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Phosphodiesterase inhibitors. Part 5: Hybrid PDE3/4 inhibitors as dual bronchorelaxant/anti-inflammatory agents for inhaled administration

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ABSTRACT

(−)-6-(7-Methoxy-2-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-4-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (KCA-1490) exhibits moderate dual PDE3/4-inhibitory activity and promises as a combined bronchodilatory/anti-inflammatory agent. N-alkylation of the pyridazinone ring markedly enhances potency against PDE4 but suppresses PDE3 inhibition. Addition of a 6-aryl-4,5-dihydropyridazin-3(2*H*)-one extension to the *N*-alkyl group facilitates both enhancement of PDE4-inhibitory activity and restoration of potent PDE3 inhibition. Both dihydropyridazinone rings, in the core and extension, can be replaced by achiral 4,4-dimethylpyrazolone subunits and the core pyrazolopyridine by isosteric bicyclic heteroaromatics. In combination, these modifications afford potent dual PDE3/4 inhibitors that suppress histamine-induced bronchoconstriction in vivo and exhibit promising anti-inflammatory activity via intratracheal administration.

Inhibition of phosphodiesterase 4 (PDE4) as a treatment option for respiratory disease has become firmly established with the recent launch of roflumilast for management of severe chronic obstructive pulmonary disease (COPD).[1] The utility of PDE4 inhibitors for treatment of COPD, and potentially also for asthma, arises from their pronounced anti-inflammatory activity coupled with bronchorelaxant properties.[2] Inhibition of PDE3 also confers bronchorelaxant activity,[3] however, and combined inhibition of PDE3 and PDE4 is thought to function additively or synergistically to induce airway smooth muscle relaxation.[4] For that reason we have been exploring the potential of dual PDE3/4 inhibitors as agents with enhanced efficacy for treatment of respiratory disease,[5] and in this context we have previously disclosed (−)-6-(7-methoxy-2-(trifluoromethyl)pyrazolo[1,5-*a*]pyridin-4-yl)-5-methyl-4,5-dihydropyridazin-3(2*H*)-one (KCA-1490, Fig. 1)[5b] as a lead compound that exhibited promising activity in pharmacological models of inflammation and bronchoconstriction.

A key challenge that hindered the clinical deployment of first generation PDE4 inhibitors, of which rolipram is the archetypal example, was their propensity for emetogenesis at doses required for effective anti-inflammatory action.[6] With second generation PDE4-selective inhibitors, such as roflumilast, that problem has been partially addressed, although treatment is still limited by nausea and emesis at high doses.[2c] In our initial pharmacological profiling, we found KCA-1490 to possess an improved therapeutic window over roflumilast, in that it exhibited a significantly reduced emetogenic liability at doses required for potent bronchodilatory and anti-inflammatory action.[5b] Unfortunately detailed toxicological evaluation of KCA-1490 in several animal models subsequently revealed that it was unsuitable as a drug development candidate. In particular, cardiovascular effects, that we attributed to PDE3 inhibition,[7] were observed in dogs and rabbits. Further development of dual PDE3/4 inhibitors for respiratory disease would be difficult without resolving this issue. One potential solution to the problem would be to develop a dual PDE3/4 inhibitor for direct topical administration to the lung by inhalation. This could allow dose sparing administration with substantially reduced systemic exposure to a compound, and it is precisely this principle that has driven the successful development of inhaled corticosteroids (ICS) for chronic application in asthma management.[8] Thus we set out to evaluate the potential of dual PDE3/4 inhibitors for inhaled delivery rather than oral administration.

