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Synthesis and Biological Evaluation of Imidazol-2-one and 2-Cyanoiminoimidazole Derivatives: Novel Series of PDE4 Inhibitors

J. Ignacio Andrés,[a,*] José M. Alonso,[a] Adolfo Díaz,[a] Javier Fernández,[a] Laura Iturrino,[a] Pedro Martínez,[a] Encarna Matesanz,[a] Eddy J. Freyne,[b] Frederik Deroose,[b] Gustaaf Boeckx,[b] Davy Petit,[b] Gaston Diels,[b] Anton Megens,[b] Marijke Somers,[b] Jean Van Wauwe,[b] Paul Stoppie,[b] Marina Cools,[b] Fred De Clerck,[b] Danielle Peeters[b] and Didier de Chaffoy[b]

[a]*Janssen-Cilag, Basic Research Centre, Jarama s/n, 45007 Toledo, Spain*
[b]*Janssen Research Foundation, B2340, Belgium*

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Abstract—This communication describes the synthesis and in vitro PDE4 inhibitory activity of a novel series of imidazol-2-one and 2-cyanoiminoimidazole derivatives. The compounds described were also tested in in vivo models to evaluate their anti-inflammatory activity after topical administration as well as their gastro-intestinal side effects. Several compounds proved to be potent PDE4 inhibitors and some 2-cyanoiminoimidazoles showed less pronounced gastro-intestinal side effects than reference compounds but maintained anti-inflammatory activity after topical administration.

Introduction

cAMP and cGMP are important second messengers with a critical regulatory role in various biological processes. The hydrolysis of these 3′,5′-cyclic nucleotides to the corresponding nucleoside 5′-monophosphates is catalyzed by a superfamily of enzymes called phosphodiesterases (PDEs).[1] PDE4 is a high-affinity cAMP specific phosphodiesterase predominantly present in virtually all immune and inflammatory cells. This PDE4 family consists of four genes (A–D) with a unique chromosome localization. Elevation of cAMP through inhibition of PDE4 results in a wide range of anti-inflammatory effects in leukocytes. Given the increased levels of cAMP in leukocytes from patients with atopic dermatitis, asthma and allergic rhinitis, inhibition of PDE4 is an attractive target for drug design, which has generated great interest.[2]

The archetypal PDE4 inhibitor rolipram (**1**) has been the starting point for many of the medicinal chemistry efforts in this field.[2] The therapeutic activity of the first generation anti-inflammatory PDE4 inhibitors is, however, limited by their side effects, especially gastro-intestinal side effects such as emesis or acid secretion. Although the anti-inflammatory activity is believed to reside, for peripheral action, on the catalytic site of PDE4, these inhibitors seem to interact with both the catalytic site and the 'high-affinity rolipram binding site' (HARBS).[3] It is thought that the interaction and activity at this HARBS is responsible for the undesirable CNS mediated side effects.[4] Strategies to overcome these side effects will depend upon the identification of PDE4 inhibitors which exhibit low affinity for the HARBS or PDE4 subtype-specific inhibitors (Fig. 1).

We have discovered a novel series of imidazol-2-one derivatives,[5] potent PDE4 inhibitors, with activities comparable to those displayed by some of the most potent reference compounds, such as RP73401 (**2**) and ariflo (**3**). Furthermore, we have prepared a series of 2-cyanoiminoimidazole derivatives that show much reduced gastro-intestinal side effects while keeping good inhibitory activity.[6]

