHz,

$CDCl_3$) δ 2.33 (s, 3H), 3.18 (t, $J = 8.0$ Hz, 2H), 4.28 (t, $J = 8.0$ Hz, 2H), 4.38 (s, 2H), 6.98 (s, 1H), 7.25 (s, 1H), 7.35−7.58 (m, 5H).

9-Methoxy-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (5c). Starting from **4c**, **5c** was finally purified by chromatography (96:4 CH_2Cl_2/acetone (NH_3 10%)): oil; quantitative yield; R_f 0.17 (96:4 CH_2Cl_2/acetone (NH_3 10%)); ^{1}H NMR (400 MHz, DMSO) δ 3.17 (t, $J = 8.6$ Hz, 2H), 3.68 (s, 3H), 4.13 (t, $J = 8.6$ Hz, 2H), 4.21 (s, 2H), 6.55 (s, 1H), 7.21 (s, 1H), 7.40−7.55 (m, 5H).

General Procedure E: Oxime Derivative Formation. 1-Phenyl-6,7-dihydro[1,4]diazepino[6,7,1-*hi*]indole-3,4-dione 3-Oxime (6a). To a solution of anhydrous THF (100 mL) and anhydrous toluene (200 mL), cooled at −5 °C, was slowly introduced **5a** (26.3 g, 0.1 mol). The mixture was maintained at 0 °C and potassium *tert*-butylate (28 g, 0.25 mol) was added leading to a red-brown solution. After 30 min, isoamyl nitrite (14 mL, 0.1 mol) was added and stirring was continued for further 30 min. Following introduction of ethyl acetate (40 mL) and water (400 mL), the organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 600 mL). The joined organic extracts were concentrated under reduced pressure, to afford after recrystallization from MeOH **6a**: 23.3 g, 80%; mp 215 °C; R_f 0.4 (90:10 CH_2Cl_2/acetone); ^{1}H NMR (400 MHz, DMSO) δ 3.22 (t, $J = 8.0$ Hz, 2H), 4.25 (t, $J = 8.0$ Hz, 2H), 7.19 (s, 1H), 7.45−7.65 (m, 6H), 11.05 (s, 1H).

9-Methyl-1-phenyl-6,7-dihydro[1,4]diazepino[6,7,1-*hi*]-indole-3,4-dione 3-Oxime (6b). Starting from **5b**, recrystallization of **6b** occurred from methanol: yellow powder; 70% yield; mp 247 °C; R_f 0.3 (60:40 ethyl acetate/cyclohexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 3.18 (t, $J = 8.2$ Hz, 2H), 4.21 (t, $J = 8.2$ Hz, 2H), 6.95 (s, 1H), 7.39 (s, 1H), 7.45−7.65 (m, 5H), 11.00 (s, 1H).

9-Methoxy-1-phenyl-6,7-dihydro[1,4]diazepino[6,7,1-*hi*]indole-3,4-dione 3-Oxime (6c). Starting from **5b**, **6b** was finally purified by chromatography (90:10 CH_2Cl_2/acetone): orange powder; 93% yield; R_f 0.17 (90:10 CH_2Cl_2/acetone); ^{1}H NMR (400 MHz, DMSO) δ 3.22 (t, $J = 8.6$ Hz, 2H), 3.68 (s, 3H), 4.26 (t, $J = 8.6$ Hz, 2H), 6.60 (s, 1H), 7.25 (s, 1H), 7.50−7.70 (m, 5H).

General Procedure F: Reduction Using H_2 and Ru/C. 3-Amino-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (7a). In a hydrogenation reactor (up to 12 bar), Ru/C (5%, 10 g) was diluted in MeOH (1 L) and **6a** (32 g, 0.11 mol) was added. Under hydrogen atmosphere (8 bar), the mixture was vigorously stirred and slowly (over 2 h) allowed to heat to 80 °C. After a further 2 h at 80 °C (end of hydrogen absorption), the reaction mixture was cooled to room temperature. The catalyst was filtered over silica gel and washed with MeOH. The resulting filtrate was concentrated. The crude material was purified by chromatography (90:10 ethyl acetate/MeOH) to afford **7a** (29 g, 94%) as an unstable oil which was immediately used in the next step: R_f 0.2 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.55 (bs, 2H), 3.13 (ddd, $J = 16.1$ Hz, 9.7 Hz, 2.0 Hz, 1H), 3.33 (dt, $J = 16.1$ Hz, 10.1 Hz, 1H), 3.97 (m, 1H), 4.38 (s, 1H), 4.65 (m, 1H), 7.05 (t, $J = 7.5$ Hz, 1H), 7.20 (d, $J = 7.5$ Hz, 1H), 7.35−7.48 (m, 4H), 7.55 (m, 2H).

3-Amino-9-methyl-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (7b). Starting from **6b**, **7b** was finally purified by chromatography (98:2 CH_2Cl_2/MeOH): off-white powder; 68% yield; mp 116 °C; R_f 0.15 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H), 2.48 (bs, 2H), 3.07 (ddd, $J = 16.1$ Hz, 9.7 Hz, 1.9 Hz, 1H), 3.29 (dt, $J = 16.1$ Hz, 10.0 Hz, 1H), 3.94 (m, 1H), 4.35 (s, 1H), 4.64 (m, 1H), 6.97 (s, 1H), 7.24 (s, 1H), 7.35−7.60 (m, 5H).

3-Amino-9-methoxy-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (7c). Starting from **6c**, **7c** was finally purified by chromatography with a gradient of 1−3% MeOH/CH_2Cl_2: 67% yield; orange brown foam; R_f 0.15 (97:3 CH_2Cl_2/MeOH); ^{1}H NMR (400 MHz, DMSO) δ 2.53 (s, 2H), 3.05 (m, 1H), 3.30 (m, 1H), 3.70 (s, 3H), 3.80 (m, 1H), 4.20 (s, 1H), 4.45 (m, 1H), 6.58 (s, 1H), 7.23 (s, 1H), 7.40−7.55 (m, 5H).