KCA-1490 exhibits moderate levels of PDE3 and PDE4 inhibition (Fig. 1). With the potential for cardiovascular effects, we had hitherto been cautious about developing a potent PDE3-inhibitory component into the compound's overall profile. However, given that an ideal compound for inhalation would have reduced systemic bioavailability, we considered that there would now be scope for improving the in vitro inhibitory activity against both PDE3 and PDE4. We describe here our strategy for modification of KCA-1490 that led to the discovery of potent dual PDE3/4

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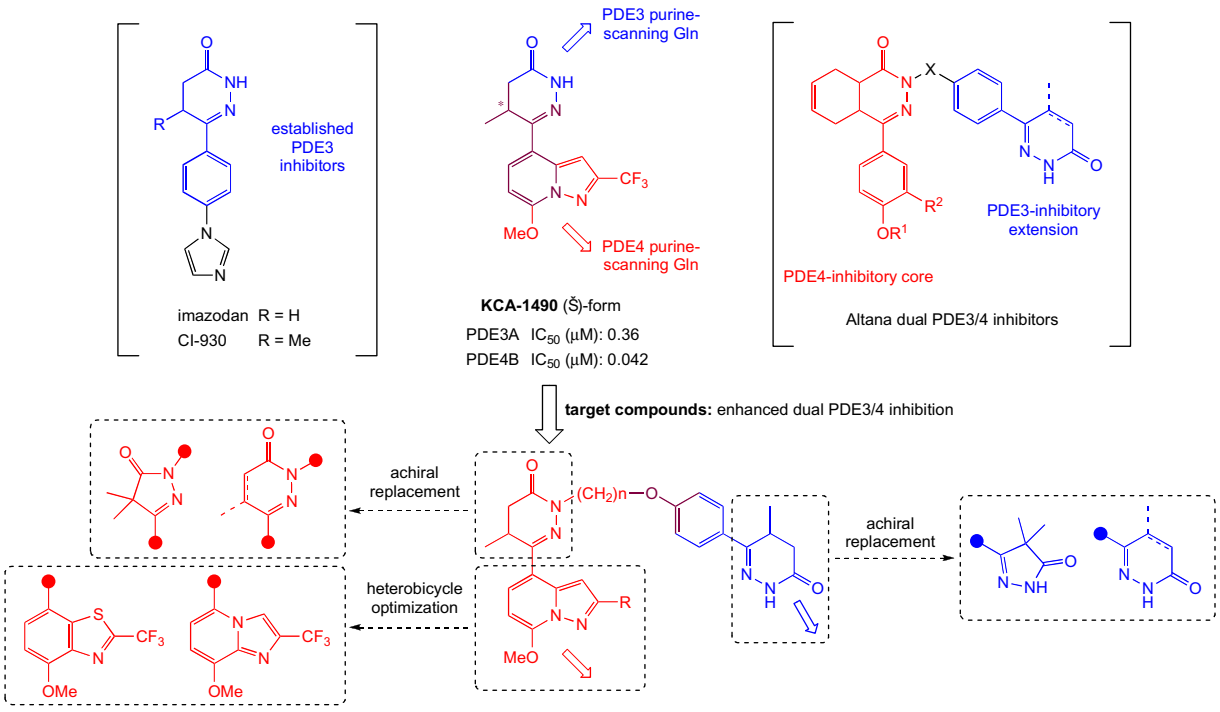


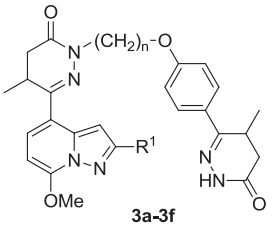
Figure 1. Planned development pathway for inhaled dual PDE3/4 inhibitors derived from KCA-1490.

inhibitors exhibiting promising anti-inflammatory activity under conditions of intratracheal administration.

