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Rhodanine derivatives as novel inhibitors of PDE4

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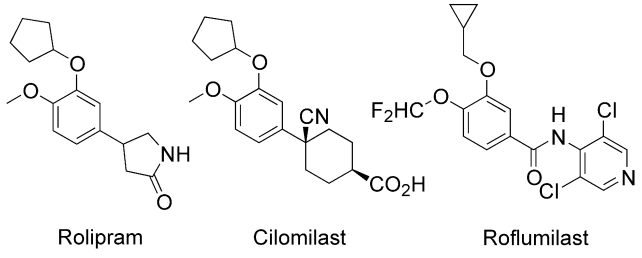
Abstract—The discovery, synthesis and in vitro activity of a novel series of rhodanine based phosphodiesterase-4 (PDE4) inhibitors is described. Structure-activity relationship studies directed toward improving potency led to the development of submicromolar inhibitors **2n** and **3i** (IC_{50} = 0.89 & 0.74 μM). The replacement of rhodanine with structurally related heterocycles was also investigated and led to the synthesis of pseudothiohydantoin **7** (IC_{50} = 0.31 μM).

The phosphodiesterase (PDE) enzymes are responsible for hydrolyzing secondary messengers cAMP and cGMP to their 5′-monophosphate counterparts. Phosphodiesterase type 4 (PDE4) is a cAMP-specific PDE expressed in inflammatory and airway smooth muscle cells. By elevating intracellular levels of cAMP, PDE4 inhibition can downregulate the inflammatory response and induce smooth muscle relaxation.[1] With anti-inflammatory and bronchodilatory activity, PDE4 inhibitors have attracted considerable interest as therapeutic agents for inflammatory diseases such as asthma and COPD (chronic obstructive pulmonary disease).[2]

However, the development of 1st generation inhibitors (e.g. rolipram) was precluded, due to the propensity of these compounds to induce dose-limiting side-effects of nausea and vomiting.[2d,3] Several 2nd generation inhibitors appear to be better tolerated, with the most advanced to date being cilomilast (Ariflo®)[4] and roflumilast (Daxas®).[5] Both of these compounds have completed phase III clinical trials and are presently awaiting regulatory approval for the treatment of asthma and/or COPD.[6]



In a search for novel inhibitors, the Scottish Biomedical lead generation library was scanned against PDE4 via high throughout screening. Prominent amongst the resultant hits was a series of derivatives containing a 5-benzylidenerhodanine[7] substructure (Fig. 1). Benzylidenerhodanines have been reported as small molecule inhibitors of numerous targets such as cyclooxygenase and 5-lipoxygenase,[8a] β-lactamase,[8b] cathepsin D,[8c] HCV NS3 protease,[8d] aldose reductase,[8e] protein mannosyl transferase[8f] and JNK-stimulating phosphatase.[8g] Herein, we report the synthesis and structure-activity relationships (SAR) for a series of benzylidenerhodanines which inhibit PDE4 in the submicromolar range.

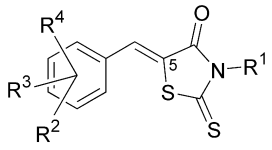


Figure 1. 5-Benzylidenerhodanine substructure.

Keywords: Rhodanine; Phosphodiesterase 4; PDE4 inhibitor; Asthma; COPD.
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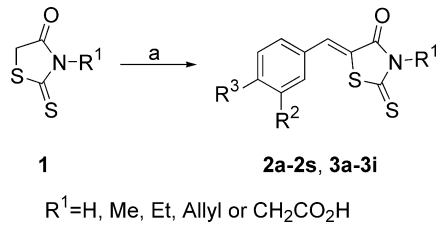
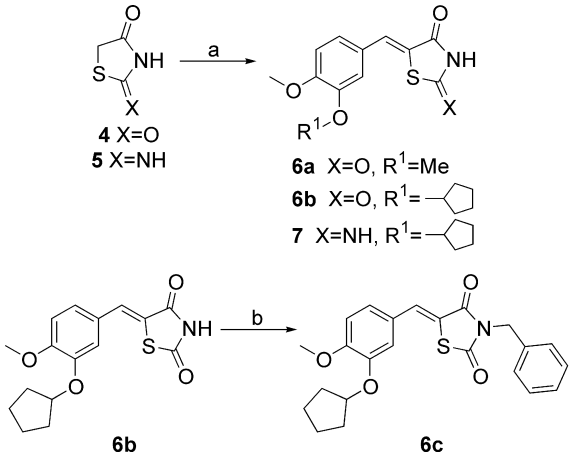


Scheme 1. Reagents and conditions: (a) RCHO, NaOAc, AcOH, reflux, 3 h or RCHO, piperidine, EtOH, reflux, 1.5 h.