General Procedure G: Optical Resolution by Salt Crystallization Using *N*-Acetyl-L-phenylalanine, *N*-Acetyl-D-phenylalanine or Di-*p*-toluoyl-D-tartaric Acid. (*R*)-3-Amino-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]-indol-4-one (8a). A solution of *N*-acetyl-L-phenylalanine (28 g, 0.135 mol) in ethyl acetate (1.2 L) was refluxed. A solution of **7a** (40 g, 0.14 mol) in ethyl acetate, previously warmed to 70 °C, was added. The solution was maintained for 5 min at reflux and allowed to cool to room temperature. After 24 h, the first crystals were filtered (ee 75%). The resulting crystals were heated in ethyl acetate (500 mL) to 70 °C overnight and then cooled to room temperature. The second crystals (50 g, 1.03 mol) were provided with ee > 99%. The crystals were dissolved in CH_2Cl_2 (1.2 L) and keeping the temperature below 5 °C, NaOH N (1 L, 1 mol) was cautiously added. The reaction mixture was extracted with CH_2Cl_2 (2 × 750 mL). Then, the combined organic layers were successively washed with water (750 mL) and brine (2 × 750 mL), dried (Na_2SO_4) and filtered to afford **8a**: 39.6 g, 99% yield; colorless oil; $[\alpha]^{20}_D$ +102° ($c = 1$, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.08−3.40 (m, 4H), 3.89 (m, 1H), 4.24 (s, 1H), 4.45 (m, 1H), 7.14 (m, 2H), 7.40−7.55 (m, 6H).

3-Amino-9-methyl-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (8b). To a stirred solution of **7b** (28.6 g, 98.1 mmol) in ethyl acetate (1 L) at reflux was added a solution of *N*-acetyl-D-phenylalanine (20.3 g, 98.1 mmol) in ethyl acetate (1 L) previously allowed to reflux. The mixture was maintained under slow stirring overnight, at room temperature. Crystals formed, were filtered and washed with cold ethyl acetate (200 mL). The salt in a mixture of water (1 L) and ethyl acetate (200 mL) was treated using concentrated NaOH (250 mL) at 0 °C. The reaction mixture was extracted with ethyl acetate (2 × 150 mL). Then, the combined organic layers were successively washed with water (150 mL) and saturated NaCl (2 × 150 mL), dried and filtered to afford **8b**: 13 g, 91%; beige powder; $[\alpha]^{20}_D$ +236° ($c = 1$, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 3H), 2.48 (bs, 2H), 3.07 (ddd, $J = 16.1$ Hz, 9.7 Hz, 1.9 Hz, 1H), 3.29 (dt, $J = 16.1$ Hz, 10.0 Hz, 1H), 3.94 (m, 1H), 4.35 (s, 1H), 4.64 (m, 1H), 6.97 (s, 1H), 7.24 (s, 1H), 7.35−7.60 (m, 5H).

3-Amino-9-methoxy-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (8c). To a strirred solution of **7c** (7.1 g, 23.1 mmol) in acetonitrile (18 mL) at reflux was added a solution of di-*p*-toluoyl-D-tartaric acid (8.9 g, 23.1 mmol) in acetonitrile (18 mL) previously heated to reflux. The reaction mixture was maintained at reflux for 5 min and was then allowed to cool to room temperature. A precipitate formed over 20 h and then was filtered, washed with cold acetonitrile (40 mL) and dried. The treatment of the salt with NaOH (1 M, 100 mL) at 0 °C was followed by the extraction with isopropyl acetate (3 × 200 mL). The joined organic extracts were washed with saturated NaCl (200 mL), dried and filtered. The solvent was removed to afford **8c**: 2.2 g, 31%; white powder; $[\alpha]^{20}_D$ +225° ($c = 3$, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 2.53 (s, 2H), 3.05 (m, 1H), 3.30 (m, 1H), 3.70 (s, 3H), 3.80 (m, 1H), 4.20 (s, 1H), 4.45 (m, 1H), 6.58 (s, 1H), 7.23 (s, 1H), 7.40−7.55 (m, 5H).

(*R*)-3-Amino-9-nitro-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (8d). To H_2SO_4 (48 mL) was slowly added over 90 min **8a** (149.7 g, 540 mmol). A solution of KNO_3 (6.55 g, 684 mmol) in H_2SO_4 (20 mL), previously cooled to 5 °C, was added dropwise maintaining the temperature below 5 °C. The reaction mixture was stirred for 40 min and allowed to warm to room temperature. The solution was poured on ice and after addition of CH_2Cl_2 (400 mL), neutralization was initiated under vigorous stirring using concentrated NH_4OH (∼188 mL). Under pHmeter control, concentrated NH_4OH was added up to pH 8. Then, CH_2Cl_2 (400 mL) was introduced under a slower stirring (avoiding emulsion) and NH_4OH addition was complete leading to pH 10−11. The aqueous layer was extracted with CH_2Cl_2 (2 × 400 mL). The combined organic extracts were washed twice with a saturated NaCl solution, dried and filtered. Concentration and chromatography by a gradient 2−4% MeOH/CH_2Cl_2 afforded **8d**: 13 g, 74%;

^{1}H NMR (400 MHz, DMSO) δ 3.15−3.45 (m, 4H), 4.04 (m, 1H), 4.38 (s, 1H), 4.49 (m, 1H), 7.44−7.55 (m, 5H), 7.94 (s, 1H), 8.37 (s,1H); MS *m*/*z* 322.

(*R*)-3,9-Diamino-1-phenyl-6,7-dihydro-3*H*-[1,4]diazepino-[6,7,1-*hi*]indol-4-one (8e). To a stirred suspension of **8d** (13 g, 0.403 mol) in EtOH (149 mL) at room temperature was added $SnCl_2 \cdot 2H_2O$ (45.3 g, 0.201 mol). The mixture was first orange and then turned dark. After stirring at 70 °C for 30 min, the solution was concentrated and solvent (∼100 mL) was removed in vacuo. Ice and water (300 mL) were poured to the mixture. Under vigorous stirring at −10 °C, concentrated NaOH was added up to pH 8. Using a slower stirring, CH_2Cl_2 (400 mL) was introduced and concentrated NaOH addition was continued up to pH 10−11. The organic layer was separated and the aqueous phase was extracted twice with CH_2Cl_2. The joined organic extracts were washed twice with a saturated NaCl solution, dried and filtered. Concentration and chromatography using a gradient 5−7% MeOH/CH_2Cl_2 afforded **8e**: mp 186−189 °C; ^{1}H NMR (400 MHz, DMSO) δ 2.57 (bs, 2H), 2.95 (ddd, J = 16.2 Hz, 9.8 Hz, 9.6 Hz, 1H), 3.77 (ddd, J = 9.9 Hz, 9.8 Hz, 9.5 Hz, 1H), 4.16 (s, 1H), 4.37 (ddd, J = 9.9 Hz, 9.6 Hz, 1.9 Hz, 1H), 5.15 (bs, 2H), 6.30 (d, J = 1.8 Hz, 1H), 6.80 (s, 1H), 7.39−7.52 (m, 5H); MS *m*/*z* 292.