Our existing structure–activity relationship (SAR) analysis in the compound series leading to KCA-1490 revealed that N-alkylation of the pyridazinone ring could substantially improve PDE4-inhibitory potency but at the expense of PDE3 inhibition, which was severely compromised.[5b,b] The reason for this divergent effect of N-alkylation is that the preferred binding mode for the pyrazolopyridine–pyridazinone core is fundamentally different in the catalytic pockets of the two phosphodiesterase families. Thus, as previously described with our binding models for KCA-1490,[5c] the pyrazolopyridine subunit engages the conserved purine-scanning glutamine in the PDE4 catalytic pocket whilst the bound orientation is reversed in PDE3 so that pyridazinone ring fulfills this role. In the PDE4-bound structure the pyridazinone NH does not hydrogen bond to the protein but is conveniently positioned so that N-alkylation extends the surface contact of the inhibitor with residues around the rim of the catalytic pocket. In contrast, binding of KCA-1490 to PDE3 exploits the pyridazinone N(1) centre as a key hydrogen bond donor for engagement of the purine-scanning glutamine, and unsubstituted lactam functionality is critical for PDE3-inhibitory activity. We decided to investigate the addition of a 6-aryl-4,5-dihydropyridazin-3(2*H*)-one extension to the *N*-alkyl group, as summarised in Figure 1, on the grounds that this should facilitate both enhancement of the PDE4-inhibitory component and restore PDE3 inhibition by reinstating a subunit with the established PDE3-inhibitory chemotype of imazodan and CI-930.[9] A similar strategy has been explored by Altana Pharmaceuticals in developing orally active phthalazinone–pyridazinone hybrid PDE3/4 inhibitors.[10]

The general synthetic route to our primary set of compounds for evaluation (**3a**–**3p**, Tables 1–3) is summarized in Scheme 1. In brief, for target compounds with trimethylene, tetramethylene and pentamethylene linkers N-alkylation of the preformed core (**1** R = CF_3 or Et)[5c] with the appropriate α, ω-dibromoalkane gave **2**. The target compounds were then completed by O-alkylation of a (*para*-hydroxyphenyl)pyridazinone or pyrazolone subunit, as exemplified by compound **4** in Scheme 1, under basic conditions. In the case of target compounds with an ethylene linker ($n = 2$), the route was modified because of a tendency for elimination of hydrogen bromide from the intermediate (**2**, $n = 2$) under basic conditions, which afforded the corresponding *N*-vinyl pyridazinone as a by-product. The modified route is illustrated for compound **3a**, and involved preparation of intermediate **5** from starting material **4** through a 4-step sequence consisting of O-alkylation with (2-bromoethoxy)(*tert*-butyl)diphenylsilane, *N*-Boc protection, removal of the TBDPS group and finally iodination of the alcohol.

Table 1



3a-3f

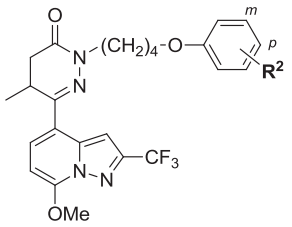
Compd[a]	R^1	*n*	Inhibition IC_{50}[b] (nM)	
			PDE3A	PDE4B
KCA-1490	CF_3	—	360	42
KCA-1450[c]	CF_3	—	2380	70
3a	CF_3	2	130	0.66
3b	CF_3	3	140	3
3c	CF_3	4	15	0.3
3d	CF_3	5	72	0.2
3e	Et	3	11	5.9
3f	Et	4	1.2	1.7

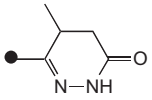
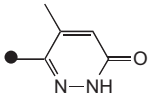
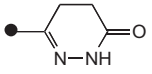
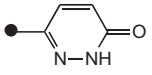
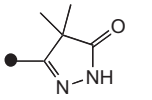
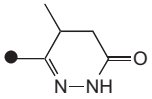
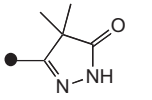
[a] Compounds **3a**–**3f** were evaluated as equimolar mixtures of all four stereoisomers.

[b] Enzyme assays were performed using the core catalytic domains from the PDE3A and PDE4B isoforms according to previously reported procedures.[11,12] Data reported are the mean of at least three experiments.

[c] Racemic form of KCA-1490.