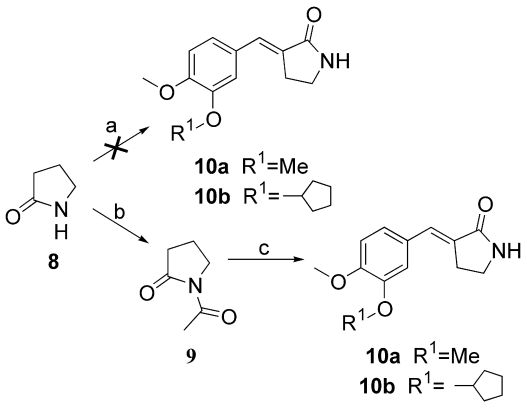


Scheme 2. Reagents and conditions: (a) RCHO, NaOAc, AcOH, reflux, 4 h; (b) Bz–Br, Cs_2CO_3, NaI, DMF, 60 °C, 19 h.

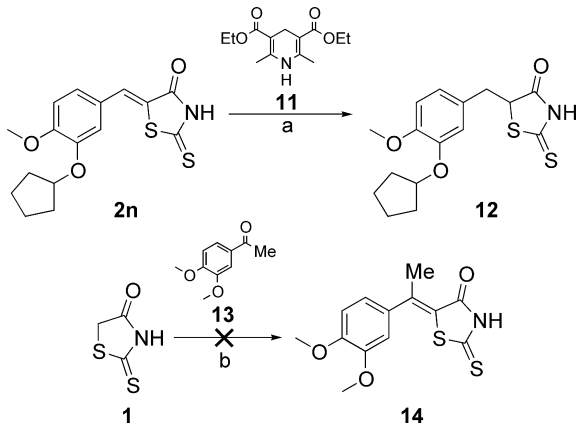
The benzylidenerhodanines in Tables 1 and 2 were synthesized in excellent yield via a Knoevenagel type condensation between the requisite rhodanine **1** and benzaldehyde (Scheme 1). Those benzaldehydes not available commercially were synthesized via well documented procedures.

Compounds **6a**, **6b** and **7** were synthesized from 2,4-thiazolidinedione **4** and pseudothiohydantoin[9] **5** in an almost identical fashion to their rhodanine counterparts (Scheme 2). The *N*3 position of **6b** was alkylated under standard conditions to afford **6c** in good yield (Scheme 2).

3-Benzylidene-2-pyrrolidinone derivatives **10a** and **10b** were synthesized from 2-pyrrolidinone **8** (Scheme 3).



Scheme 3. Reagents and conditions: (a) MeONa or EtONa, RCHO, toluene; (b) Ac_2O, reflux, 1.5 h; (c) RCHO, NaH, THF, 0–10 °C.



Scheme 4. Reagents and conditions: (a) activated silica gel 60, toluene, 80–85 °C, 24 h, in dark; (b) NH_4OH, NH_4Cl, EtOH, reflux.

However, due to the weak acidity of its α-hydrogens, **8** could not be directly condensed with the requisite benzaldehyde. Instead, this compound was initially reacted with acetic anhydride to generate **9**. The *N*-acetyl group is electron-withdrawing and facilitates the condensation reaction by enhancing the acidity of the α-hydrogens. This activating group was easily removed from the products in situ by using an excess of base during the condensation.[10]

Modification of the exocyclic alkene linker was of obvious interest for SAR studies (Scheme 4). Reduction of the alkene moiety in **2n** (yielding **12**) was achieved in good yield using Hantzsch ester **11**.[11] Despite employing conditions previously reported as suitable for condensing rhodanine with ketones,[12] we were unable to synthesize compound **14** from rhodanine **1** (R_1=H) and 3,4-dimethoxyacetophenone **13**. The exact reason for the failure of this reaction is unknown although steric and electronic factors may be involved.

All compounds prepared were evaluated for their in vitro activity against PDE4B and PDE3A using the enzymatic assay described previously.[13] The respective IC_{50} values are shown in Tables 1–3.