(*R*)-3-Amino-1-phenyl-9-pyrrolidin-1-yl-6,7-dihydro-3*H*-[1,4]diazepino[6,7,1-*hi*]indol-4-one (8f). Following the general procedure A, **8d** (13 g, 40.3 mmol) provided the Boc-aminodiazepinoindole derivative (17 g, 40.2 mmol). In a hydrogenation reactor (up to 12 bar), Ru/C (5%, 4 g) was diluted in MeOH (1 L) and the previous crude material (11 g, 0.26 mmol) was added. Under hydrogen atmosphere (8 bar), the mixture was allowed to heat to 80 °C and vigorously stirred for 6 h. The reaction mixture was cooled to room temperature. The catalyst was filtered and washed with MeOH. The resulting filtrate was concentrated. The crude material was purified by chromatography eluting with 1−2% MeOH/CH_2Cl_2 providing the amino derivative (8.8 g, 22.4 mmol) immediately used in the next step. To a stirred solution of the previous residue (6 g, 15.2 mmol) in dried DMF (150 mL) were added 1,4-dibromobutane (3.6 mL, 30.4 mmol) and sodium hydrogen carbonate (6.4 g, 76 mmol). The reaction mixture was stirred at 60 °C for 7 h and was concentrated. Water (500 mL) was introduced and the aqueous layer was extracted with CH_2Cl_2 (3 × 500 mL). The organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by chromatography eluted with 1% MeOH/CH_2Cl_2 provided the pyrrolidine derivative (4.1 g, 9.2 mmol) which was deprotected following the general procedure B. Purification by chromatography eluting with 0−5% MeOH/CH_2Cl_2 provided **8f** (3 g, 8.7 mmol): powder; 49% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (m, 4H), 2.38 (s, 3H), 2.95−3.05 (m, 1H), 3.00−3.20 (m, 4H), 3.15−3.20 (m,1H), 3.80−3.90 (m, 1H), 4.35 (s, 1H), 4.50−4.60 (m, 1H), 6.15 (s, 1H), 6.68 (s, 1H), 7.25−7.40 (m, 3H), 7.60 (m, 2H).

General Procedure H: Coupling Reaction with Pentafluorophenyl Ester Derivatives. *N*-((*R*)-4-Oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (9a). To a stirred solution of isonicotinic acid (1.85 g, 15 mmol) in CH_2Cl_2 (80 mL) were added pentafluorophenol (5.5 g, 30 mmol) and 4-(dimethylamino)-pyridine *p*-toluenesulfonate (1.2 g, 3.8 mmol). After being stirred for 15 min at room temperature, *N*′-(3-(dimethylamino)-propyl)-*N*-ethylcarboimide hydrochloride (6.5 g, 34 mmol) was added and stirring was maintained overnight. The solvent was removed in vacuo, the residue (7 g) was diluted in CH_2Cl_2 (80 mL) and **8a** (1.5 g, 5.47 mmol) was introduced. The reaction mixture was stirred overnight and concentrated. Purification by chromatography (95:5 CH_2Cl_2/acetone then 98:2 CH_2Cl_2/MeOH) provided **9a**: yellow solid; 43% yield; mp 234 °C; $[\alpha]^{20}_D$ +23° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.15 (dd, J = 16.5 Hz, 9.9 Hz, 1H), 3.48 (m, 1H), 3.95 (m, 1H), 4.49 (m, 1H), 5.50 (d, J = 7.9 Hz, 1H), 7.20 (m, 2H), 7.40−7.55 (m, 5H), 7.62 (m, 1H), 7.93 (d, J = 4.5 Hz, 2H), 8.76 (d, J = 4.5 Hz, 2H), 10.0 (d, J = 7.9 Hz, 1H). Anal. ($C_{23}H_{18}N_4O_2 \cdot {}^3/_{10}CH_2Cl_2 \cdot {}^1/_2H_2O$) H, N; C: calcd, 67.12; found, 67.60.

***N*-((*R*)-9-Methoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (9c).** Starting from **8c**, **9c** was finally purified by chromatography (98:2 CH_2Cl_2/MeOH): white powder; 54.7% yield; mp 220−224 °C; $[\alpha]^{20}_D$ +2.4° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.12 (dd, J = 16.3 Hz, 8.1 Hz, 1H), 3.38 (dt, J = 16.3 Hz, 10.0 Hz, 1H), 3.75 (s, 3H), 4.0 (m, 1H), 4.68 (m, 1H), 5.60 (d, J = 7.4 Hz, 1H), 6.70 (d, J = 2.3 Hz, 1H), 7.10 (s, 1H), 7.35−7.65 (m, 5H), 7.80 (d, J = 6,0 Hz, 2H), 8.13 (d, J = 7.4 Hz, 1H), 8.80 (d, J = 6.0 Hz, 2H); MS *m*/*z* 412.

Quinoline-3-carboxylic Acid ((*R*)-9-Methoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (16c). Starting from **8c**, **16c** was finally purified by recrystallization from MeOH: white powder; 77% yield; mp 112 °C; $[\alpha]^{20}_D$ +0.3° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.14 (dd, J = 16.4 Hz, 8.1 Hz, 1H), 3.39 (dt, J = 16.4 Hz, 9.9 Hz, 1H), 3.75 (s, 3H), 4.02 (m, 1H), 4.70 (m, 1H), 5.68 (d, J = 7.4 Hz, 1H), 6.72 (d, J = 2.3 Hz, 1H), 7.12 (s, 1H), 7.35−8.25 (m, 10H), 8.75 (d, J = 2.1 Hz, 2H), 9.45 (d, J = 2.1 Hz, 1H). Anal. ($C_{28}H_{22}N_4O_3 \cdot {}^1/_2H_2O$) C, H, N, O.

Isoquinoline-3-carboxylic Acid *N*-((*R*)-4-Oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-amide (17a). Starting from **8a, 17a** was finally purified by chromatography with a gradient of 3−4% acetone/CH_2Cl_2: white powder; 64% yield; mp 250 °C; $[\alpha]^{20}_D$ +10° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.15 (dd, J = 16.2 Hz, 7.9 Hz, 1H), 3.39 (dt, J = 16.2 Hz, 10.0 Hz, 1H), 4.02 (m, 1H), 4.70 (m, 1H), 5.70 (d, J = 8.2 Hz, 1H), 7.13 (t, J = 7.7 Hz, 1H), 7.25−7.80 (m, 9H), 7.95−8.10 (m, 2H), 8.64 (s, 1H), 9.29 (s, 1H), 9.86 (d, J = 8.2 Hz, 1H). Anal. ($C_{27}H_{20}N_4O_2 \cdot {}^3/_{20}CH_2Cl_2 \cdot {}^3/_{10}H_2O$) C, H, N,O, H.