Table 2



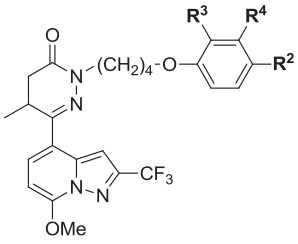
Compd[a]	R^2	Position	Inhibition IC_{50}[b] (nM)	
			PDE3A	PDE4B
3c		*p*	15	0.3
3g		*p*	40000	6
3h		*p*	360	0.1
3i		*p*	38,000	20
3j		*p*	270	0.5
3k		*m*	590	0.5
3l		*m*	1730	0.064

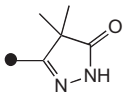
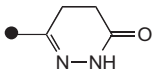
[a] Compounds **3c** and **3k** were evaluated as equimolar mixtures of all four stereoisomers; compounds **3g**, **3h**, **3i**, **3j**, and **3l** were tested in racemic form.
[b] See footnote in Table 1.

Use of iodide **5** for N-alkylation of the preformed pyrazolopyridine–pyridazine core (**1**, $R^1 = CF_3$), which in this case was the racemic form (KCA-1450) of KCA-1490, gave the required target after final Boc deprotection. Target compounds **3q**–**3w** (Tables 4 and 5) with tetramethylene linkers were prepared by similar strategies to the route outlined in Scheme 1, but replacing the pyrazolopyridine–pyridazinone starting material (**1**) with an alternative preformed core. The synthesis of the appropriate core starting materials has been described in our earlier work.[5c,d]

We first needed to select the optimum linker length for our proposed hybrid PDE3/4 inhibitor structures. For this purpose we evaluated a series of alkylidine linkers [$(CH_2)_n$; $n = 2–5$] in compounds **3a**–**3f** (Table 1), where a chiral 5-methyl-3,4-dihydropyridazinone subunit was used in both the core and extension. For simplicity we constructed these compounds from racemic building blocks and tested them as equimolar mixtures of all four stereoisomers; unsurprisingly no diastereoselective discrimination had arisen during the assembly of the structures. Although the formation of stereoisomeric mixtures in this compound subset was a complicating factor, we planned to remove at least one and preferably both chiral subunits during subsequent development. The compounds were assessed for inhibitory activity against the core catalytic domains from PDE3A and PDE4B using previously reported protocols.[11,12] As expected, the hybrid compounds exhibited substantial improvement in PDE4-inhibitory potency. Thus, in the trifluoromethyl substituted series (**3a**–**3d**; $R^1 = CF_3$) 23- to 350-fold enhancements were seen in PDE4 inhibition relative to the parent structure (KCA-1450). Moreover, the 6-aryl-4,5-dihydropyridazin-3(2*H*)-one extension was clearly effective as a PDE3-inhibitory subunit. Optimal PDE3 inhibition ($IC_{50} = 15$ nM) was found with the tetramethylene linker (compound **3c**), whereas the PDE4-inhibitory activity was essentially the same with both pentamethylene and tetramethylene linkers (**3d**, **3c**). We also assessed two ethyl-substituted pyrazolopyridine analogues (**3e**, **3f**) in this compound subset because during the development of KCA-1490 both ethyl and trifluoromethyl groups at the pyrazolopyridine 2-position supported activity. With compounds **3e** and **3f** optimal activity against both PDE3 and PDE4 was seen with the tetramethylene linker, where **3f** showing essentially equipotent inhibition.