To establish basic SAR information, we initially synthesized simple rhodanine derivatives with either a mono- or di-substituted benzylidene ring. The only active PDE4 inhibitor to be generated via this strategy was the 3′,4′-dimethoxy derivative **2a** (IC_{50} = 17.90 μM). In addition to PDE4, **2a** displayed activity against PDE3 (IC_{50} = 10.50 μM), an enzyme whose inhibition has been linked with cardiotoxicity.[1] In an effort to increase both potency and selectivity toward PDE4, the main structural areas of **2a** (i.e. rhodanine ring, catechol subunit and alkene linker) were subjected to chemical modification.

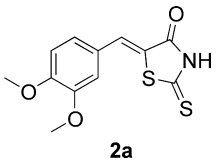
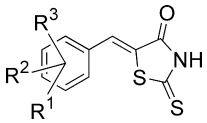


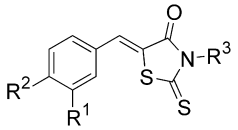
Table 1. Modification of the catechol subunit



Compound	R^1	R^2	R^3	PDE4B IC_{50}[a] (μM)	PDE3A IC_{50}[a] (μM)
2a[15]	3-OMe	4-OMe	H	17.90	10.50
2b[15]	H	H	H	NI	NT
2c	3-OMe	H	H	NI	NT
2d[15]	H	4-OMe	H	NI	NT
2e[16]	3-OH	4-OH	H	NI	NT
2f[17]	3-OH	4-OMe	H	NI	NT
2g[15]	3-OMe	4-OH	H	NI	NT
2h[15]	2-OMe	3-OMe	H	54.65	NT
2i[18]	3-OMe	4-OMe	5-OMe	64.82	NT
2j	3-OMe	4-OEt	H	6.91	9.46
2k	3-OEt	4-OMe	H	5.30	39.81
2l[15]	3-OEt	4-OEt	H	6.51	80
2m	3-OAllyl	4-OMe	H	3.08	NI
2n	3-OcPent	4-OMe	H	0.89	10.83
2o	3-OMe	4-OcPent	H	30.42	28.50
2p	3-OcHex	4-OMe	H	1.94	NI
2q[19]	3-OBz	4-OMe	H	NI	NT
2r	3-OcPent	4-OEt	H	1.85	24.99
2s[15]	3,4-OCH_2O–		H	NI	NT
Rolipram				2.00	NI

NI, no inhibition.
NT, not tested.
[a] Mean of at least two experiments.

Table 2. Effect of *N*-alkyl substitution



Compound	R^1	R^2	R^3	PDE4B IC_{50}[a] (μM)	PDE3A IC_{50}[a] (μM)
3a[23]	OMe	OMe	Me	NI	NT
3b	OMe	OMe	Et	NI	NT
3c	OMe	OMe	Allyl	NI	NT
3d	OMe	OMe	CH_2CO_2H	NI	NT
3e	OcPent	OMe	Me	2.87	NI
3f	OcPent	OMe	Allyl	4.13	NI
3g	OcPent	OMe	CH_2CO_2H	3.07	NI
3h	OH	OMe	Et	11.20	NI
3i[24]	OMe	OH	Et	0.74	NI

NI, no inhibition.
NT, not tested.
[a] Mean of at least two experiments.

The first area that we focused on was the catechol subunit. Modifications to this region (Table 1) established a SAR similar to that previously elucidated for the catechol subunit in rolipram.[14] Briefly, both methoxy groups were essential for activity with a 3′,4′-dialkoxy substitution pattern proving optimal. At the 3′-position, increasing the size of the alkoxy substituent significantly enhanced activity with the highest potency residing in the cyclopentyloxy derivative **2n**. In contrast, a methoxy proved optimal at the 4′-position with the introduction of larger alkoxy groups resulting in marked decreases in inhibitor potency.

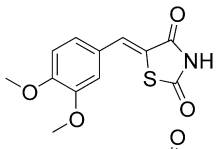
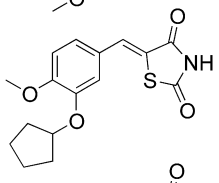
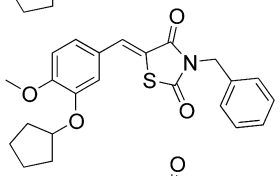
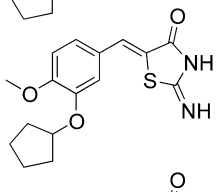
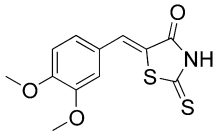
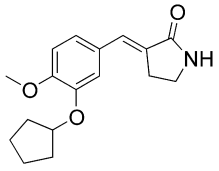
To investigate the binding role of the catechol oxygens we synthesized the 3′,4′-methylenedioxy derivative **2s**. The alkoxy oxygens in **2a** have the ability to act as hydrogen bond acceptors (HBAs), meaning the spatial orientation of the lone pairs on these atoms and the subsequent dipoles they create will be critical to the success of any such interaction with the active site. Like rolipram,[20] the dipoles created by the catechol lone pairs in **2a** appear to be orientated in the same general direction (Fig. 2).