Isoquinoline-3-carboxylic Acid *N*-((*R*)-9-Methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (17b). Starting from **8b, 17b** was finally purified by chromatography (98:2 CH_2Cl_2/acetone): white powder; 93% yield; mp 130 °C; $[\alpha]^{20}_D$ +25° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.10 (dd, J = 16.1 Hz, 9.3 Hz, 1H), 3.35 (dt, J = 16.1 Hz, 10.1 Hz, 1H), 4.0 (m, 1H), 4.70 (m, 1H), 5.69 (d, J = 8.2 Hz, 1H), 7.05 (s, 1H), 7.30 (s, 1H), 7.35−7.50 (m, 3H), 7.65−7.85 (m, 2H), 7.95−8.15 (m, 2H), 8.65 (s, 1H), 9.30 (s, 1H), 9.88 (d, J = 8.2 Hz, 1H); MS *m*/*z* 446.

Isoquinoline-3-carboxylic Acid *N*-((*R*)-9-Methoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]-indol-3-yl)amide (17c). Starting from **8c**, **17c** was finally purified by chromatography (97:3 CH_2Cl_2/MeOH): white powder; 87% yield; mp 211 °C; $[\alpha]^{20}_D$ +0.3° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10 (m, 1H), 3.35 (m, 1H), 3.75 (s, 3H), 4.0 (m, 1H), 4.70 (m, 1H), 5.70 (d, J = 7 Hz, 1H), 6.70 (s, 1H), 7.10 (s, 1H), 7.25−7.85 (m, 7H), 7.95 (d, J = 7 Hz, 1H), 8.20 (d, J = 7 Hz, 1H), 8.25 (d, J = 7 Hz, 1H), 8.75 (m, 1H), 9.75 (m, 1H); MS *m*/*z* 462. Anal. ($C_{28}H_{22}N_4O_3$) H, O; C: calcd, 72.71; found, 71.64; N: calcd, 12.11; found, 11.48.

General Procedure I: Coupling Reaction Using Acidic Chloride. *N*-((*R*)-9-Methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (9b). To a stirred solution of **8b** (5 g, 17.2 mmol) in pyridine (100 mL) at 0 °C was slowly introduced isonicotinoyl chloride·HCl (4.58 g, 25.7 mmol). The reaction mixture was then stirred at room temperature overnight. After introduction of CH_2Cl_2 (50 mL) and H_2O (50 mL), the organic layer was separated and the aqueous phase was extracted with further CH_2Cl_2 (50 mL). The combined organic extracts were dried and concentrated. After purification by chromatography (98:2 CH_2Cl_2/MeOH) followed by recrystallization from ethyl acetate, **9b** (6.8 g, 83%) was obtained: white powder; 83% yield; mp 238−240 °C; $[\alpha]^{20}_D$ +55° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.3 5 (s, 3H), 3.12 (dd, J = 16.1 Hz, 9.6 Hz, 1H), 3.35 (dt, J = 16.1 Hz, 10.2 Hz, 1H), 4.00 (m, 1H), 4.67 (m, 1H), 5.59 (d, J = 7.4 Hz, 1H), 7.05 (s, 1H), 7.30 (s, 1H), 7.35−7.57 (m, 5H), 7.80 (d, J = 6.1 Hz, 2H), 8.05 (d, J = 7.4 Hz, 1H), 8.79 (d, J = 6.1 Hz, 2H). Anal. ($C_{24}H_{20}N_4O_2$) C, H, N, O.

3-Methyl-*N*-((*R*)-4-oxo-1-phenyl-9-pyrrolidin-1-yl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (9f). Obtained from coupling with **8h** and using proce-

dure in pyridine (see **9b**), **9f** was finally purified by chromatography with a gradient of 2−4% MeOH/CH_2Cl_2: yellow powder; 23% yield; mp >250 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (m, 4H), 3.02 (m, 1H), 3.14 (m, 4H), 3.28 (m, 1H), 3.89 (m, 1H), 4.55 (m, 1H), 5.53 (m, 1H), 6.20 (s, 1H), 6.70 (s, 1H), 7.25−7.45 (m, 3H), 7.57 (m, 2H), 7.72 (m, 2H), 8.08 (m, 1H), 8.73 (m, 2H).

***N*-((*R*)-9-Nitro-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-yl)nicotinamide (10d).** Instead of pyridine, the amino intermediate **8d** (0.7 g, 2.17 mmol) was dissolved in CH_2Cl_2 (20 mL) and triethylamine (0.42 mL, 2.4 mmol) was added. The reaction was stirred overnight at room temperature and concentrated. After purification by chromatography (98:2 CH_2Cl_2/MeOH), **10d** (0.8 g, 95%) was obtained: yellow powder; mp 172−176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.32 (dd, J = 16.8 Hz, 9.9 Hz, 1H), 3.50 (dt, J = 16.8 Hz, 10.0 Hz, 1H), 4.17 (m, 1H), 4.75 (m, 1H), 5.70 (d, J = 7.6 Hz, 1H), 7.40−7.57 (m, 6H), 7.85 (d, J = 7.6 Hz, 1H), 8.25 (m, 2H), 8.33 (s, 1H), 8.80 (d, J = 4.7 Hz, 1H), 9.20 (s, 1H); MS m/z 427.

2-Methoxy-*N*-((*R*)-9-methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)benzamide (13b). Instead of pyridine, the amino intermediate **8b** was treated in CH_2Cl_2 and triethylamine (see synthesis of **10d**). **13b** was purified by chromatography (97:3 CH_2Cl_2/MeOH): white powder; 80% yield; mp 123−125 °C; $[\alpha]^{20}_D$ +34° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.10 (dd, J = 16.0 Hz, 9.6 Hz, 1H), 3.33 (dt, J = 16.0 Hz, 10.1 Hz, 1H), 3.98 (m, 1H), 4.07 (s, 3H), 4.68 (m, 1H), 5.67 (d, J = 7.5 Hz, 1H), 7.00−7.13 (m, 3H), 7.28 (s, 1H), 7.32−7.60 (m, 6H), 8.25 (dd, J = 7.8 Hz, 1.8 Hz, 1H), 9.80 (d, J = 7.5 Hz, 1H). Anal. ($C_{26}H_{23}N_3O_3 \cdot {}^1/_2H_2O$) C, H, N.

