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New PDE4 inhibitors based on pharmacophoric similarity between papaverine and tofisopam

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ABSTRACT

Pharmacophoric comparison between papaverine and tofisopam led to identify three new series of micro- to sub-micromolar inhibitors of phosphodiesterase-4, including 7,8-dialkoxy-2,3-benzodiazepin-4-one derivatives, 7,8-dialkoxy-1,4-benzodiazepin-2-one derivatives, and dialkoxybenzophenone derivatives.

Cyclic nucleotide phosphodiesterases (PDEs) represent an important class of enzymes for the cellular regulation, by hydrolyzing the intracellular cyclic AMP (cAMP) and cyclic GMP (cGMP). Among all the PDE families,[1] increasing interest has been focused on cAMP-specific PDE4 family, which appears as a potential target for the development of new anti-asthmatic and anti-inflammatory drugs.[2,3]

PDE inhibitors are generally hydrophobic compounds with relatively large chemical diversity. Among them, the alkaloid papaverine **1a** is presenting micromolar IC_{50} towards PDE4,[4,5] while its diethoxy-derivative ethaverine **1b** is exhibiting a sub-micromolar activity (Table 1).[6] In terms of pharmacophoric fragments, papaverine **1a** presents a H-bond acceptor system (imine nitrogen) flanked by two dimethoxy phenyl rings. A drug repositioning strategy allowed recently identifying tofisopam **2**, clinically used as anxiolytic drug, as a sub-micromolar PDE4 inhibitor.[7] While belonging to the class of 2,3-benzodiazepines, tofisopam exhibits common pharmacophoric pattern with papaverine.

Based on the hypothesis of a common pharmacophoric pattern between papaverine and tofisopam, this work describes the SAR analysis of three novel series, 7,8-dialkoxy-2,3-benzodiazepinones (series **I**), 7,8-dialkoxy-1,4-benzodiazepinones (series **II**) and 4,5-dialkoxybenzophenones (series **III**) as phosphodiesterase-4 inhibitors (Fig. 1).

We first initiated the synthesis of 7,8-dialkoxy-2,3-benzodiazepine compounds, using a convenient synthetic pathway leading to both tofisopam-related analogs and 2,3-benzodiazepin-4-one derivatives **I** (Scheme 1).[8] The commercially available 3,4-dialkoxy-phenylacetic acids were esterified in presence of methanol upon acidic catalysis, before to be regioselectively acylated at the 6-position of the phenyl ring, in presence of tin(II) chloride. The cyclization was performed with hydrazine hydrate upon acidic catalysis, leading to 2,3-homophthalazin-4-ones **4**. A treatment of **4** with sodium hydride in presence of methyl iodide led to derivatives **5**. However, the amide function of **4** could also be activated into imino-chloride, which could further be methylated to yield tofisopam-related analogs **6a–c**.

Next, we investigated the hypothesis to remove the alkoxy groups at the 7- and 8-positions of the 2,3-benzodiazepin-4-one scaffold **I**, while keeping them at the 3- and 4-positions of the free-rotating ring. In the absence of alkoxy groups at the 7- and 8-positions of benzodiazepinone, another synthetic pathway has been developed (Scheme 2). Commercially available 2-iodobenzoic acid was activated into the corresponding aroyl chloride, before to be involved in a Friedel–Crafts reaction in presence of dialkoxybenzene. Then, a copper-catalyzed reaction was performed on the resulting 2-iodobenzophenone **8**, leading to the substitution of the iodine atom by a diethylmalonate moiety.[9]

After alkaline hydrolysis and in situ decarboxylation, the 2,3-benzodiazepin-4-ones **9** were obtained by condensation with hydrazine hydrate. N-methylation of the amide function was eventually performed with methyliodide to give the compounds **10**.