Table 3

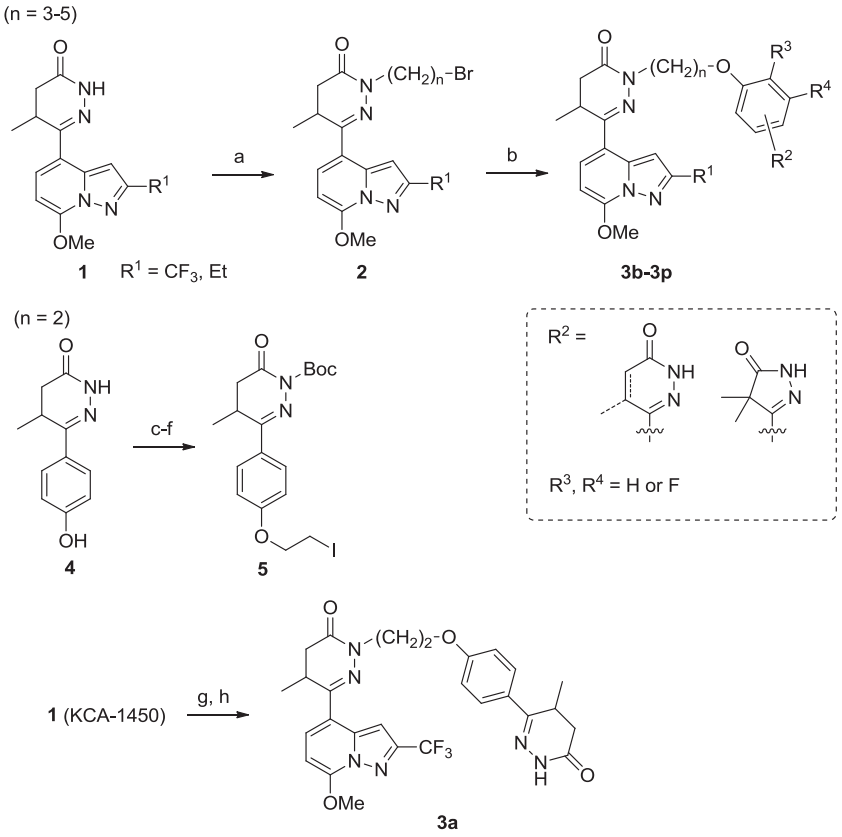


Compd[a]	R^2	R^3	R^4	Inhibition IC_{50}[b] (nM)	
				PDE3A	PDE4B
3j		H	H	270	0.5
3m		F	H	1520	1.4
3n		H	F	58	0.3
3o		F	F	15	3.1
3p		F	F	5.1	2.2

[a] These compounds were tested in racemic form.
[b] See footnote of Table 1.

Having identified the tetramethylene chain as the most promising linker, we next explored options for replacement of the terminal pyridazinone unit in the PDE3-inhibitory extension of the compound series. We had initially retained a chiral 5-methyl-3,4-dihydropyridazinone in this extension for the compound subset in Table 1 because this subunit was well established in PDE3 inhibitors such as CI-930 (Fig. 1) and had also been successfully exploited by Merck[13] for the development of a series of PDE3-selective inhibitors. However, we have recently shown that the 5-methyl-3,4-dihydropyridazinone ring of KCA-1490 and analogs can be replaced effectively by an achiral 4,4-dimethylpyrazolone subunit.[5d] We therefore sought to evaluate this and other achiral pyridazinone-based options in our hybrid PDE3/4 inhibitor series, compounds **3g**–**3j** (Table 2). Assessment of this series revealed that replacement of the dihydropyridazinone ring in parent structure **3c** by fully unsaturated pyridazinone subunits (**3g**, **3i**) severely reduced PDE3-inhibitory potency; with 4,5-dihydropyridazin-3(2*H*)-one and 4,4-dimethyl-1*H*-pyrazol-5(4*H*)-one replacements (compounds **3j** and **3h** respectively) activity loss against PDE3 was somewhat less profound. These latter two compounds retained similar levels of PDE4-inhibitory activity to the parent compound (**3c**), though a significant reduction in PDE4 inhibition was again observed with the unsaturated pyridazinone subunits (**3g**, **3i**).