However, the cyclic framework in **2s** will undoubtedly force the dipoles in this compound to be directed away from each other (Fig. 2). [21] This modification abolishes inhibitory activity and suggests that the alkoxy oxygens are indeed participating in hydrogen bonding.

The preceding modifications suggest that when the benzylidenerhodanines interact with PDE4, the alkoxy groups bind within two hydrophobic pockets while the catechol oxygens participate in hydrogen bonding with a suitable donor residue (Fig. 3). This hypothesized binding mode is in agreement with published crystal structures of PDE4 in complex with rolipram and other catechol ether inhibitors.[22]

After the catechol subunit, we turned our attention to the rhodanine ring. Two modifications were made to this area with the first being the introduction of *N*-alkyl substitution (Table 2). As shown, the *N*-methyl, *N*-ethyl, *N*-allyl and *N*-carboxymethyl derivatives of **2a** were all devoid of activity (**3a**–**3d**). The introduction of *N*-alkyl substitution was also detrimental to **2n**, although it is interesting to note that the resultant compounds (**3e**–**3g**) were inactive against PDE3. The poor activity of the *N*-alkylated derivatives suggests that the NH proton may be participating in hydrogen bonding. Whilst detrimental to the preceding compounds, *N*-alkyl substitution proved hugely beneficial to **2f** and **2g**. For example, the introduction of an *N*-ethyl group converted **2f** into an active, albeit relatively weak, PDE4 inhibitor (**3h**). The increase was even more dramatic for **2g** with an *N*-ethyl moiety transforming this previously inactive compound into a submicromolar inhibitor (**3i**). The activity displayed by these compounds was surprising and difficult to explain considering the inactivity of their parent structures and that *N*-alkyl substitution had proved detrimental to **2a** and **2n**. However, it is possible that the *N*-ethyl groups may allow the rhodanine moieties in **3h** and **3i** to mimic the binding mode of the catechol subunit in **2n**. In this theoretical binding mode, the *N*-ethyl group would occupy the same space as the 3′-cyclopentyloxy moiety whilst the rhodanine carbonyl would mimic the hydrogen-bonding role of the catechol oxygens (Fig. 4). An interesting point to note is that methylation of the hydroxyl groups in **3h** and **3i** (generating **3b** in each case) abolishes all activity. This observation provides strong evidence that the

Table 3. Replacement of rhodanine heterocycle

Compound		PDE4B IC_{50}[a] (μM)	PDE3A IC_{50}[a] (μM)
6a[25]		14.10	NI
6b		1.09	NI
6c		NI	NT
7		0.31	NI
10a[10]		28.30	NI
10b		4.01	NI

NI, no inhibition.
NT, not tested.
[a] Mean of at least two experiments.

hydroxyl groups in these compounds are acting as hydrogen-bond donors (HBDs).

Next, we replaced rhodanine with different heterocyclic moieties (Table 3). Whilst thiazolidinedione derivatives **6a** and **6b** were equipotent with their rhodanine counterparts, pseudothiohydantoin **7** was 3-fold more active than **2n** and some 58-fold more potent than original lead **2a**. Interestingly, all three compounds were inactive against PDE3. The *N*-benzyl derivative

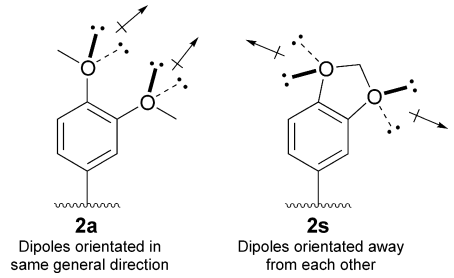


Figure 2. Dipole orientation in **2a** and **2s**.