General Procedure J: Coupling Reaction with Carboxylic Acid Derivatives and TOTU. *N*-((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]-indol-3-yl)nicotinamide (10e). To a stirred solution of **8e** (35 g, 0.119 mol) in CH_2Cl_2 (1.3 L), were introduced acid isonicotinic (16.2 g, 0.131 mol) and *O*-[(ethoxycarbonyl)cyanomethyleneamino)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TOTU) (39.3 g, 0.119 mol). The suspension was cooled to 0 °C and diisopropylethylamine (41.7 mL, 0.239 mol) was added. The reaction mixture was stirred for 15 min and allowed to warm to room temperature. After being stirred overnight, the mixture was filtered, washed twice with H_2O (500 mL), HCl and H_2O (250 mL). After neutralization with concentrated NaOH, the aqueous phase was extracted by CH_2Cl_2. The combined organic layers were washed by H_2O, dried and filtered. The solvent was removed in vacuo and the crude material was purified by chromatography eluting with a MeOH gradient in CH_2Cl_2 to afford **10e**: yellow powder; 24.1 g, 50%; ee >99%; mp >250 °C; R_f 0.7 (95:5 CH_2Cl_2/MeOH); $[\alpha]^{20}_D$ +61° (c = 1, H_2O); ^{1}H NMR (400 MHz, DMSO) δ 3.02 (dd, J = 16.1, 8.5 Hz, 1H), 3.25 (m, 1H), 3.85 (m, 1H), 4.42 (m, 1 H), 5.27 (bs, 2H), 5.48 (d, J = 8.0 Hz, 1H), 6.40 (s, 1H), 6.95 (s, 1H), 7.45−7.65 (m, 6H), 8.37 (dt, J = 8.0 Hz, 1.8 Hz, 1H), 8.75 (dd, J = 4.8 Hz, 1.6 Hz, 1H), 9.20 (d, J = 2.1 Hz, 1H), 9.82 (d, J = 8.0 Hz, 1H). Anal. ($C_{23}H_{19}N_5O_2$) C, H, N, O.

***N*-((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (9e).** Starting from **8e**, **9e** was finally purified by chromatography, first eluting with CH_2Cl_2 then eluting with 98:2 CH_2Cl_2/MeOH. The pure residue was dissolved in hot 25:75 CH_2Cl_2/MeOH, CH_2Cl_2 was removed in vacuo and a yellow precipitate formed within 1 h at 0 °C (19 g, 52%). To the yellow precipitate (13.9 g, 34.8 mmol) dissolved in MeOH (415 mL) at room temperature was added dropwise concentrated sulfuric acid (5.5 mL). A red coloration and a slight exotherm were observed. After stirring for 10 min, absolute EtOH (690 mL) was added stepwise while MeOH was evaporated in vacuo. The mixture allowed to cool at 0 °C. Maintaining the temperature 1 h, a red precipitate formed. The precipitate was filtered and dried at 50 °C for 2 h and then triturated in hot MeOH (50 mL). EtOH (550 mL) was slowly added and the mixture was stirred at 50 °C for 15 min and at 0 °C for 1 h providing **9e**: 22.5 g, 100%; pale beige powder; mp 220−225 °C; $[\alpha]^{20}_D$ +94° (c = 1, DMSO); ^{1}H NMR (400 MHz, DMSO) δ 3.15 (dd, J = 16.3 Hz, 8.7 Hz, 1H), 3.37 (m, 1H), 3.95 (m, 1H), 4.49 (m, 1H), 5.52 (d, J = 7.8 Hz, 1H), 6.91 (s, 1H), 7.34 (s, 1H), 7.42−7.58 (m, 5H), 8.10 (d, J = 6.0 Hz, 2H), 8.90 (d, J = 6.0 Hz, 2H), 10.13 (d, J = 7.8 Hz, 1H). Anal. ($C_{23}H_{19}N_5O_2 \cdot {}^3/_2H_2SO_4 \cdot {}^{11}/_{10}H_2O \cdot {}^1/_3EtOH$) C, H, N, S: O: calcd, 26.0; found, 25.33.

***N*-((*R*)-4-Oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino-[6,7,1-*hi*]indol-3-yl)nicotinamide (10a).** Starting from **8a,** a precipitate formed and **10a** was filtered and washed with CH_2Cl_2: a white solid; 69% yield; mp 253 °C; $[\alpha]^{20}_D$ +53° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.19 (m, 1H), 3.43 (m, 1H), 3.98 (m, 1H), 4.54 (m, 1H), 5.54 (d, J = 8.0 Hz, 1H), 7.24 (m, 2H), 7.43−7.70 (m, 7H), 8.40 (dt, J = 8.0 Hz, 1.8 Hz, 1H), 8.78 (dd, J = 4.8 Hz, 1.8 Hz, 1H), 9.19 (d, J = 1.8 Hz, 1H), 9.95 (d, J = 8.0 Hz, 1H). Anal. ($C_{23}H_{18}N_4O_2$) C, H, N.

3,5-Dichloro-*N*-((*R*)-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (11a). Starting from **8a, 11a** was finally purified by chromatography with a gradient of 0−2% MeOH/CH_2Cl_2 followed by recrystallization from diethyl ether/petroleum ether: white powder; 20% yield; mp 182 °C; $[\alpha]^{20}_D$ +136.5° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.15 (dd, J = 16.4 Hz, 8.2 Hz, 1H), 3.42 (dt, J = 16.4 Hz, 9.8 Hz, 1H), 3.95 (m, 1H), 4.54 (m, 1H), 5.43 (d, J = 8.3 Hz, 1H), 7.22 (m, 2H), 7.40−7.58 (m, 6H), 8.72 (s, 2H), 10.26 (d, J = 8.3 Hz, 1H); MS m/z 451.

3,5-Dichloro-*N*-((*R*)-9-methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)isonicotinamide (11b). Starting from **8b, 11b** was finally purified by chromatography with a gradient of 0−5% acetone/CH_2Cl_2 followed by recrystallization from diethyl ether/hexane: white powder; 22% yield; mp 147−198 °C; $[\alpha]^{20}_D$ +166° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 2.53 (s, 3H), 3.10 (dd, J = 16.0 Hz, 8.4 Hz, 1H), 3.40 (m, 1H), 3.93 (m, 1H), 4.52 (m, 1H), 5.42 (d, J = 8.3 Hz, 1H), 7.05 (s, 1H), 7.40−7.60 (m, 6H), 8.70 (s, 2H), 10.23 (d, J = 8.3 Hz, 1H). Anal. ($C_{24}H_{18}Cl_2N_4O_2 \cdot {}^1/_{10}C_6H_{12} \cdot {}^1/_{10}H_2O$) C, H, N, O, Cl.

***N*-((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-yl)-3,5-dichloroisonicotinamide (11e)**. Starting from **8e, 11e** was finally purified by chromatography with a gradient of 0−2% MeOH/CH_2Cl_2 followed by recrystallization from ethyl acetate/hexane: yellow powder; 33% yield; mp 166−220 °C; $[\alpha]^{20}_D$ +175° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.00 (dd, J = 16.0 Hz, 8.9 Hz, 1H), 3.30 (m, 1H), 3.82 (m, 1H), 4.45 (m, 1H), 5.28 (bs, 2H), 5.39 (d, J = 8.3 Hz, 1H), 6.38 (s, 1H), 6.88 (s, 1H), 7.40−7.60 (m, 5H), 8.71 (s, 2H), 10.18 (s, 1H). Anal. ($C_{23}H_{17}Cl_2N_3O_2 \cdot {}^2/_5C_4H_8O_2 \cdot {}^1/_{10}C_6H_{12}$) C, H, O, Cl; N: calcd, 13.73; found, 14.60.