Given the loss in PDE3-inhibitory activity observed for **3g**–**3j**, we decided to assess the impact of repositioning the heterocycle on the phenoxy ring of the extension. To do this we prepared compounds **3k** and **3l** for comparison with **3c** and **3j** (Table 2).



Scheme 1. Reagents and conditions: (a) $Br(CH_2)_nBr$, NaH/DMF, rt; (b) phenol derivatives, K_2CO_3, KI/DMF, 60 °C; (c) $Br(CH_2)_2OTBDPS$, K_2CO_3/DMF, rt to 60 °C (80%); (d) $(Boc)_2O$, DMAP/MeCN, rt (quant.); (e) TBAF/THF, rt (81%); (f) I_2, Ph_3P, imidazole/THF, rt (98%); (g) **5**, NaH/DMF, 60–80 °C (48%); (h) TFA/CH_2Cl_2, rt (48%).

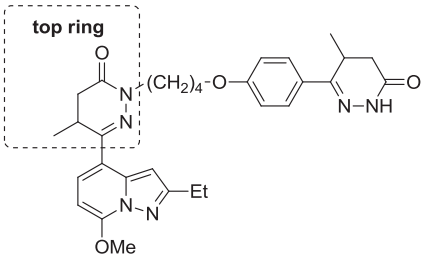
Repositioning the 5-methyl-3,4-dihydropyridazinone ring in **3c** from the *p*-position to the *m*-position (**3k**) had little impact on PDE4 inhibition but severely reduced PDE3 inhibition. With the dimethylpyrazolone, as an achiral surrogate for the 5-methyl-3,4-dihydropyridazinone, further weakening of PDE3-inhbitory activity was observed on altering the extension connectivity from *p*-**3j** to *m*-**3l**. The latter compound showed further gains in PDE4-inhibitory performance, however, and proved to be a very potent inhibitor for PDE4 with 4 orders of magnitude selectivity over PDE3.

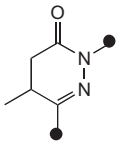
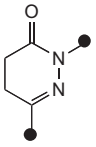
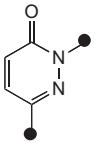
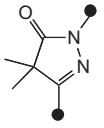
Based on the data in Table 2, the two most promising rings as achiral subunits for the PDE3-inhibitory extension of our hybrid PDE3/4 inhibitor series appeared to be the 4,5-dihydropyridazin-3(2*H*)-one and 4,4-dimethyl-1*H*-pyrazol-5(4*H*)-one in compounds **3h** and **3j**. In an effort to restore the PDE3-inhibitory activity of these compounds to levels comparable to **3c**, we next considered introduction of substituents on the aryl ring of the extension. Merck had previously demonstrated that fluorination of the aryl ring in their PDE3-selective 6-aryl-5-methyl-4,5-dihydropyridazin-3(2*H*)-one series strongly enhanced PDE3 inhibition.[13a] Given this precedent we focused on the impact of monofluorination at the 2- and 3-positions of the phenoxy ring in compound **3j** and on 2,3-difluorination (Table 3). Compound **3m** (R^3 = F, R^4 = H) showed an activity loss against PDE3 but **3n** (R^3 = H, R^4 = F) was about fivefold more potent than **3j** as a PDE3 inhibitor. The difluorinated compound (**3o**) was 18-fold more potent than **3j**, however, and proved to be equipotent with **3c** as a PDE3-inhibitor. Finally, replacement of the 4,4-dimethylpyrazolone ring in **3o** with the 4,5-dihydropyridazinone (**3p**) resulted in a further slight gains in potency against PDE3 (IC_{50} 5.1 nM) whilst retaining respectable levels of activity against PDE4 (IC_{50} 2.2 nM).

