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Synthesis and Structure–Activity Relationships of 4-Oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]indoles: Novel PDE4 Inhibitors

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Abstract—A novel series of benzodiazepine derivatives have been discovered as inhibitors of PDE4 enzymes. We have found that our compounds are selective versus other PDE enzymes, and that the activity can be modulated by specific structural modifications. One compound exhibited a strong eosinophilic infiltration inhibiting action on sensitized Brown-Norway rats (compound **9,** 5.1 mg/kg p.o.), moreover this compound is not emetic at 3 mg/kg i.v.

Phosphodiesterases (PDE) are important enzymes, participating in cellular regulation of the second messengers cAMP or cGMP. Following the classification proposed by Beavo,[1] the different PDE isoforms can be grouped in seven different families.

PDE4 enzymes are cAMP specific, the inhibition of which has attracted increasing interest as a possible treatment of an allergic disease such as asthma.[2,3] Among the PDE4 inhibitors that have been synthesised, most compounds are derivatives of Rolipram (Fig. 1).[4] Others are derivatives of xanthines, or pyridopyrimidines.[5] Rolipram is a selective inhibitor at the catalytic site, but also binds stereoselectively (*R*-Rolipram) to a separate site called high affinity Rolipram binding site (HARBS). The IC_{50} of Rolipram on HARBS is 0.005 μM. This site is thought to be responsible for the CNS in vivo activity of Rolipram[6] and has been implicated in the side effects observed, such as emesis.[7,8]

Recently we have discovered a new series of PDE4 inhibitors in the benzodiazepine family, represented by compound **1**. Several of these compounds exhibit potent phosphodiesterase inhibiting properties with high selectivity towards PDE4 and low affinity for the HARBS. In this paper we report the structure–activity relationships and pharmacological activities of a novel series of benzodiazepine PDE4 inhibitors.

Chemistry

Several years ago, we studied cholecystokinin receptor antagonists and synthesised a series of derivatives of aminobenzodiazepine. By testing this library against PDE4 enzymes extracted from human U937 cells, we found that a compound of this series (**1**) had good inhibitory activity.We subsequently carried out a structure–activity study around this compound in efforts to increase activity.

All compounds in this series were synthesised according to Scheme 1, where a representative example (**1**) is shown.

Starting aminobenzodiazepines,[9] aminopyrrolobenzodiazepines[10] or aminoquinolodiazepines[11] have been described previously. Enantiomeric separation of the

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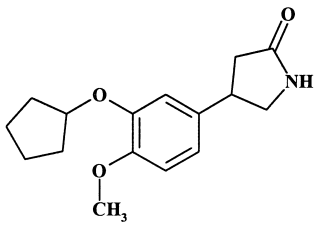


Figure 1. Rolipram.

aminopyrrolobenzodiazepines was carried out by crystallisation of their salts with *N*-acetylphenylalanine in *n*-propanol or ethylacetate. Condensations between amines and different acids were carried out by classical methods: acid chloride and amine in an inert solvent in presence of a base, dicyclohexylcarbodiimide and hydroxybenzotriazole, pentafluorophenyl ester of the acid with the amine in an inert solvent, and *O*-[(ethoxycarbonyl)-cyanomethylene-amino]-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TOTU) with the acid and the amine in an inert solvent in presence of diisopropylethylamine.

Pharmacology

The inhibitory activities of these compounds have been tested (Table 1) on different PDE isoenzymes extracted from human U937 cells (PDE4),[12] dog aorta smooth muscle cells (PDE3) and guinea-pig trachea smooth muscle cells (PDE1/5). IC_{50}'s have been determined by assaying the cleavage of [^{3}H]cAMP or [^{3}H]cGMP to its corresponding radiolabeled nucleoside 5′-monophosphate.

One of the most potent compounds (**8**) displayed an IC_{50} on PDE4 of 0.71 ± 0.166 μM and showed selectivity for PDE4 against PDE3 ($IC_{50} \geq 100$ μM) and PDE1/5 ($IC_{50} \geq 100$ μM). Binding to the HARBS has been determined by displacing radioactive [3H]-Rolipram from brain membrane suspensions from Wistar rats.

In vivo activity has been assessed by measuring inhibition of antigen-induced lung eosinophilia in ovalbumin-sensitized Brown-Norway rats after oral administration.[13] In this model, the active compounds **9** and **10** have ED_{50}s of 5.1 mg/kg p.o. [3.2–6.9] and 10.7 mg/kg p.o. [3.44–33.26], respectively.