We next considered 7,8-dialkoxy-1,4-benzodiazepin-2-ones **II** as valuable carba-azaisosteres of 2,3-benzodiazepin-4-ones **I** (Fig. 1). From a synthetic point of view, we first synthesized the

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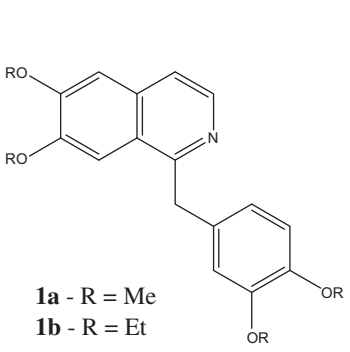
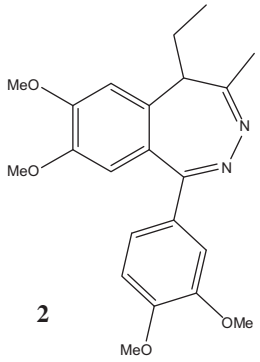
Table 1
PDE4 inhibition of reference compounds





Compound	Name	IC_{50} (μM)
1a	Papaverine	1.1
1b	Ethaverine	0.4
2	Tofisopam	0.68

1,3-benzoxazine-2,4-diones **11** following standard procedures (Scheme 3).[10] The 1,4-benzodiazepin-2-ones **12** were obtained by condensation with methyl glycinate, then the benzamide function was activated into imino-chloride **13** using $POCl_3$ in presence of dimethylaniline.[11] Starting from compounds **13**, we were able to introduce a diversity of decorations at the 5-position of the 1,4-benzodiazepin-2-one, using either pallado-catalyzed cross-coupling reactions (Suzuki–Miyaura reaction (**14a–f**), Sonogashira reaction (**14h–i**)), or amination reaction (**14g**). Eventually, we synthesized a series of benzophenones **15a–j** (series **III**), as seco derivatives of benzodiazepinones following classical Friedel–Crafts reaction (Scheme 4). Amination step was performed on 4-bromobenzophenone derivatives using Buchwald cross coupling reaction.[12] All the synthesized compounds were tested as PDE4 inhibitors following standard procedure.[13] IC_{50} values are summarized in Tables 2–5.

The tofisopam-analog girisopam **6a**,[14] another well-known anxiolytic agent, appeared to be five-fold less active on PDE4 than its parent drug **2**. tofisopam and girisopam exhibit two main structural differences: the first one is the presence of two H-bond acceptor methoxy groups in 3- and 4-position of the exocycle for tofisopam, in comparison with a withdrawing chlorine atom at position 3 for girisopam; the second one is the presence of a small hydrophobic ethyl group at 5-position of the tofisopam seven-member ring. Based on our working hypothesis dealing with pharmacophoric pattern analogy between papaverine **1a** and tofisopam **2**, the ethaverine-diethoxy groups were introduced into girisopam analogs **6b** and **6c**. In spite of the presence of a chlorine atom on the exocycle, affinities of both compounds for PDE4 exhibited a gain of one order of magnitude in comparison with girisopam, with IC_{50} values of 0.4 and 0.2 μM, respectively. This result validates our working hypothesis dealing with pharmacophoric pattern analogy between papaverine and tofisopam.

