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Otimização Estrutural de 6-Arilpiridazin-3-onas como Potentes Inibidores da PDE4

Resumo: A síntese e a análise da relação estrutura-atividade (REA) de uma série de derivados 4,5-di-hidropiridazin-3-onas como inibidores de PDE4 foi descrita. Explorações topológicas na posição N-2 do anel piridazina permitiu identificação de interações adicionais no sítio de ligação da enzima PDE4, levando a compostos significativamente mais potentes (**10v**, IC_{50} ~20 nM) com aumento da solubilidade em água.

Palavras-chave: Fosfodiesterase-4; 4,5-di-hidropiridazin-3-onas; Relações Estrutura-Atividade.

Abstract

The synthesis and structure-activity relationship (SAR) analysis of series of 4,5-dihydropyridazin-3-one derivatives as PDE4 inhibitors are described. Topological explorations at the position N-2 of the pyridazine ring allowed identification of additional interactions with PDE4 binding site, leading to significantly more potent compounds (**10v**, IC_{50} ~20 nM) with increased water solubility.

Keywords: Phosphodiesterase-4; 4,5-dihydropyridazin-3-ones; Structure-Activity Relationships.

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Structural Optimization of 6-aryl Pyridazin-3-ones as Novel Potent PDE4 Inhibitors

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1. Introduction

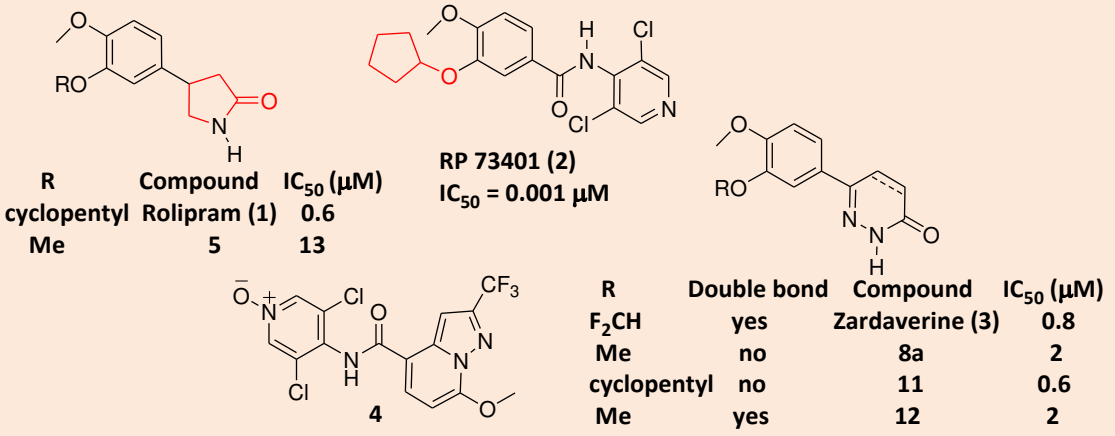
The different eleven major subclasses of cyclic nucleotide phosphodiesterases (PDE) constitute attractive targets for the design of new therapeutic agents.[1,2] In particular the phosphodiesterase-4 (PDE4) is responsible for specific hydrolysis of cyclic adenosine monophosphate (cAMP), and is mainly located in airway smooth muscles, in immune and inflammatory cells, but also in the brain.[3,4]

The antidepressant rolipram (**1**) was identified as the first selective PDE4 inhibitor with a µM range IC_{50} value, and served as a lead structure for further optimizations. SAR analyses clearly highlighted the role played

by the lipophilic cyclopentyloxy group (Figure 1) in the structure of rolipram,[5] and constituted a key pharmacophoric requirement for more potent PDE4 inhibitors structurally-related to rolipram such as RP 73401 (**2**).[6]

Zardaverine (**3**) is a 6-aryl-pyridazinone with antiinflammatory properties, that are related with its PDE4/PDE3 inhibitory profile.[7] In the past, other 6-aryl pyridazinones were identified as inhibitors of cardiac phosphodiesterase 3 (PDE3) that present potent cardiotonic activity.[8-12] Recently, other classes of compounds that showed interaction with PDE4 were developed, such as the pyrazolo[1,5-*a*]pyridines, among which the compound **4**, showed an IC_{50} of 0.27 nM for PDE4 and presented anti-inflammatory properties in animal models.[13]

The aim of this work was to better characterize substituent effects on both potency and selectivity of a series of differently substituted 6-aryl-pyridazinones.