*Corresponding author. Fax: +34-925-23-2603; e-mail: iandres@prdes.jnj.com

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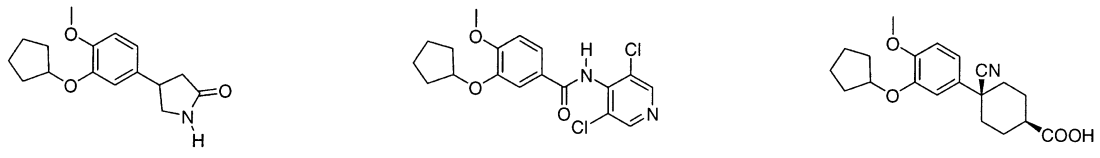
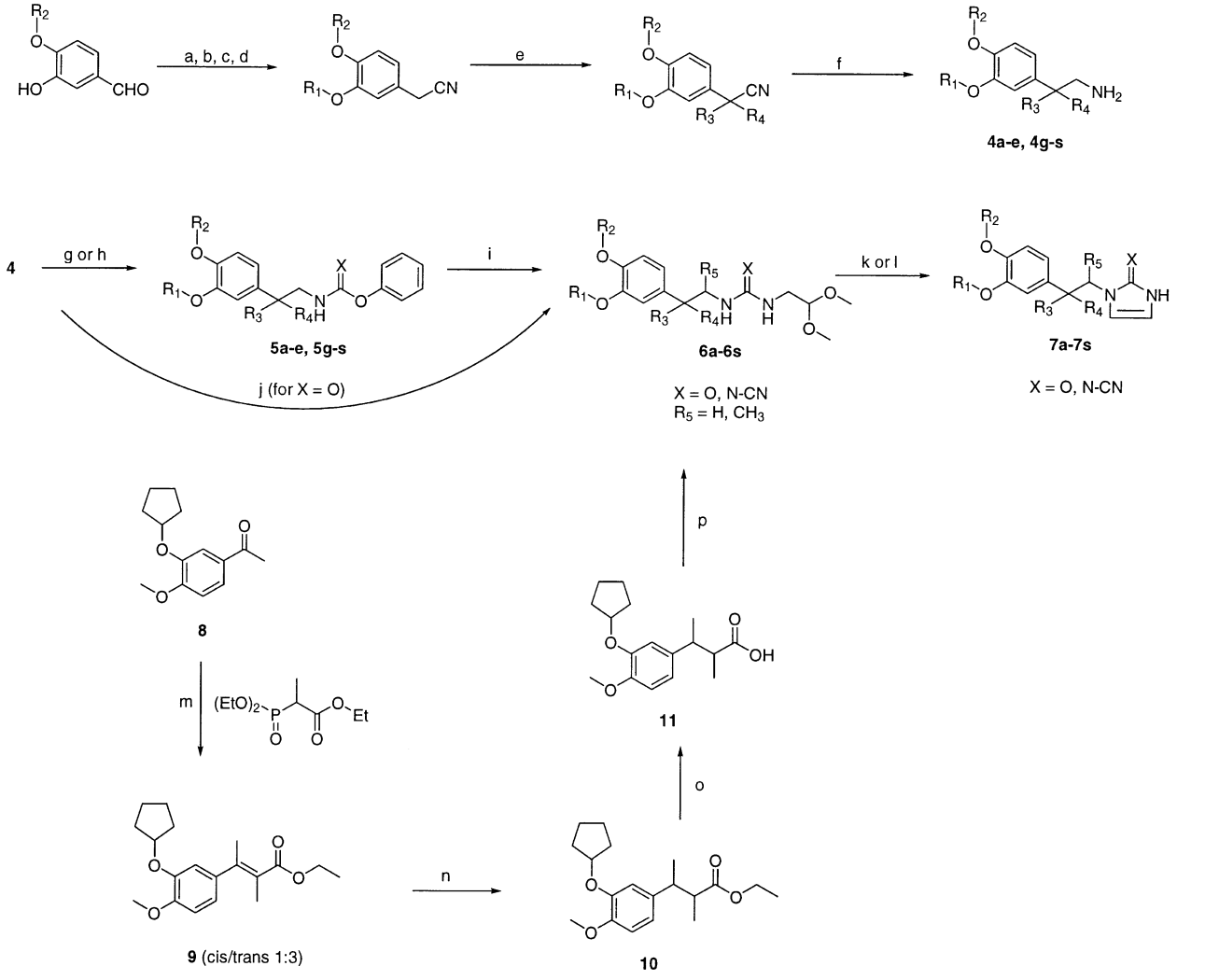