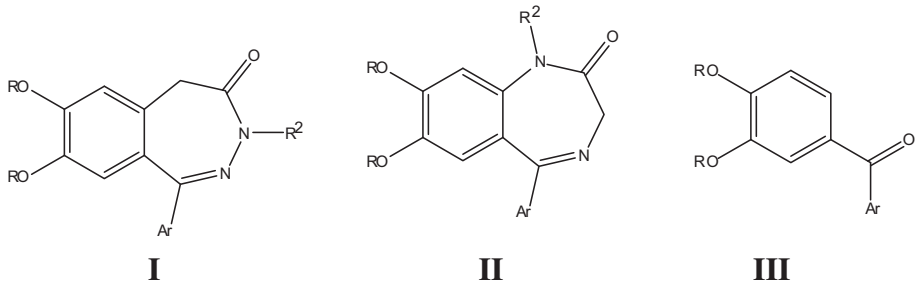
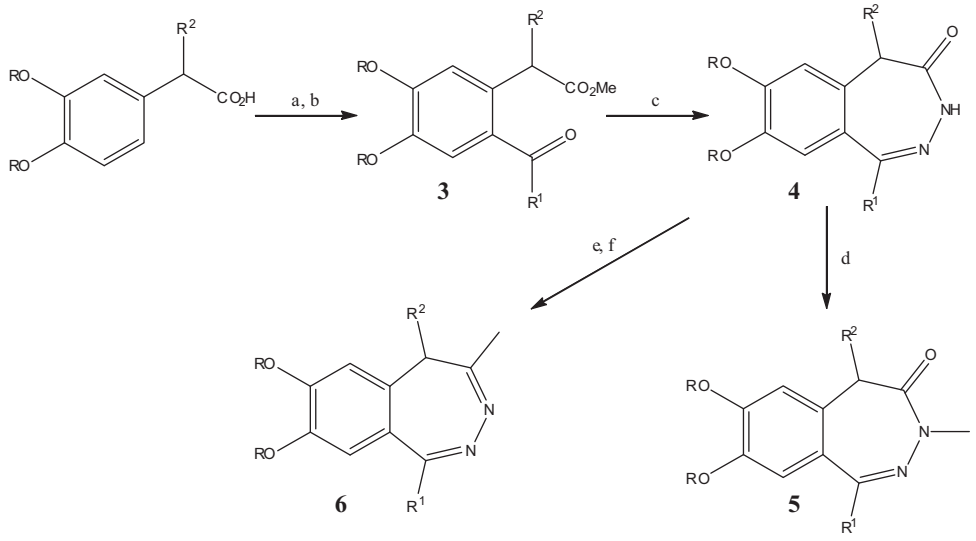
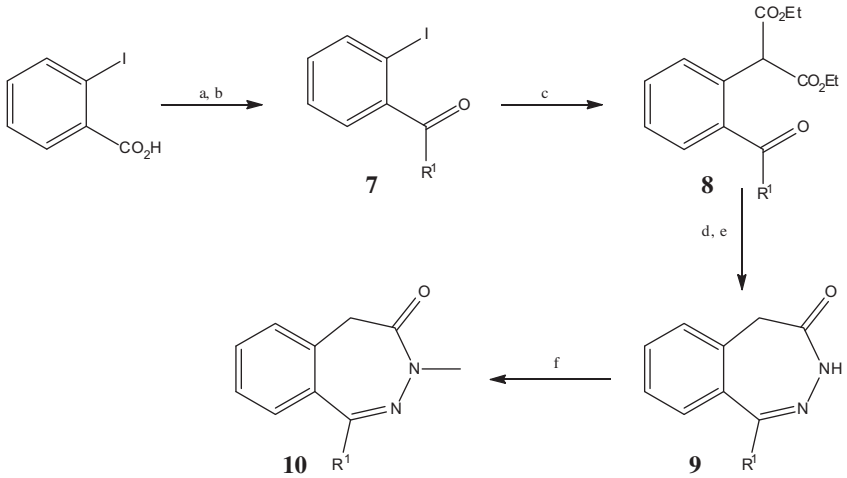


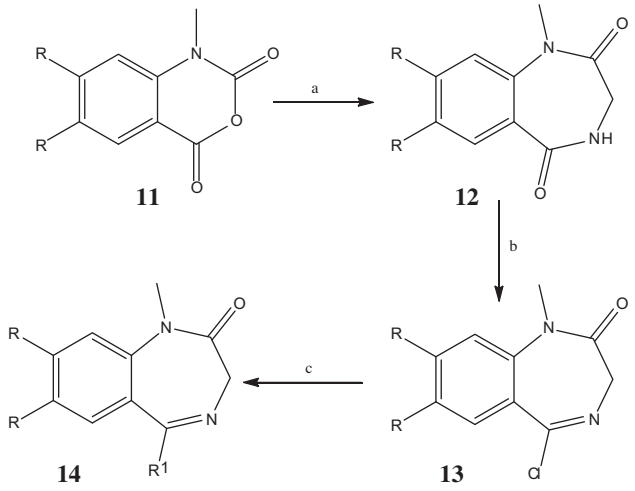
Figure 1. Novel classes of PDE4 inhibitor.