R	Compound	IC_{50} (µM)
cyclopentyl	Rolipram (1)	0.6
Me	5	13

R	Double bond	Compound	IC_{50} (µM)
F_2CH	yes	Zardaverine (3)	0.8
Me	no	8a	2
cyclopentyl	no	11	0.6
Me	yes	12	2

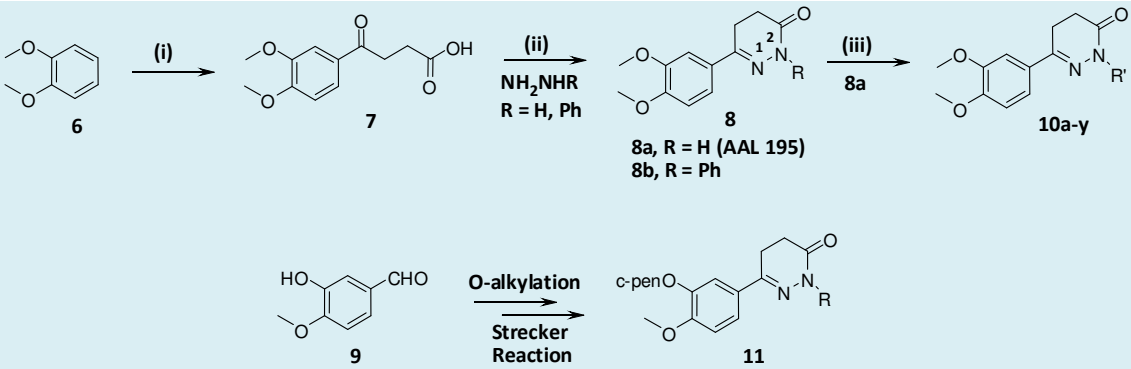
Figure 1. Rolipram and structurally-related compounds as PDE4 inhibitors

2. Materials and Methods

The NH free 6-(3,4-dimethoxyphenyl)-dihydropyridazinone derivative **8a** was used as internal reference for SAR analysis, whereas the unsaturated pyridazinone **12** was proved to present the same potency on PDE 4. Moreover the N-2 nitrogen was used as an anchor point for further topological explorations. Applying homology and molecular diversity concepts, a limited number of lengths and chemical functions (cations, anions, H bond acceptor-donor systems, aromatics) were easily introduced at the N-2 nitrogen.

The selected dihydropyridazinones **10a-h** were prepared by refluxing the corresponding β-benzoyl propionic acid intermediate **7** with hydrazines. Moreover further N-substitutions could be obtained from the unsubstituted pyridazinone **8a** in presence of NaH as a base and various functionalized halides.

Two different approaches were chosen for preparing the β-benzoyl propionic acids. Classical Friedel-Crafts reaction[14] using veratrol in presence of succinic anhydride and $AlCl_3$ afforded the dimethoxyphenyl derivative **7**. The Strecker reaction[15] starting from the correctly substituted benzaldehyde **9** was also used for building the meta alkoxy derivative **11** (Scheme 1). The final compounds have been purified by flash chromatography. The chromatographically pure compounds gave NMR spectra in agreement with their expected structures. Detailed experimental procedures and characterizations will be given elsewhere.