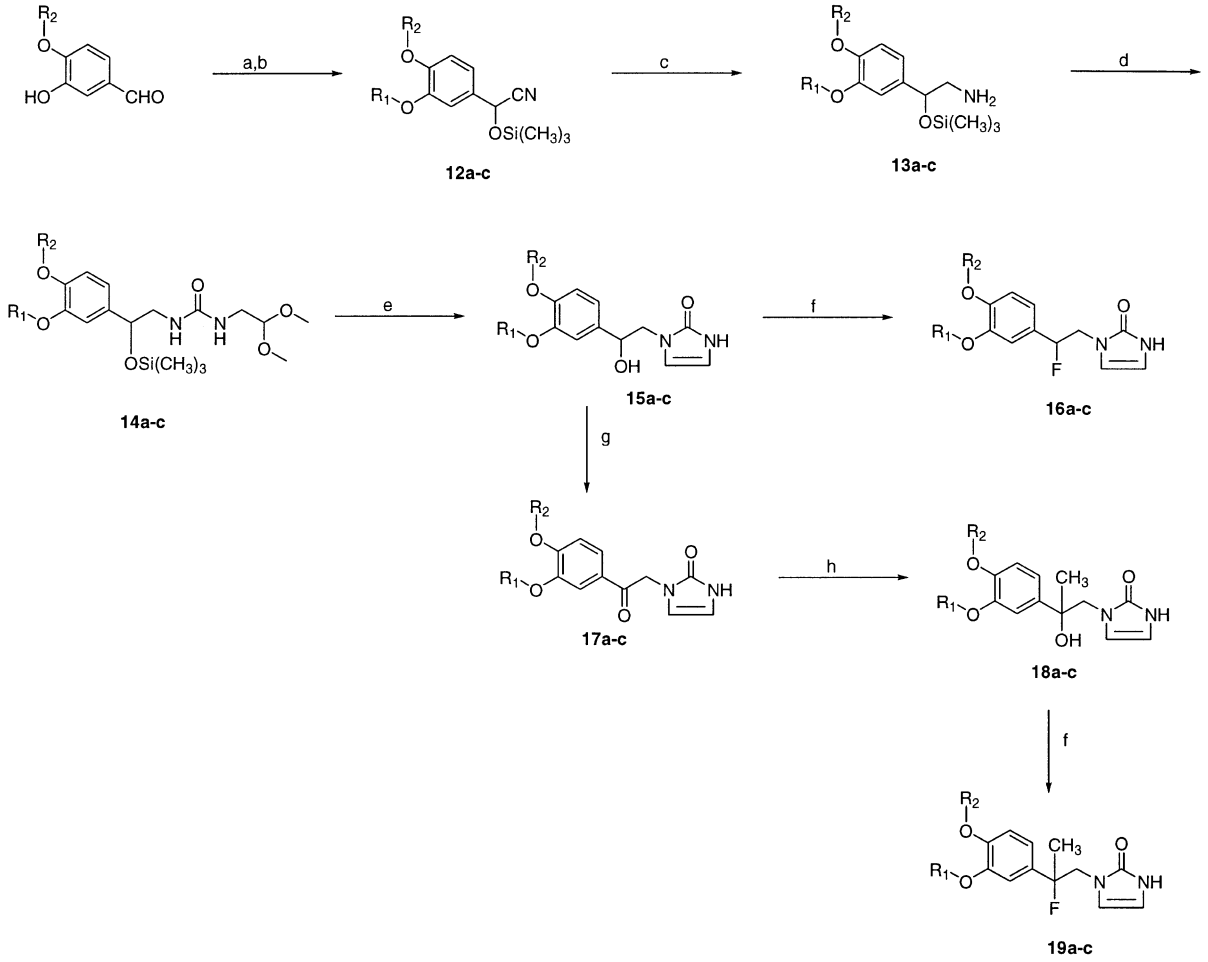
Figure 1.