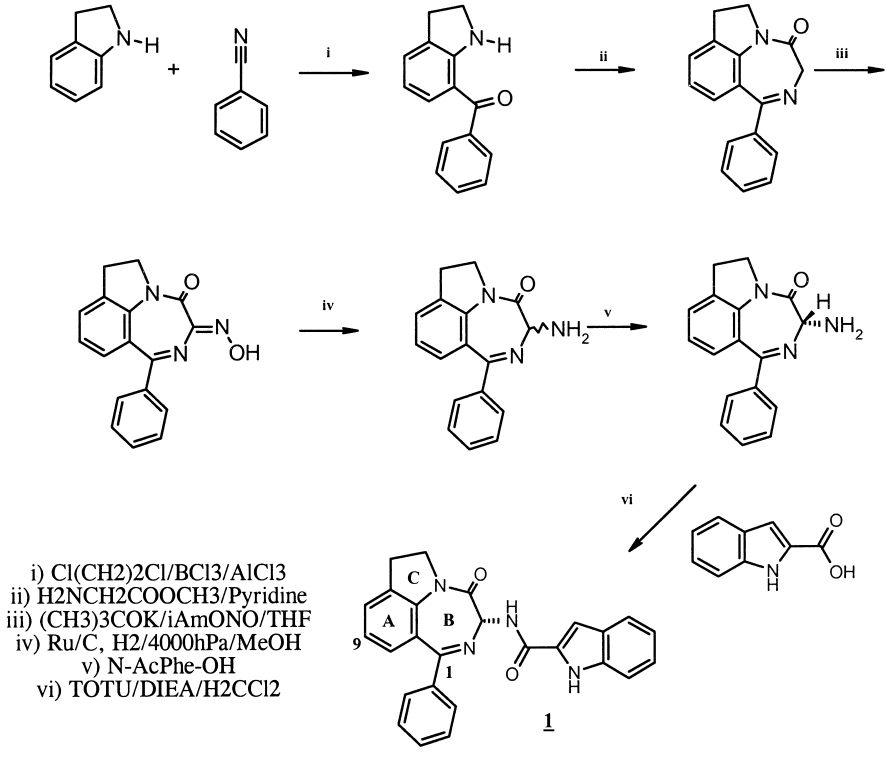
Furthermore, these two compounds have been tested for their emetic potential in dogs in comparison with Rolipram. In this model, both compounds did not show any emetic effect up to 3 mg/kg i.v. although rolipram is strongly[14] emetic at the dose of 0.03 mg/kg i.v.

Results and Discussion

We have systematically modified several regions of the parent compound, (3*R*)-3-(2-indolyl)-carboxylamino-4-oxo-1-phenyl-3,4,6,7-tetrahydro-[1,4]diazepino[6,7,1-*hi*]-indole (**1**), to elucidate important features for activity.

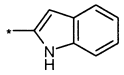
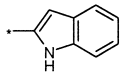
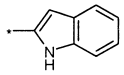
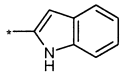
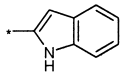
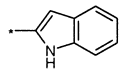
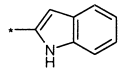
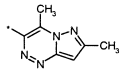
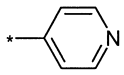
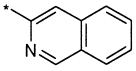
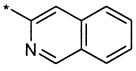
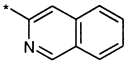
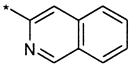
Size of the fused ring C (Fig. 2)

We explored the importance of the ring structure fused to the benzodiazepine nucleus by synthesis of compounds **3** and **4** (Table 1). Both the non-cyclic benzodiazepine **3** and



Scheme 1. Synthesis of benzodiazepine derivatives.

Table 1. Phosphodiesterase inhibitory activity and HARBS binding of benzodiazepine derivatives