Scheme 1. Reagents and conditions: (i) succinic anhydride (1.0 equiv.), $AlCl_3$ (5.0 equiv.), nitrobenzene, rt, 24 h, 66%; (ii) hydrazine hydrate or phenyl hydrazine (2.0equiv.), n-BuOH, 85 °C, 2h, 96%; (iii) NaH (1.1 equiv., in 60% oil suspension), RBr (1.1 equiv.), DMF, rt, 0.25-0.5 h, 53-98%

The choice of representatives for exploring the molecular diversity was partially driven by the easy transformations between chemical moieties (i.e. $CO_2Et \rightarrow CO_2H \rightarrow CONR_1R_2$, $OH \rightarrow NR_1R_2$, $NHBoc \rightarrow NH_2 \rightarrow NHCOR$). In general three different lengths were chosen for the linker, representing short (n = 1 or 2), medium (n = 3 or 4), and large (n = 5 or 6) distance interactions between the main pyridazine scaffold and the functional group FG.

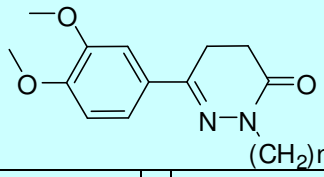
Cytosolic PDE4 isoforms (PDE1, PDE3, PDE4, and PDE5) were purified by anion-exchange chromatography from the medial layer of bovine aorta and PDE2 was isolated from human platelets as previously described.[16] PDE activities were measured by radio enzymatic assay at a substrate concentration of 1μM cAMP in the presence of 15000 cpm [^{3}H]-cAMP as a tracer.[17] The buffer solution was of the following composition: 48 mM Tris-HCl pH 7.5, 2 mM magnesium acetate, and 1 mM ethylene glycol bis(β-aminoethylether) *N,N,N,N'*-tetracetic acid (EGTA). To prevent the influence of cross-contamination with PDE3 the study was always carried out in the presence of 50 μM cGMP. The compounds were dissolved in DMSO. The final concentration of DMSO in the assay (1%) did not affect PDE activity. The concentration of compounds that produced 50% inhibition of substrate hydrolysis (IC_{50}) values was calculated by non linear regression analysis from concentration-response curves (Prism software) and represented the mean value of three determinations.[17]

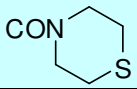
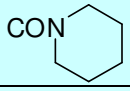
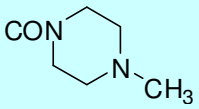
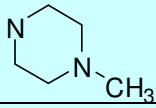
3. Results and Discussion

Compounds were first tested as PDE4-inhibitors. The value of the percentage of inhibition ≥ 95% at 10 μM allowed us to select the most active compounds, for which both an IC_{50} value and a selectivity profile towards other PDE isoforms could be determined. Results are given in Tables 1 and 2.

Following this procedure, it is interesting to notice that both rolipram and the unsubstituted pyridazinone **11** presented similar potencies with IC_{50} of 0.6 μM, most probably as the result of the strong structural similarities between both representatives which share a common pharmacophoric pattern[5].

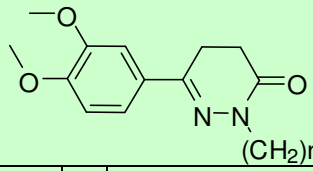
Table 1. Inhibition of PDE4 by dihydropyridazinones



Compounds	Functional Group (FG)	n	PDE4 IC_{50}[a] µM, or (% of inhibition[b])		Compounds	Functional Group (FG)	n	PDE4 IC_{50}[a] µM, or (% of inhibition[b])
Rolipram			0.6		**10k**	$CONH_2$	1	(58%)
RP 73401			0.001		**10l**	DMB[c]	1	0.76
5			53.0		**10m**	DMB[c]	3	0.60
11			0.63		**10n**		1	(88%)
8a	H	0	2.0		**10o**		3	(86%)
8b	Ph	0	0.55		**10p**		3	(85%)
10a	Ph	1	0.06		**10q**	NHBOC[d]	3	0.48
10b	Ph	3	0.02		**10r**	NHBOC[d]	6	0.06
10c	OH	3	(79%)		**10s**	NHCOPh	6	0.03
10d	OH	6	0.25		**10t**	DMB[c]	6	(90%)
10e	CO_2Et	3	0.27		**10u**		6	(16%)
10f	CO_2Me	4	0.17		**10v**	NH_2	6	0.02
10g	CO_2H	1	(26%)		**10w**		6	0.18
10h	CO_2H	3	(70%)		**10x**		6	0.21
10i	CO_2H	4	1.4		**10y**		6	0.39
10j	CONHOH	4	3.3					