Chemistry

The general synthetic pathway for the preparation of the target compounds is illustrated in Schemes 1 and 2. There are very few literature references describing the synthesis of 2-cyanoiminoimidazoles.[7] We designed a new synthetic pathway for 2-cyanoiminoimidazoles based on the well known synthesis of imidazol-2-ones,[8] so that we could use the same starting materials. The starting 3,4-dialkyloxybenzaldehydes were converted into the required phenylethylamines **4** using standard procedures (Scheme 1). Reaction of amines **4** either with phenylchloroformate or diphenyl *N*-cyanocarbonimidate led to the corresponding intermediates **5** which, after reaction with 2,2-dimethoxyethylamine, yielded the requisite ureas or *N*-cyanoguanidines **6**.[9] The ureas **6** (X = O) could also be obtained directly from amines **4** by reaction with *N*-(2,2-dimethoxyethyl)-1-imidazol-1-carboxamide in THF at reflux temperature. The imidazol-2-one derivatives **7** (X = O) were prepared by cyclization of ureas **6**, in the presence of dilute hydrochloric acid in a mixture of methanol/water at room temperature.[8] The



Scheme 1. Reagents and conditions: (a) R_1X, K_2CO_3, DMF, 80 °C or R_1OH, DIAD, PPh_3, THF, rt; (b) $NaBH_4$, CH_3OH–THF, rt; (c) $SOCl_2$, DMF (cat.), toluene; (d) KCN, C_2H_5OH, H_2O, DMF, 60 °C, 2 h; (e) for R_4 = H: LDA, THF, R_3X, −78 °C to rt or 1) $C_2H_5OCOOC_2H_5$, $NaOC_2H_5$, toluene-H_2O, 110 °C; 2) CH_3X, rt; 3) NaOH, 60 °C; for R_3, R_4 = c-alkyl: $BrCH_2CH_2Br$, NaOH 50%, BTEAC, 60–70 °C; (f) H_2, RaNi, CH_3OH–NH_3, rt; (g) PhOCOCl, $(C_2H_5)_3N$, CH_2Cl_2, rt; (h) $(PhO)_2CNCN$, C_2H_5OH, rt; (i) $NH_2CH_2CH(OCH_3)_2$, $(C_2H_5)_3N$, DMAP, 1,4-dioxane, reflux; (j) $ImCONHCH_2CH(OCH_3)_2$, THF, reflux; (k) 0.5 N HCl (1.5 eq.), CH_3OH/H_2O (2:1, v/v), rt; (l) 0.5 N HCl (1.5 eq.), THF, reflux, 1 h; (m) NaH, THF, reflux, 90%; (n) H_2, Pd/C (10%), EtOH, rt, 92%; (o) 1 N aq. NaOH, CH_3OH/THF (1:1, v/v), reflux, 52%; (p) 1) $(C_6H_5O)_2P(O)N_3$, Et_3N, toluene, 90 °C; 2) $NH_2CH_2CH(OCH_3)_2$, rt, 82%.



Scheme 2. Reagents and conditions: (a) R_1X, K_2CO_3, DMF, 80 °C or R_1OH, DIAD, PPh_3, THF, rt; (b) TMSCN, ZnI_2, CH_2Cl_2, rt; (c) H_2, RaNi, CH_3OH/NH_3; (d) $Im(CO)NHCH_2CH(OCH_3)_2$, THF, reflux; (e) 1 N HCl, CH_3OH/H_2O, rt; (f) DAST, CH_2Cl_2, −78 °C; (g) $ClCOCOCl$, $(C_2H_5)_3N$, DMSO, CH_2Cl_2, −70 °C to rt; (h) CH_3MgCl, THF, 10 °C.

best conditions found for the cyclization of *N*-cyanoguanidines **6** to the 2-cyanoiminoimidazole derivatives **7** (X = N–CN) were the treatment with 1.5 equivalents of dilute HCl in THF and heating for not more than 1 h (Scheme 1). Longer reaction times resulted in extensive decomposition. Compound **7f** was synthesized by a different pathway starting from the acetophenone derivative **8**,[10] which was transformed in three steps into the acid derivative **11**. Curtius rearrangement of this compound to the corresponding isocyanate derivative, followed by in situ reaction with 2,2-dimethoxyethylamine, yielded the urea derivative **6f**, which upon cyclization by the standard procedure afforded imidazol-2-one derivative **7f**. The enantiomers of **7p** were obtained by chiral HPLC separation of the racemic compound.