No	*n*	Sructure	Configuration	R1	R2	R3	R4 Or Heterocycle	R5	R6	PDE4	PDE3 IC_{50}[a],μM	PDE1/5	HARBS Binding IC_{50}[a], μM
1	1	I	R	H	H					4	≥100	≥100	ND[c]
2	1	I	R	H	F					≥100	≥100	ND	ND
3	>[b]	I	R,S	H	H					≥100	≥100	ND	ND
4	2	I	R,S	H	H					≥100	≥100	≥100	ND
5	1	I	S	H	H					100	≥100	100	ND
6	1	I	R,S	Cl	F					≥100	≥100	ND	ND
7	1	I	R	MeO	H					0.65	≥100	100	ND
8	1	I	R	Me	H					0.71	≥100	≥100	1.09
9	1	I	R	Me	H					1.14	≥100	≥100	≥3
10	1	I	R	Me	H					2.6	≥100	≥100	≥3
11	1	I	R	H	H					4.2	≥100	≥100	≥3
12	1	I	R	MeO	H					1.62	82	65.3	> 3
13	1	I	R	OH	H					0.41	≥3.5	≥100	ND
14	1	II	R	Me	H	H	H	Cl	H	1.25	27	> 100	≥3
15	1	II	R	MeO	H	H	H	Cl	H	1.8	62	40	≥3
16	1	II	R	Me	H	H	Cl	H	Cl	1.15	43.7	> 100	ND
17	1	II	R	Me	H	Cl	H	Cl	H	0.76	17.5	> 100	≥3
18	1	II	R	Me	H	MeO	H	H	H	0.76	> 100	71.7	≥3
19	1	II	R	Me	H	MeO	H	MeO	H	0.96	7.85	ND	≥3
20	1	II	R	H	H	H	Cl	NH2	Cl	0.63	> 100	> 100	≥2
21	1	II	R	Me	H	H	Cl	NH2	Cl	0.33	17.5	> 100	ND

[a]IC_{50} values are average of three independent determinations.
[b]Compound without C ring.
[c]Not determined.

the compound **4** ($n = 2$) having a 6 membered ring are not active (Table 1). Only compounds originally selected, with a five membered ring have the desired activity. This is of great importance for the conformation of the molecule because the five membered ring imposes a considerable rigidity to the structure, and only two conformations are possible for the multi-ring nucleus.

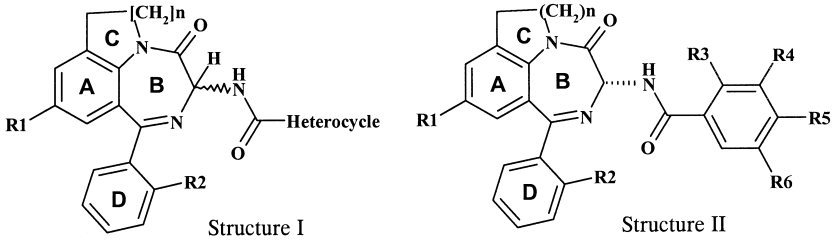


Figure 2. Structures of benzodiazepine derivatives in Table 1.

Stereochemistry

The active isomer is of *R* configuration, the *S* isomer being completely devoid of inhibitory activity against PDE4 (**5** *versus* **1**).

Derivatives of the phenyl ring D in position 1 of the main ring B (Fig. 2)

The ortho fluoro analogue **2** showed that a slight modification of the **D** phenyl ring induces a total withdrawal of the PDE4 inhibiting activity.

Ring

Substitutions of ring A showed an influence on activity. Thus, the introduction of a methoxy or a methyl group at the 9-position increases activity (compounds **7** versus **1, 12** versus **11**, **10** versus **11,** and **21** versus **20**). The PDE4 potency is further enhanced by the introduction of a hydroxyl group at the same position (compound **13**).

Side chain modifications

The nature and substitution of the aromatic ring on the side chain modulate the inhibitory activity of the compounds. Among the derivatives of benzoic acid, the 4-chloro substituted analogue **14** is active, and the dichloro compounds are equivalent **16** or better **17** than **14**. The monomethoxy substituent in the ortho position (**18**) shows among the best activities, but the dimethoxy substitution gives no improvement (**19**). The best derivative is the tri-substituted 4-amino-3,5-dichlorobenzoic analogue **21**.

Compounds substituted with heterocyclic moieties (Table 1) show different activities according to the nature and substitution of the heterocycle. Among heterocycles, a pyrazolotriazine is the best compound in vitro (**8**). The 4-pyridine derivative **9** was found very promising in further pharmacological studies and was selected for clinical investigations. In summary, a novel series of benzodiazepine derivatives has been discovered as inhibitors of the enzyme PDE4. We have shown that our compounds are selective versus other PDE isoenzymes. Compound **9** was selected for further biological evaluation. This compound shows significant in vivo activity in a model of antigen induced eosinophilia in the Brown-Norway rat. In addition, compound **9** shows no emetic activity at therapeutic doses unlike Rolipram and numerous inhibitors related to Rolipram. Since many of the first generation inhibitors have limited potential due to side effects, this series of compounds offers a new class with significant potential for the treatment of inflammatory diseases. More detailed SAR and pharmacological activity will be communicated at a later date.

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