***N*-((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-yl)-2-methoxybenzamide (13e).** Starting from **8e, 13e** was finally purified by chromatography with a gradient of 0−2% MeOH/CH_2Cl_2: yellow powder; 65% yield; mp 234 °C; $[\alpha]^{20}_D$ +39.5° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.00 (dd, J = 16.2 Hz, 9.5 Hz, 1H), 3.25 (dt, J = 16.2 Hz, 9.9 Hz, 1H), 3.70 (bs, 2H), 3.90 (m, 1H), 4.05 (s, 3H), 4.60 (m, 1H), 5.65 (d, J = 7.3 Hz, 1H), 6.45 (d, J = 2.1 Hz, 1H), 6.80 (s, 1H), 6.95−7.10 (m, 2H), 7.30−7.50 (m, 4H), 7.56 (dd, J = 7.0 Hz, 1.4 Hz, 2H), 8.23 (dd, J = 7.9 Hz, 1.9 Hz, 1H), 9.81 (d, J = 7.3 Hz, 1H). Anal. ($C_{25}H_{22}N_4O_3$) C, H, N, O.

4-Amino-3,5-dichloro-*N*-((*R*)-9-methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-benzamide (14b). Starting from **8b**, **14b** was finally purified by chromatography (98:2 CH_2Cl_2/MeOH) followed by recrystallization from Et_2O: white powder; 56.3% yield; mp 244 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 3.10 (dd, J = 16.2 Hz, 7.9 Hz, 1H), 3.35 (dt, J = 16.2 Hz, 9.9 Hz, 1H), 3.97 (m, 1H), 4.65 (m, 1H), 4.80 (bs, 2H), 5.55 (d, J = 7.6 Hz, 1H), 7.04 (s, 1H), 7.28 (s, 1H), 7.34−7.57 (m, 5H), 7.77 (d, J = 7.6 Hz, 1H), 7.84 (s, 2H). Anal. ($C_{25}H_{20}Cl_2N_4O_2 \cdot H_2O$) C, H; N: calcd, 11.27; found, 10.85.

4-Amino-*N*-((*R*)-9-amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-3,5-dichlorobenzamide (14e). Starting from **8e, 14e** was finally purified by chromatography (98:2 CH_2Cl_2/MeOH) followed by recrystallization from EtOAc: yellow powder; 43% yield; mp >280 °C;

$[\alpha]^{20}_D$ +48° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 2.00 (dd, J = 15.9 Hz, 9.2 Hz, 1H), 3.27 (m, 1H), 3.83 (m, 1H), 4.42 (m, 1H), 5.32 (bs, 2H), 5.45 (d, J = 8.0 Hz, 1H), 6.10 (bs, 2H), 6.36 (s, 1H), 6.88 (s, 1H), 7.37−7.60 (m, 5H), 8.0 (s, 2H), 9.43 (d, J = 8.0 Hz, 1H). Anal. ($C_{24}H_{19}Cl_2N_5O_2 \cdot {}^1/_6C_3H_8O$) C, H, N.

[4-((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-ylcarbamoyl)phenyl]carbamic Acid *tert*-Butyl Ester (15e). 15e was synthesized from **8e** and using the 4-*tert*-butoxycarbonylaminobenzoic acid prepared by Boc protection of 4-aminobenzoic acid as described in general procedure A and **15e** was finally purified by chromatography (80:20 ethyl acetate/hexane) followed by recrystallization from 2-propanol/hexane: yellow powder; 52% yield; mp 215−225 °C; $[\alpha]^{20}_D$ +47° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 9H), 3.05 (dd, J = 16.2 Hz, 9.3 Hz, 1H), 3.29 (dt, J = 16.2 Hz, 9.9 Hz, 1H), 3.70 (bs, 2H), 3.93 (m, 1H), 4.63 (m, 1H), 5.62 (d, J = 7.5 Hz, 1H), 6.46 (d, J = 2.0 Hz, 1H), 6.72 (bs, 1H), 6.84 (s, 1H), 7.34−7.50 (m, 5H), 7.57 (m, 2H), 7.92 (m, 3H). Anal. ($C_{29}H_{29}N_5O_4$) H, N; C: calcd, 68.09; found, 66.43; O: calcd, 12.51; found, 13.96.

Quinoline-3-carboxylic Acid ((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (16e). Starting from **8e**, **16e** was finally purified by chromatography with a gradient of 0−4% MeOH/CH_2Cl_2: yellow powder; 43.5% yield; mp >260 °C; $[\alpha]^{20}_D$ +75.5° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.02 (dd, J = 15.9, 8.9 Hz, 1H), 3.28 (m, 1H), 3.85 (m, 1H), 4.45 (m, 1H), 5.28 (bs, 2H), 5.55 (d, J = 8.0 Hz, 1H), 6.39 (s, 1H), 6.90 (s, 1H), 7.40−7.60 (m, 5H), 7.72 (t, J = 7.9 Hz, 1H), 7.90 (t, J = 7.1 Hz, 1H), 8.15 (m, 2H), 9.07 (d, J = 2.0 Hz, 1H), 9.40 (d, J = 2.0 Hz, 1H), 9.92 (d, J = 8.0 Hz, 1H). Anal. ($C_{27}H_{21}N_5O_2 \cdot {}^1/_2H_2O \cdot {}^1/_4CH_3OH$) C, H, N, O.

Isoquinoline-3-carboxylic Acid ((*R*)-9-Nitro-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (17d). Starting from **8d**, **17d** was finally purified by crystallization from acetone. The crystals were dissolved in CH_2Cl_2 and the solvent was removed in vacuo: white powder; 54% yield; mp 185−190 °C; $[\alpha]^{20}_D$ +229° (c = 0.2, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.25 (m, 1H), 3.45 (m, 1H), 4.13 (m, 1H), 4.56 (m, 1H), 5.60 (d, J = 7.7 Hz, 1H), 7.30−7.60 (m, 5H), 7.80−7.96 (m, 2H), 8.05 (s, 1H), 8.20−8.35 (m, 2H), 8.45 (s, 1H), 8.65 (s, 1H), 9.50 (s, 1H), 9.72 (d, J = 7.7 Hz, 1H). Anal. ($C_{27}H_{19}N_5O_4 \cdot {}^3/_5H_2O$) H, N; C: calcd, 62.70; found, 61.76.