[a]The IC_{50} was calculated by linear regression (correlation coefficient r = 0.095) and represents the mean value of three determinations. The experimental error is about 15%. [b]At 10 µM of final drug concentration. [c]DMB = 2,4 dimethoxybenzyl. [d]BOC = tert-butyloxycarbonyl

Table 2. Selectivity of the most potent PDE4-inhibitors derived from dihydropyridazinones versus PDE1, PDE2, PDE3 and PDE5



Compounds	FG	n	IC_{50} [a] (μM) or (%) of inhibition at 10 μM[b]				
			PDE1	PDE2	PDE3	PDE4	PDE5
8a	H	0	(2%)	(9%)	(55%)	2.0	(10%)
10a	Ph	1	(23%)	(26%)	(58%)	0.06	(20%)
10b	Ph	3	(24%)	(23%)	(58%)	0.02	nd
10r	NHBoc	6	(12%)	nd	(65%)	0.065	(16%)
10s	NHCOPh	6	(25%)	(46%)	(60%)	0.032	(33%)
10v	NH_2	6	(32%)	(47%)	(57%)	0.023	(27%)

[a]The IC_{50} was calculated by linear regression (correlation coefficient r = 0.095) and represents the mean value of three determinations. The experimental error is about 15%. nd = not determined.

Examination of Table 1 shows that introduction of phenyl ring onto the N-2 nitrogen of the pyridazine ring increased significantly the inhibitory activity (comparison of **8b** with **8a**). Moreover superior homologues were more potent, as illustrated by the potent N-phenylpropyl compound **10b**, which presented an IC_{50} value of 20 nM. In general, introduction of various H bond acceptor-donor groups (OH, carboxamides, N-acyl groups, etc.) at various distances (n = 3 to 6) was also promising. In particular the N-benzoyl derivative **10s** (n=6) showed an IC_{50} of 30 nM. However, all these compounds are fairly soluble in water. Finally, the unsubstituted amino derivative **10v** (n=6) presented a similar IC_{50} value (20 nM), but have showed increased water solubility.

When we checked the selectivity profile of the most interesting compounds listed in Table 2. They all showed a good selectivity on PDE4 towards other PDE isoforms (2-3 orders of magnitude less potency). Previous papers have also shown that N-substitution of pyridazinone derivatives was beneficial for PDE4 activity.[10-13,18]

The 6-aryl pyridazinones and rolipram behave as potent and selective inhibitors. The initial SAR analysis dealing with rolipram series allowed identification of a first pharmacophoric pattern highlighting a typical dialkoxyphenyl group bearing in meta position a lipophilic substituent. By another hand, a carbonyl dipole at a specific distance was critical for activity[5]. Further works based on the 3D crystal structure of PDE4 showed this carbonyl dipole was located close to Mg^{2+} ion present in the catalytic site of the enzyme.

Similar SAR analysis could be found within the pyridazinone series. The analysis of 3D structure of PDE4 co-crystallized with zardaverine, another 6-aryl-pyridazone presenting sub-micromolar IC_{50} value, showed that it was located in the catalytic site in a similar manner[19]. In addition easy N-substitution of pyridazinone **8a** allowed a first topological exploration in a new site which may accept diverse functional groups at different distances from the main binding pocket. In particular, additional hydrophobic interaction turned the starting μM compound **8a** into a potent PDE4 inhibitor **10b** (IC_{50} = 20 nM). In addition, when a flexible spacer (n=6) was used, the introduction of a primary

amino group (cpd 10v) was also beneficial, once it was able to increase not only its potency (IC_{50} = 20 nM), but also its water solubility.

4. Conclusion

In conclusion, this work opens a large avenue to further topological explorations in the vicinity of the catalytic site, allowing the design of novel PDE4 inhibitors with original pharmacological properties (water-soluble PDE4 inhibitors, dual-acting compounds, etc.).

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