Scheme 1. Reagents and conditions: (a) $H_2SO_{4(cat)}$, MeOH, reflux; (b) R_1-COCl, $SnCl_2$, rt; (c) NH_2–NH_2·H2O, EtOH, 150 °C, 3 h, then AcOH, EtOH, reflux, 30 min; (d) MeI, NaH, THF; (e) $POCl_3$, dimethylaniline, $CHCl_3$, 120 °C; (f) MeLi, THF, −78 °C.



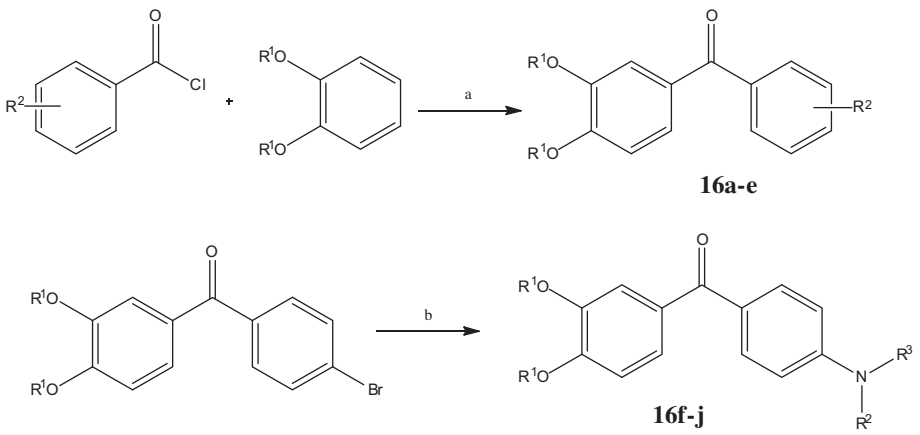
Scheme 2. Reagents and conditions: (a) $SOCl_2$, DMF, 60 °C; (b) $AlCl_3$, DCE, $(EtO)_2$benzene; (c) $CH(CO_2Et)_2$, CuI, picolinic acid, Cs_2CO_3, dioxane, 90 °C; (d) LiOH, MeOH, H_2O, reflux; (e) NH_2–NH_2·H_2O, EtOH, 150 °C, 3 h, then AcOH, EtOH, reflux, 30 min; (f) MeI, NaH, THF.



Scheme 3. Reagents and conditions: (a) Methyl glycinate hydrochloride, pyridine, reflux, 6 h, then add AcOH, 130 °C, 12 h; (b) $POCl_3$, dimethylaniline, $CHCl_3$, 120 °C; (c) **14a–f**: R_1-$B(OH)_2$, K_3PO_4, $Pd(PPh_3)_4$, DMF, 100 °C, 12 h; **14g**: piperidine, EtOH, 110°, 48 h; **14h**: phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, PPh_3, 55 °C, 3 h; **14i**: **14h**, Pd/C, MeOH, DCM, H_2 (70 psi), 48 h.

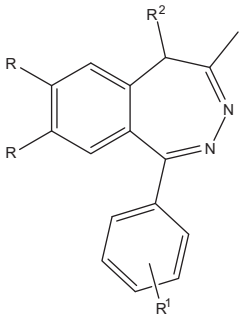
We next investigated the replacement of the girisopam methylimine moiety by an amide group, leading to compound **5c**, and an IC_{50} of 7.7 μM (Table 3). As observed with **6c**, a chlorine atom at 4-position (**5d**) appeared to be more active with an IC_{50} of 1.5 μM. The substitution of the phenyl group by a 2-benzothienyl ring led to **5g** with a micromolar affinity. Then the N-methylation of the amide function led to **5e** with a 10-fold increase in affinity (IC_{50} = 0.3 μM). The same series of compound was synthesized in the diethoxy-series leading to compounds **5i–m**. All of them appeared to be more potent towards PDE4 than their corresponding dimethoxy analogs **5a–h**. Especially, **5m** was found the most potent inhibitor of PDE4 with an IC_{50} of 0.09 μM, about seven-fold better than tofisopam.