Having developed a successful strategy for eliminating the chiral subunit in the PDE3-inhibitory extension of our Table 1 compound subset, we next turned our attention to achiral replacements for the core 5-methyl-3,4-dihydropyridazinone ring. For this work we used compound **3f** as our reference point and compared the activity of analogs **3q**, **3r** and **3s** (Table 4), possessing, respectively, 4,5-dihydropyridazin-3(2*H*)-one, pyridazin-3(2*H*)-one and 4,4-dimethyl-1*H*-pyrazol-5(4*H*)-one rings as achiral surrogates for the core. Pleasingly, both compounds **3q** and **3s** exhibited comparable levels of dual PDE3/4-inhibitory potency to the parent (**3f**); compound **3r** showed a 12-fold loss in activity against PDE4. Although ultimately our intention is to reduce systemic exposure through inhaled delivery, we tested the potent, well-balanced dual PDE3/4 inhibitors of Table 4 for their capacity to suppress histamine-induced bronchoconstriction in guinea pigs under iv administration (dosed at 0.1 mg/kg). As expected, **3f** and **3s** showed excellent bronchodilatory activity. Compound **3r** was moderately effective but analog **3q**, with the 4,5-dihydropyridazin-3(2*H*)-one core, displayed comparatively weak bronchodilatory activity. At present the reason for the poorer performance of **3q** is unclear.

With strategies now in place for eliminating both of the chiral 5-methyl-3,4-dihydropyridazinone subunits from our initial compound subset (Table 1), we next focused on bringing the two approaches together for preparation of potent dual PDE3/4 inhibitors lacking any stereogenic centers (Table 5). Given the well-balanced dual PDE3/4-inhibitory activity and strong bronchodilatory performance of compound **3s**, we decided to take the dimethylpyrazolone ring forward from Table 4 as the subunit for the core of the structure. From the compounds in Table 3, both dimethylpyrazolone (**3o**) and dihydropyridazinone (**3p**) rings, coupled to a difluorinated phenoxy subunit, were considered as good candidates for the PDE3-inhibitory extension of the hybrid structure. However, we also considered replacing the pyrazolo [1,5-*a*]pyridine subunit of the core. We had previously shown that

Table 4



Compd[a]	Top ring	Inhibition IC_{50} (nM) [b]		Inhibitory effect on bronchoconstriction 0.1 mg/kg iv[c] (%)
		PDE3A	PDE4B	
3f		1.2	1.7	96
3q		0.27	2.7	28
3r		2.7	20	71
3s		1.7	0.6	92

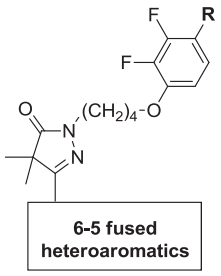
[a] Compound **3f** was evaluated as an equimolar mixture of all four stereoisomers; compounds **3q**–**3s** were tested in racemic form.
[b] See footnote in Table 1.
[c] The inhibitory activity of compounds on histamine-induced bronchoconstriction in guinea pigs was measured according to an established procedure.[14]

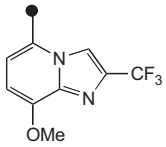
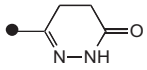
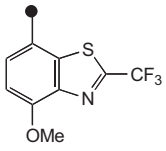
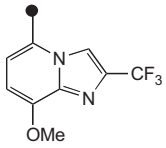
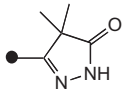
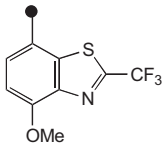
isosteric imidazopyridine and benzothiazole replacements for the pyrazolopyridine in combination with a dimethylpyrazolone ring were particularly effective in achiral analogs of KCA-1490 (Fig. 2).[5d] Thus, imidazopyridine **7** exhibited well-balanced dual PDE3/4-inhibitory activity and excellent bronchodilatory activity under both iv and po administration; benzothiazole **8** was equally effective in suppressing histamine-induced bronchoconstriction under iv administration but showed no effect via the oral route.[5d] This is significant because for minimal systemic exposure an inhaled agent should have negligible oral bioavailability. Thus with ICS 40–90% of administered drug may be deposited in the pharynx, resulting in ingestion and systemic steroid exposure by absorption from the gastrointestinal tract.[15] We therefore designed and evaluated four achiral hybrid PDE3/4 inhibitors (**3t**–**3w**) that included imidazopyridine and benzothiazole subunits as the core heterobicycle (Table 5). (Synthetic details for these compounds[16] are provided in the Supplementary data.)