Isoquinoline-3-carboxylic Acid ((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (17e). Starting from **8e**, a precipitate formed and **17e** was finally filtered and washed with CH_2Cl_2: yellow powder; 46% yield; mp >280 °C; $[\alpha]^{20}_D$ +16° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 3.03 (dd, J = 15.7 Hz, 9.1 Hz, 1H), 3.25 (m, 1H), 3.88 (m, 1H), 4.45 (m, 1H), 5.30 (bs, 2H), 5.40 (d, J = 7.7 Hz, 1H), 6.40 (s, 1H), 6.90 (s, 1H), 7.38−7.55 (m, 5H), 7.82−7.95 (m, 2H), 8.20−8.35 (m, 2H), 8.65 (s, 1H), 9.50 (s, 1H), 9.67 (d, J = 7.7 Hz, 1H). Anal. ($C_{27}H_{21}N_5O_2$) C, H, N.

4,7-Dimethylpyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylic Acid ((*R*)-9-Methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (18b). Starting from **8b, 18b** was finally purified by chromatography (98:2 CH_2Cl_2/acetone): yellow powder; 71% yield; mp 130 °C; $[\alpha]^{20}_D$ +19.6° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 2.58 (s, 3H), 3.02 (m, 1H), 3.25 (m, 4H), 3.90 (m, 1H), 4.60 (m, 1H), 5.57 (m, 1H), 6.95 (s, 1H), 7.0 (s, 1H), 7.20 (s, 1H), 7.25−7.55 (m, 5H), 9.80 (m, 1H). Anal. ($C_{26}H_{23}N_7O_2 \cdot {}^1/_2H_2O$) C, H, N.

4,7-Dimethyl-3,4-dihydropyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylic Acid *N*-((*R*)-9-Methoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (18c). Starting from **8c**, **18c** was finally purified by chromatography (96:4 CH_2Cl_2/acetone): yellow powder; 61% yield; mp 95 °C; $[\alpha]^{20}_D$ +19.6° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.65 (s, 3H), 3.12 (dd, J = 16.2 Hz, 8.0 Hz, 1H), 3.30 (s, 3H), 3.35 (m, 1H), 3.72 (s, 3H), 4.00 (m, 1H), 4.70 (m, 1H), 5.67 (d, J = 7.7 Hz, 1H), 6.70 (d, J = 2.3 Hz, 1H), 7.09 (s, 2H), 7.35−7.50 (m, 3H), 7.62 (m, 2H), 9.88 (d, J = 7.7 Hz, 1H); MS m/z 481.

2,4,7-Trimethylpyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylic Acid ((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (18e). Starting from **8e**, **18e** was finally purified by chromatography with a gradient of 0−1% MeOH/CH_2Cl_2: orange powder; 44% yield; mp 182−185 °C; $[\alpha]^{20}_D$ −20° (c = 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58 (s, 3H), 3.02 (dd, J = 16.2 Hz, 8.0 Hz, 1H), 3.20−3.37 (m, 4H), 3.60−3.85 (m, 2H), 3.92 (m, 1H), 4.64 (m, 1H), 5.64 (d, J = 7.6 Hz, 1H), 6.46 (d, J = 2.0 Hz, 1H), 6.82 (s, 1H), 7.05 (s, 1H), 7.30−7.45 (m, 3H), 7.59 (d, J = 7.0 Hz, 2H), 9.83 (d, J = 7.7 Hz, 1H). Anal. ($C_{25}H_{22}N_8O_2 \cdot {}^1/_2H_2O \cdot {}^3/_{20}CH_2Cl_2$) C, H, O, N.

4,7-Dimethylpyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylic Acid ((*R*)-4-Oxo-1-phenyl-9-pyrrolidin-1-yl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)amide (18f). Starting from **8h**, **18f** was finally purified by successive chromatography, first eluting with 0−2.5% MeOH/CH_2Cl_2, then eluting with 95:5 CH_2Cl_2/acetone and followed by recrystallization in ethyl acetate: yellow powder; 61% yield; mp 95 °C; $[\alpha]^{20}_D$ +19.6° (c = 1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (m, 4H), 2.60 (s, 3H), 3.02 (m, 1H), 3.15 (m, 4H), 3.25 (s, 3H), 3.30 (m, 1H), 3.90 (m, 1H), 4.62 (m, 1H), 5.63 (m, 1H), 6.25 (s, 1H), 6.72 (s, 1H), 7.02 (s, 1H), 7.25−7.43 (m, 3H), 7.60 (m, 2H), 9.83 (m, 1H).

General Procedure K: Coupling Reaction with Carboxylic Acid Derivatives and PyBrop. [4-((*R*)-4-Oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-ylcarbamoyl)pyridin-3-yl]carbamic Acid *tert*-Butyl Ester (12a). To a stirred solution of **8a** (555 mg, 2 mmol) in DMF (15 mL) were introduced 3-*tert*-butoxycarbonylaminoisonicotinic acid (477 mg, 2 mmol) previously prepared by protection of 3-aminoisonicotinic acid (see general procedure A), bromotris(pyrrolidino)phosphonium hexafluorophosphate (PyBrop) (1.2 g, 2.4 mmol) and triethylamine (0.83 mL, 6 mmol). After being stirred at room temperature for 12 h, the solvent was removed under reduced pressure and CH_2Cl_2 (100 mL) was introduced. The organic extract was washed with H_2O (100 mL), HCl and H_2O (250 mL). After neutralization with concentrated NaOH, the aqueous phase was extracted by CH_2Cl_2 (2 × 150 mL). The combined organic layers were washed with H_2O, dried and filtered. The solvent was removed in vacuo and the crude material was purified by chromatography eluting with 98:2 CH_2Cl_2/MeOH to afford **12a**:670 mg, 67%; yellow powder; ^{1}H NMR (400 MHz, DMSO) δ 1.45 (s, 9H), 3.15 (m, 1H), 3.40 (m, 1H), 3.97 (m, 1H), 4.51 (m, 1H), 5.45 (d, J = 7.6 Hz, 1H), 7.22 (m, 2H), 7.40−7.68 (m, 6H), 7.92 (d, J = 5 Hz, 1H), 8.40 (d, J = 5.0 Hz, 1H), 9.33 (s, 1H), 9.87 (s, 1H), 10.15 (d, J = 7.6 Hz, 1H); MS m/z 498.