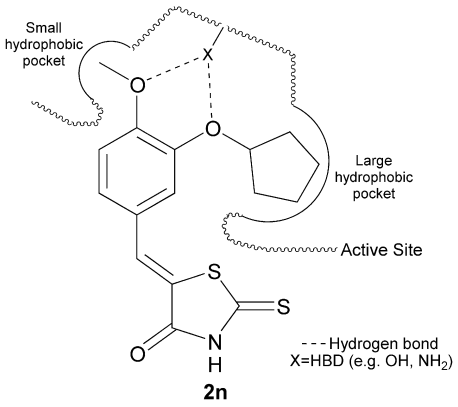


Figure 3. Proposed binding of **2n** with PDE4 active site.

6c, which was synthesized to further explore the space surrounding the *N*3 position, was inactive. Since the thioketone in **2a** and **2n** could be replaced without adversely affecting activity, we decided to investigate the complete removal of this moiety. Interestingly, 2-pyrrolidinone derivatives **10a** and **10b** were 1.6- and 4.5-fold weaker than their rhodanine counterparts. This was somewhat surprising as the activities of **6a**, **6b** and **7** suggested that the thioketone was not essential for inhibitory activity. However, the drop in potency may simply stem from **10a** and **10b** adopting a slightly altered binding conformation due to a steric clash between the H4 and H2′ hydrogens.

The alkene linker between the catechol and heterocyclic rings was the final area to undergo modification (Scheme 4). Reduction of the alkene moiety in **2n** yielded benzylrhodanine **12**, which was some 2.5-fold weaker than its unsaturated parent (IC_{50} = 2.20μM). This drop in activity was not particularly surprising as the greater flexibility of the benzyl linker in **12** will allow this compound to adopt more binding conformations than its rigid benzylidene counterpart. However, it is also possible that the drop in activity is due to a loss in conjugation between the catechol and heterocyclic rings. For example, the 4′-methoxy group in **2n** can increase the electron density on the carbonyl oxygen through resonance (Fig. 5), thereby enhancing this group's ability to act as a HBA. Reducing the alkene linker will prevent this movement of electron density and thus weaken any

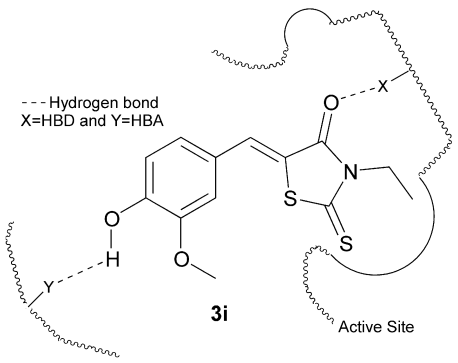


Figure 4. Proposed binding of **3i** with PDE4 active site.

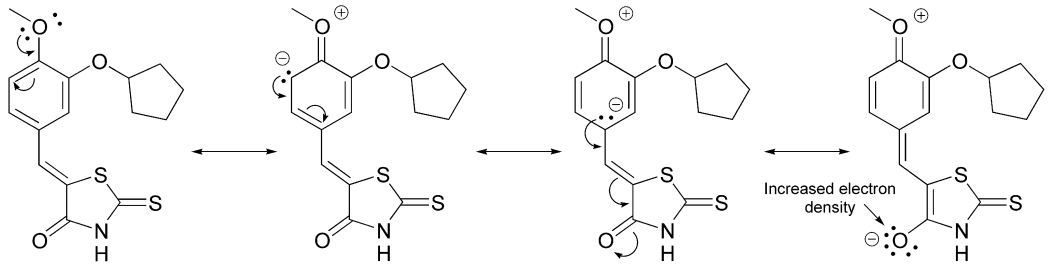


Figure 5. Movement of electron density in **2n**.

hydrogen bond between the carbonyl oxygen in **12** and the PDE4 active site. The conjugation effect in question is clearly observed by comparing the carbonyl stretching frequencies for the two compounds. For example, the υC=O for **2n** appears at 1688 cm^{-1} with the corresponding absorption for **12** occurring some 40 cm^{-1} higher at 1728 cm^{-1}.

In conclusion, we have identified a series of rhodanine derivatives as novel inhibitors of PDE4. Structures **2n** and **3i** displayed the most significant activity of the compounds synthesized, being some 20- and 24-fold more potent than lead compound **2a**. Replacing the rhodanine ring system with different heterocycles also generated active inhibitors with the most potent of these being pseudothiohydantoin **7**. Further research into PDE4 inhibitors structurally related to compound **7** is currently on-going and will be published in due course.

Acknowledgments

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