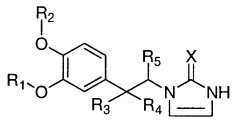
Target compounds in which R_3 = F or OH were synthesized as shown in Scheme 2. Reaction of the starting substituted benzaldehydes with trimethylsilyl cyanide produced the corresponding silylated cyanohydrines **12**, which after reduction of the nitrile group and further reaction with *N*-(2,2-dimethoxyethyl)-1-imidazol-1-carboxamide yielded the required ureas **14**. Cyclization as described in Scheme 1 provided the imidazol-2-ones **15**, containing a hydroxyl group on the spacer, which could be transformed into the fluoro analogues **16** upon treatment with DAST.[11] Oxidation of the hydroxyl group of **15** to the corresponding ketone **17**, and subsequent reaction with methylmagnesium chloride, yielded methyl-hydroxy derivatives **18**, which provided the target methyl-fluoro substituted compounds **19** upon treatment with DAST.

In principle compounds containing the cyanoiminoimidazole moiety could exist as two tautomeric ('imino' and 'amino') structures. We only observed one unique form for each compound. The structure of this cyanoiminoimidazole ring was elucidated by ^1H NMR NOE experiments. The NOE effects observed between the protons of the ring and the protons of the carbon linked to the heterocyclic nitrogen led us to conclude that only the 'imino' tautomer was present. Further NMR experiments aimed at elucidating the *E*:*Z* isomery of the iminic double bond were unsuccessful.

Biological Results and Discussion

Compounds were tested for their inhibitory potencies against recombinant PDE4 B from human mononuclear lymphocytes, which is a PDE4 subtype broadly

Table 1. IC_{50}s (μM) for inhibition of PDE4 B and LADs (mg/kg, po; $n = 3$ per dose level) for inhibition of gastric emptying[12]



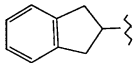
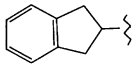
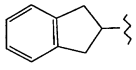
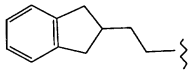
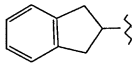
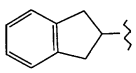
Compd	R_1	R_2	R_3	R_4	R_5	X	PDE4 B IC_{50} (μM)	Gastric emptying (mg/kg)
7a	Cyclopentyl	H_3C	H	H	H	O	0.1096	0.08
7b	Cyclopentyl	H_3C	H_3C	H	H	O	0.0241	0.01
7c	Cyclopentyl	F_2HC	H_3C	H	H	O	<0.01	0.02
7d	Cyclopentyl	F_3C	H_3C	H	H	O	>1	1.25
7e	Cyclopentyl	H_3C	H_3C	H_3C	H	O	0.367	0.63
17a	Cyclopentyl	H_3C	O		H	O	0.486	1.25
7f	Cyclopentyl	H_3C	H_3C	H	CH_3	O	0.277 0.0858	n. t. 0.01
7g		H_3C	H_3C	H	H	O		
7 h		H_3C	H_3C	H	H	O	0.241	0.02
7i		H_3C	H_3C	H	H	O	0.021	0.01
7j	Cyclopentyl	H_3C	$–CH_2$-$CH_2–$		H	O	0.341	0.63
7k	Cyclopentyl	F_2HC	$–CH_2$-$CH_2–$		H	O	0.0392 0.399	0.08 0.31
15b		H_3C	HO	H	H	O		
16b		H_3C	F	H	H	O	0.0243	0.08
18c		H_3C	HO	H_3C	H	O	>1	0.31
19c		H_3C	F	H_3C	H	O	0.327	0.31
7l		H_3C	H_3C	H	H	N-CN	0.0565	1.25
7m		F_2HC	H_3C	H	H	N-CN	0.0224	0.08
7n	Cyclopentyl	H_3C	H_3C	H	H	N-CN	0.03 0.196	1.25 >1.25
7o		H_3C	H_3C	H	H	N-CN		
7p		H_3C	H_3C	H	H	N-CN	0.019	2.5
7q		H_3C	H_3C	H	H	N-CN	0.0676	>1.25
7r	$Ph–(CH_2)_5–$	H_3C	H_3C	H	H	N-CN	0.0361	>1.25
7s	$Ph–(CH_2)_5–$	F_2HC	H_3C	H	H	N-CN	0.0284	>1.25
***R*-(+)-7p**		H_3C	H_3C	H	H	N-CN	0.019	2.5
***S*-(−)-7p**		H_3C	H_3C	H	H	N-CN	0.057	>5
Rolipram							0.741	0.04
RP 73401							0.0002	0.5
Ariflo							0.071	5