So far, we keep as a constant the presence of alkoxy groups at 7- and 8-positions of the bicycle. However, tofisopam and papaverine exhibit the same alkoxy moieties at the 3- and 4-positions of the free-rotating ring. So, we investigated the hypothesis to remove the alkoxy groups at the 7- and 8-positions of the 2,3-benzodiazepin-4-one scaffold, while keeping them at the 3- and 4-positions of the exocycle. Diethoxy analog **9** appeared to be 4-fold more potent than **5i**, which carries the diethoxy moieties at 7- and 8-positions. As previously observed, N-methylation of the amide led to a better affinity (**10**, IC_{50} = 0.14 μM). This last result seems to show that among the two pairs of dialkoxy groups exhibited by papaverine



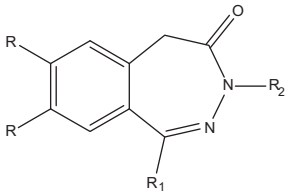
Scheme 4. Reagents and conditions: (a) $AlCl_3$, nitrobenzene, reflux, 12 h; (b) $Pd(OAc)_2$—5%, BINAP—6%, Cs_2CO_3, dioxane, 90 °C, 7 h.

Table 2
PDE4 inhibition of tofisopam-related derivatives



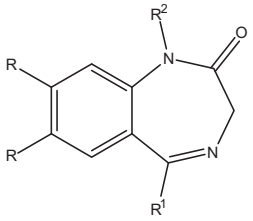
Compound	R	R_1	R_2	IC_{50} (μM)
Tofisopam	OMe	3,4-MeO	Et	0.68
6a (Girisopam)	OMe	3-Cl	H	3.2
6b	OEt	3-Cl	Et	0.39
6c	OEt	4-Cl	Et	0.21

Table 3
PDE4 inhibition of 2,3-benzodiazepin-4-ones



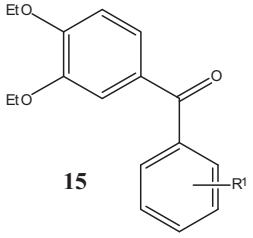
Compound	R	R_1	R_2	IC_{50} (μM)
5a	OMe	Ph	H	7.70
5b	OMe	Ph	Me	5.1
5c	OMe	3-Cl-Ph	H	7.7
5d	OMe	4-Cl-Ph	H	1.5
5e	OMe	3,4-Cl_2-Ph	H	1.64
5f	OMe	2-Naphtyl	H	1.50
5g	OMe	2-Benzothienyl	H	1.20
5h	OMe	2-Benzothienyl	Me	0.30
5i	OEt	Ph	H	0.80
5j	OEt	3-Cl-Ph	H	1.4
5k	OEt	4-Cl-Ph	H	0.8
5l	OEt	2-Benzothienyl	H	0.26
5m	OEt	2-Benzothienyl	Me	0.087
9	H	3,4-EtO_2-Ph	H	0.19
10	H	3,4-EtO_2-Ph	Me	0.14

Table 4
PDE4 inhibition of 1,4-benzodiazepin-2-ones



Compound	R	R_1	R_2	IC_{50} (μM)
13a	OMe	Ph	H	>20
14a	OMe	Ph	Me	18
14b	OMe	3-Cl-Ph	Me	>20
14c	OMe	4-Cl-Ph	Me	>20
14b	OEt	Ph	H	0.95
14d	OEt	Ph	Me	0.86
14e	OMe	2-Benzothienyl	Me	2.3
14h	OMe	Ph-C≡C-	Me	1.6
14f	OMe	Ph-CH=CH-	Me	8.4
14i	OMe	Ph-$(CH_2)_2$-	Me	>20
14g	OMe	1-piperidinyl	Me	>20

Table 5
PDE4 inhibition of benzophenone derivatives



15

Compound	R	R_1	IC_{50} (μM)
15	OEt	—	2.9
15a	OEt	2-Cl-Ph	1.4
15b	OEt	4-Cl-Ph	>20
15c	OEt	3,4-Cl_2-Ph	7.9
15d	OEt	1-Naphtyl	4.3
15e	OEt	2-Naphtyl	5.0
15f	OMe	1-Piperidino-	9.6
15g	OEt	1-Piperidino-	3.6
15h	OEt	4-Ph-piperazin-1-yl-	17.0
15i	OEt	4-Bn-piperazin-1-yl-	2.3
15j	OEt	N–Me–N–Bn-4-amino	2.2

and tofisopam, the one carried by the free-rotating ring may be more important for affinity.