[4-((*R*)-9-Amino-4-oxo-1-phenyl-3,4,6,7-tetrahydro[1,4]-diazepino[6,7,1-*hi*]indol-3-ylcarbamoyl)pyridin-3-yl]carbamic Acid *tert*-Butyl Ester (12e). Starting from **8e** in THF (instead of DMF), **12e** was purified by successive chromatography with a gradient of 0−2% MeOH/CH_2Cl_2 then using ethyl acetate: yellow powder; 23% yield; mp 221−225 °C; $[\alpha]^{20}_D$ −24.5° (c = 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.00−3.80 (m, 2H), 3.04 (dd, J = 16.3 Hz, 8.2 Hz, 1H), 3.28 (dt, J = 16.3 Hz, 9.9 Hz, 1H), 3.94 (m, 1H), 4.60 (m, 1H), 5.52 (d, J = 7.1 Hz, 1H), 6.46 (d, J = 2.0 Hz, 1H), 6.84 (s, 1H), 7.33−7.60 (m, 6H), 8.18 (t, J = 7.1 Hz, 1H), 8.37 (d, J = 5.1 Hz, 1H), 9.70 (s, 1H), 9.80 (s, 1H). Anal. ($C_{28}H_{28}N_6O_4 \cdot {}^2/_3H_2O \cdot {}^1/_{20}CH_2Cl_2$) C, H, N, O.

General Procedure L: Acetylation of the Amino Group. *N*-((*R*)-9-Acetylamino-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-4-amino-3,5-dichloro-2-methylbenzamide (14h). To a stirred solution of **14e** (1.5 g, 3.1 mmol) in pyridine (10 mL) was added acetic anhydride (6.2 mL, 6.5 mmol). The reaction mixture was stirred overnight at room temperature. Water (65 mL) was introduced and stirring was maintained further 4 h. The aqueous layer was extracted with EtOAc (3 × 75 mL). The joined organic layers were washed with saturated $NaHCO_3$ (2 × 75 mL), dried,

filtered and concentrated in vacuo. Purification by chromatography eluted with a gradient of 0−40% acetone/CH_2Cl_2 provided **14h:** 0.7 g, 43%; beige powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 3H), 3.10 (dd, J = 16.3 Hz, 8.3 Hz, 1H), 3.32 (dt, J = 16.3 Hz, 10.0 Hz, 1H), 3.95 (m, 1H), 4.60 (m, 1H), 4.82 (bs, 2H), 5.45 (d, J = 7.4 Hz, 1H), 7.00 (d, J = 1.5 Hz, 1H), 7.30−7.52 (m, 5H), 7.71 (bs, 1H), 7.75 (d, J = 7.4 Hz, 1H), 7.80 (s, 2H), 7.96 (s, 1H); MS m/z 521. Anal. ($C_{26}H_{21}Cl_2N_5O_3 \cdot {}^3/_{10}C_2H_5O \cdot {}^3/_5H_2O$) C, H, N, O, Cl.

***N*-((*R*)-9-Acetylamino-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-3-methylisonicotinamide (9h).** Starting from **9e, 9h** was finally purified by successive chromatography, first eluting with a gradient of 0−40% acetone/CH_2Cl_2 then eluting with a gradient of 5−10% MeOH/CH_2Cl_2: beige powder; 72% yield; mp 204−205 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 3H), 3.07 (dd, J = 16.4 Hz, J = 8.4 Hz, 1H), 3.27 (dt, J = 16.4 Hz, 10.0 Hz, 1H), 3.92 (m, 1H), 4.56 (m, 1H), 5.44 (d, J = 7.3 Hz, 1H), 6.97 (d, J = 1.3 Hz, 1H), 7.24−7.46 (m, 5H), 7.72 (m, 3H), 7.93 (s, 1H), 8.04 (d, J = 7.3 Hz, 1H), 8.71 (d, J = 5.9 Hz, 2H); MS m/z 439. Anal. ($C_{25}H_{21}N_5O_3 \cdot {}^1/_{10}CH_2Cl_2 \cdot {}^3/_4H_2O$) C, H, N, O.

General Procedure M: Pyrrolidine Formation. 4-Amino-3,5-dichloro-2-methyl-*N*-((*R*)-4-oxo-1-phenyl-9-pyrrolidin-1-yl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-benzamide (14f). To a stirred solution of **14e** (1.5 g, 3.1 mmol) in acetonitrile (50 mL) was added 1,4-dibromobutane (0.56 mL, 4.7 mmol). The reaction mixture was stirred overnight at reflux and then was concentrated. Water (200 mL) was introduced and, keeping the temperature around 0 °C, concentrated NaOH was slowly added dropwise up to pH 12. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by chromatography eluted with a gradient of 0−40% acetone/CH_2Cl_2 provided **14f**: 0.6 g, 36%; yellow powder; mp 218−220 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (m, 4H), 3.07 (dd, J = 16.0 Hz, 9.2 Hz, 1H), 3.21 (m, 4H), 3.34 (dt, J = 16.0 Hz, 9.8 Hz, 1H), 3.95 (m, 1H), 4.63 (m, 1H), 4.80 (bs, 2H), 5.57 (d, J = 7.4 Hz, 1H), 6.28 (s, 1H), 6.76 (s, 1H), 7.32−7.47 (m, 3H), 7.62 (d, J = 7.7 Hz, 2H), 7.84 (m, 3H); MS m/z 533. Anal. ($C_{28}H_{25}Cl_2N_5O_2 \cdot {}^1/_2H_2O$) C, H, N, O, Cl.

General Procedure N: Dimethylation of the Amino Group. Aminodichloro-*N*-((*R*)-dimethylaminooxophenyl-3,4,6,7-tetrahydro[1,4]diazepino[6,7,1-*hi*]indol-3-yl)-*C*-methylbenzamide (14 g). To a stirred solution of **14e** (2 g, 4.2 mmol) in acetonitrile (100 mL) were successively added 37% formaldehyde in water (3.43 mL, 42 mmol), sodium cyanoborohydride (0.8 g, 12.5 mmol) and glacial acetic acid (0.5 mL). The reaction mixture was stirred at room temperature for 2 h and further glacial acetic acid (0.5 mL) was introduced. After stirring 15 min, the mixture was pooled into diethyl ether (350 mL), a white precipitate formed and was filtered. The organic layer was successively washed with NaOH (1 N, 2 × 100 mL) and saturated NaCl (2 × 100 mL), then dried, filtered and concentrated in vacuo. Purification by successive chromatography, first eluting with a gradient of 0−1% MeOH/CH_2Cl_2, then eluting with a gradient of 0−20% acetone/CH_2Cl_2 afforded **14g**: 0.65 g, 31%; orange powder; mp 192 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 (s, 6H), 3.07 (dd, J = 16.1 Hz, 8.1 Hz, 1H), 3.33 (dt, J = 16.1 Hz, 9.9 Hz, 1H), 3.93 (m, 1H), 4.62 (m, 1H), 4.77 (bs, 2H), 5.55 (d, J = 7.5 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 6.92 (s, 1H), 7.30−7.45 (m, 3H), 7.59 (d, J = 7.1 Hz, 1H), 7.8 (m, 3H); MS m/z 507. Anal. ($C_{26}H_{23}Cl_2N_5O_2 \cdot {}^1/_4C_2H_{10}O \cdot {}^1/_3H_2O$) C, H, N, O, Cl.

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