Table 2. Results obtained in the TPA-induced ear inflammation model

Dose	**7a**	**7g**	**7i**	**7j**	**7l**	**7m**	**7n**	**7o**	**7p**	**7r**	**7s**	(+)-**7p**	(−)-**7p**	Rolipram	RP 73401	Ariflo
2 mpk	81	74	90	66	78	52	84	71	82	77	69			43	69	88
1 mpk	32	56	34	18	38	22	50	38	51	36	41	56	54	6	43	69

% mean values of inhibition of inflammation at 2 mg/kg (50 μg) and 1 mg/kg (25 μg) after treatment of 6–8 animals with each compound.[13]

Table 3. Inhibition of [^{3}H]-rolipram binding to rat forebrain membranes[16]

Compd	K_i (nM)
7g	3.5
Rolipram	11
RP73401	26
***R*-(+)-7p**	63
7p	180
***S*-(−)-7p**	220

distributed in tissues. In order to evaluate their gastro-intestinal side effects, compounds were tested in vivo for their inhibitory effect on gastric emptying in rats.[12] Table 1 summarizes the results of a selection of some compounds in order to discuss the structure–activity relationships. The most potent compounds were also tested against the other PDE4 isozymes (A, C and D) as well as against PDE1, 3 and 5. All the compounds tested were selective PDE4 inhibitors, while none of them were PDE4 subtype-selective inhibitors. The topical anti-inflammatory activity of several compounds was also evaluated in the TPA induced ear inflammation model to determine their potential as anti-inflammatory agents for topical treatment.[13]

In general, the alkoxy substitution confirmed the known structure–activity relationship of rolipram-derived PDE4 inhibitors.[14,15] Substitution of the methyl group at R_2 by a difluoromethyl group resulted in a significant increase in PDE4 inhibitory activity. By contrast, introduction of a trifluoromethyl group led to a complete loss of activity. Among substituents at R_1 position a variety of bulky substituents are allowed. Thus cyclopropylmethyl, bicyclo[2.2.1]hept-2-yl, phenylpentyl and indan-2-yl proved to give compounds of the same order of activity as cyclopentyl moiety, while tetrahydrofuran-3-yl showed a tenfold decrease in potency. Regarding the modifications in the spacer, the presence of a methyl group at R_3 benzylic position gave more potent compounds than those in which $R_3 = R_4 = R_5 = H$ but the introduction of further methyl groups at R_4 and/or R_5 positions proved to be detrimental for inhibitory activity. Other functional groups such as ketone or hydroxyl instead of methyl at R_3 proved to give less active compounds as well, while the introduction of a fluorine atom at that position led to compounds showing comparable activity to those with $R_3 = CH_3$.

Regarding gastro-intestinal side effects it can be concluded that the substitution of the imidazol-2-one ring by a 2-cyanoiminoimidazole reduced to a larger degree the gastro-intestinal side effects in vivo without affecting the PDE4 inhibitory potency in vitro. This more pronounced decrease in side-effect potential might greatly improve the therapeutic margin of these products. Compared with reference compounds many of the imidazol-2-ones and 2-cyanoiminoimidazoles showed good PDE4 inhibitory activities, while these latter derivatives displayed gastro-intestinal side effects in the same range as the best reference compound, ariflo, and the enantiomer *S*-(−) of one of the best compounds, **7p**, showed even a better activity/gastro-intestinal side effects ratio.