We next considered 7,8-dialkoxy-1,4-benzodiazepin-2-ones **II** as valuable carba-azaisosteres of 2,3-benzodiazepin-4-ones **I** (Fig. 1). Structurally-related diazepinoindoles CI-1018 have already been reported by Burnouf et al. as potent PDE4 inhibitors, but this compound does not exhibit any of the alkoxy groups, characteristic of the papaverine or tofisopam derivatives.[15] Globally, 1,4-benzodiazepin-2-one derivatives appeared to be less potent on PDE4 than their corresponding 2,3-benzodiazepin-4-one isosteres (Table 4). For example, the 2-benzothienyl derivative **14e** (IC_{50} = 2.3 μM) was found 10-fold less potent than its isostere **5h** (IC_{50} = 0.30 μM). However, the diethoxy substituents led again to a better affinity than their corresponding dimethoxy substituted derivatives.

The results obtained in both series of benzodiazepinones **I** and **II**, along with tofisopam and papaverine, highlight the hypothesis of a common pharmacophoric pattern resumed into the dialkoxybenzophenonimine scaffold. In order to validate this pharmacophoric hypothesis, we considered benzophenones **III** as a series of seco benzodiazepinones derivatives (Table 5). Most of the benzophenones exhibited a range of inhibition between 1 and 10 μM, slightly less active than the reference compound papaverine. The poor inhibition observed with compound **15b** appeared to be very interesting in the way where it is the seco-derivative of girisopam-related **6c** (IC_{50} = 0.21 μM). One hypothesis to explain this drastic loss of inhibition may rely on the position of the diethoxy substituents. We previously observed that affinity is increased with the diethoxy substituents on the free rotating aromatic ring (**9**). Following this hypothesis applied to benzophenone would localize the chlorine atom of **15b** at the 7-position of girisopam-related **6c**, resulting in a drastic loss of affinity. This hypothesis could then explain the good affinity of **15a**, in which the chlorine atom at 2-position may induce some specific features (geometric, lipophilic) mimicking the seven-member ring closure of tofisopam. This last result allowed us to definitively link the different series **I**, **II** and **III** through a common pharmacophoric pattern represented in Figure 2. This pharmacophoric pattern can also be observed into

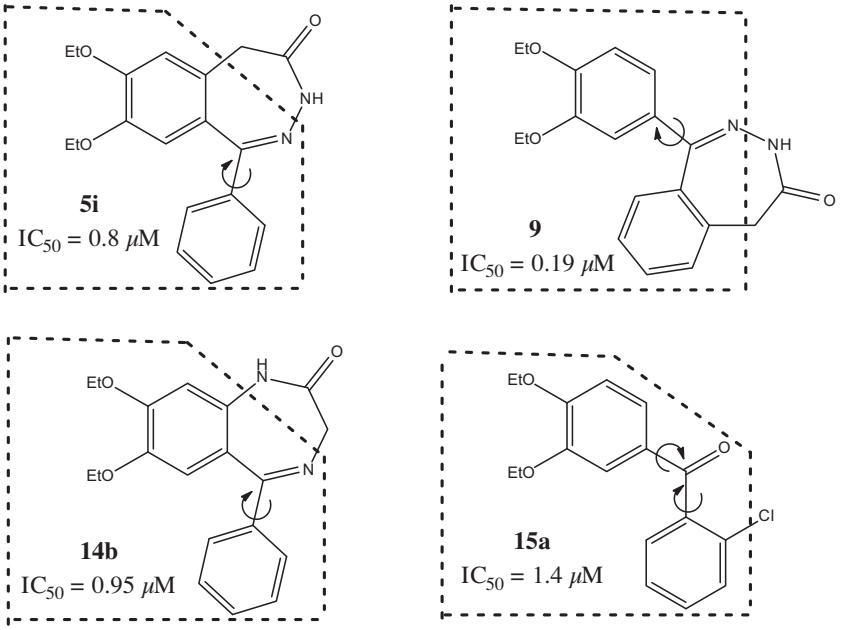


Figure 2. Common pharmacological pattern between series **I**, **II** and **III**.