Compounds **3v** and **3w**, with the terminal dimethylpyrazolone subunit, were less effective as PDE3 inhibitors than the cognate dihydropyridazinones (**3t** and **3u** respectively), though each compound pair showed comparable levels of PDE4-inhibitory activity. We evaluated the performance of all four compounds in suppressing histamine-induced bronchoconstriction under iv administration (0.1 mg/kg). Both compounds with the terminal dihydropyridazinone subunit (**3t** and **3u**) showed potent activity. In contrast, the corresponding dimethylpyrazolone analogs (**3v** and **3w**) displayed moderate to low potency, perhaps reflecting a weaker PDE3-inhibitory component in their activity profiles. Given the strong bronchodilatory performance of imidazopyridine **3t** and benzothiazole **3u**, we took these compounds forward for evaluation of anti-inflammatory activity under conditions of intratracheal administration (see Supplementary data). Both **3t** and **3u** were effective for suppression of LPS-induced inflammation (56%, 51% at 100 μg/kg, respectively), thus confirming in vivo activity as inhaled agents.

In conclusion, we have developed a series of potent hybrid PDE3/4 dual-inhibitors by rational design based on an understanding of the distinct PDE3- and PDE4-binding modes of lead compound, KCA-1490. N-alkylation of the pyridazinone ring in KCA-1490 strongly enhances PDE4-inhibitory potency but compromises PDE3 inhibition by disrupting hydrogen bonded engagement of the purine-scanning glutamine in the PDE3 catalytic pocket. However, by incorporation of a PDE3-inhibitory extension into the *N*-alkyl group it is possible both to enhance levels of PDE4 inhibition over KCA-1490 and instill potent PDE3 inhibition. Strategies were developed both to eliminate the chiral 5-methyl-3,4-dihydropyridazinone in the PDE4-inhibitory core of the hybrid structure and avoid the need for a similar chiral subunit in the PDE3-inhibitory extension. In this way we were able to develop achiral hybrid structures with low nanomolar dual PDE3/4-inhibitory activity that potently suppress histamine-induced bronchoconstriction in vivo under iv administration. We have further demonstrated that two of the compounds, imidazopyridine **3t** and benzothiazole **3u**, show promising anti-inflammatory activity under conditions of

Table 5



Compd	R	6–5 Fused heteroaromatics	Inhibition IC_{50}[a] (nM)		Inhibitory effect on bronchoconstriction 0.1 mg/kg iv[b] (%)
			PDE3A	PDE4B	
3t			5	6.7	87
3u			6.9	0.77	96
3v			40	3.2	76
3w			63	0.38	36

[a] See footnote in Table 1.
[b] See footnote in Table 4.

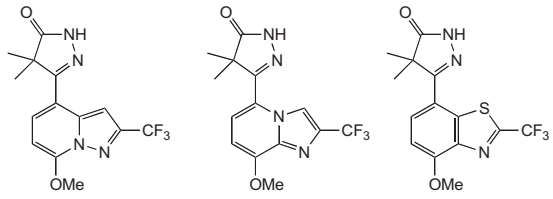


Figure 2. Achiral dimethylpyrazolone analogs of KCA-1490.[5d]

intratracheal administration, thereby laying a foundation for the potential development of these compounds as inhaled agents for respiratory disease. Further evaluation of this hybrid PDE3/4 inhibitor series will be reported in due course.

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Supplementary data

Supplementary data (preparation of **3a–3w**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.121.

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