The cyanoiminoimidazoles and some imidazol-2-one derivatives were also tested in the TPA model to evaluate their anti-inflammatory activity after topical treatment. As can be deduced from the data shown in Table 2 the compounds tested showed more than 50% inhibitory effect in the TPA-induced ear inflammation model, after topical administration of 2 mg/kg of compound. Even more, some of them kept over 50% inhibitory activity after 1 mg/kg application, being comparable in that respect to the reference compound ariflo.

In order to evaluate a possible correlation between the inhibition of gastric emptying and the affinity for the high-affinity rolipram binding site (HARBS), a few compounds were also evaluated for their ability to displace [^{3}H]-rolipram from its high-affinity binding site in rat brain membranes.[16] As can be deduced from the data shown in Table 3 there is a certain degree of correlation between the results obtained in both tests. Compound ***S*-(−)-7p** showed the lowest affinity for HARBS while showing the least gastro-intestinal side effects in our test.

In conclusion, we have reported the synthesis of a series of imidazol-2-one derivatives and of 2-cyanoiminoimidazole derivatives which showed PDE4 inhibitory activities comparable to reference compounds. Those compounds were topically active in an in vivo model of anti-inflammatory activity and in the case of the 2-cyanoiminoimidazole series with much reduced gastro-intestinal side effects. Further pharmacological evaluation of compound ***S*-(−)-7p** will be reported elsewhere.

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12. Inhibition of gastric emptying in rats: a 2-mL caloric test meal (commercial Shak ISO from Roussel UCLA/nutrition France; 1 kcal /mL) was given to male rats (200–250 g) pretreated orally for 30 min with test compound or solvent. The weight of the stomach content was measured as the difference in weight between the full and empty stomach 1 h after the administration of the meal. Stomachs containing food residues were discarded. Criterion for drug-induced inhibition of gastric emptying: stomach contents >1.15 g (0% false positive control rats). Compounds were prepared as solutions in polyethyleneglycol 200 (PEG200) and administered in a volume of 1.25 mL/kg (higher volumes of PEG200 given orally inhibit gastric emptying per se). For each compound, the LAD (lowest active dose) was determined, that is, the lowest dose that induced inhibition of gastric emptying in at least two out of three tested rats.
13. Female CD1 mice (20–22 g) were divided in groups of 6–8 animals. Solutions of tetradecanoyl phorbol acetate (TPA, 100 μg/mL) with or without experimental compound (5 mg/mL) were prepared in 200 μL of acetone. Ten μL quantities (containing 1 μg TPA and 50 μg compound) were applied to the inner surface of the left ear. The right ear received 10 μL of acetone and served as the control. Three hours later, the ears were removed and an 8-mm punch was taken. The degree of ear edema was calculated by substracting the weight of the right (control) ear punch from that of the left (treated) ear in each treatment group. Differential ear weights were then compared between the TPA and TPA + compound groups to determine the percentage of inhibition.
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16. Inhibition of [^{3}H]-rolipram binding to rat forebrain membranes by selected compounds was tested as follows: to a mix of 50 μM adenosine monophosphate and 0.05% BSA in 50 mM Tris buffer, pH 7.5, containing 5 mM $MgCl_2$, [^{3}H]-rolipram racemate (Amersham TRK1055, 85Ci/mmol, 2.35 μM) and compound were added. The reaction was started by adding membranes (1.45 mg/mL protein content) and incubating the mixture for 30 min at 25 °C. The reaction was stopped by filtering the mixtures over GF/B filters (Whatman), presoaked in 1 mg/mL BSA. The filtered protein was washed, dried and the radioactivity on the filters quantified by liquid scintilliation counting. Membranes were prepared from 12-g forebrains from 10 rats of 250 g this is sufficient to do all the compounds different times.