the 4-(2,3-dimethoxyphenyl)-phthalazin-1-one derivatives, described by Van der Mey et al. as submicromolar PDE4 inhibitors.[16]

One of the main negative properties of benzophenone scaffold is its lack of aqueous solubility, so we tried to circumvent this problem by introducing some water-solubilizing groups, based on a substituted nitrogen atom at para-position. The electronic doublet of nitrogen has the capability to be a hydrogen bond acceptor, as well as an alkoxy group. Indeed, compounds **15i–j** were found as micromolar inhibitors of PDE4, with IC_{50} about 2 μM.

Inhibitory activities of these new PDE4 inhibitors were screened on several phosphodiesterases (Table 6). While tofisopam **2** exhibits submicromolar to micromolar inhibition towards PDE2, PDE3 and PDE4, 2,3-benzodiazepin-4-ones **5i** and **9**, but also benzophenone **15a** appeared to be more selective towards PDE4 in comparison with other PDE (IC_{50} >10 μM). Moreover, kinetic experiments performed with compounds **9** and **15a** confirmed a mechanism by competitive inhibition for both kinds of inhibitors (Fig. 3)

Table 6
Inhibitory activities towards several phosphodiesterases

Compound	IC_{50} (μM)				
	PDE1	PDE2	PDE3	PDE4	PDE5
Tofisopam **2**	nd	0.90	5.90	0.68	36% [a]
Girisopam **6a**	nd	4.00	22% [a]	3.20	12.5
6b	nd	44% [a]	nd	0.39	nd
6c	nd	38% [a]	4% [a]	0.21	39% [a]
5i	37% [a]	5% [a]	30% [a]	0.80	30% [a]
9	3% [a]	27% [a]	12% [a]	0.19	17% [a]
15a	21% [a]	46% [a]	24% [a]	1.40	4% [a]

[a] Percentage of inhibition at 10 μM; nd: not determined.

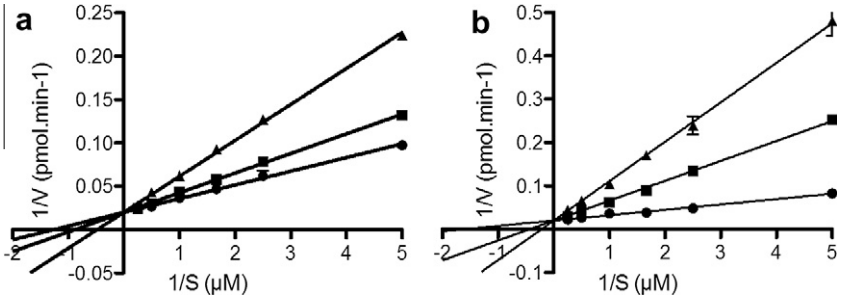


Figure 3. Lineweaver–Burk plots of compounds **10** and **15a** inhibitory effects on purified PDE4 activity (using 0.25–4 μM cAMP as substrate). Apparent K_m value for PDE4 = 0.71 μM. (a) **10** K_i = 0.066 μM (●: 0 M; ■: 3.10^{-8} M; ▲: 1.10^{-7} M); (b) **15a** K_i = 0.42 μM (●: 0 M; ■: 1.10^{-6} M;▲: 3.10^{-6} M).

In conclusion, we have described SAR studies, identifying the dialkoxybenzophenonimine scaffold as the pharmacophoric pattern common to both PDE4 inhibitors papaverine and tofisopam. This result led us to design three novel series of potent and selective PDE4 inhibitors exhibiting micromolar to sub-micromolar IC_{50} values.

Acknowledgments

JChem for Excel was used for structure database management, search and prediction, Instant JChem 5.4.0.411, 2009, ChemAxon (http://www.chemaxon.com).

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