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Discovery of thiadiazoles as a novel structural class of potent and selective PDE7 inhibitors. Part 1: Design, synthesis and structure–activity relationship studies

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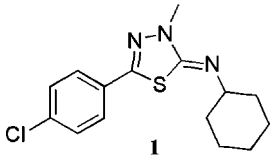
Abstract—The synthesis and SAR studies of a series of structurally novel small molecule inhibitors of PDE7 are discussed. The best compounds from the series displayed low nanomolar inhibitory activity and are selective versus PDE4.

1. Introduction

The family of phosphodiesterases (PDE's) regulate levels of secondary messengers adenosine and guanosine 3′,5′-cyclic monophosphates (cAMP and cGMP, respectively) via hydrolysis to their corresponding inactive 5′-monophosphate nucleotides.[1] Inhibition of cAMP-specific PDE activity causes an increase in concentrations of cAMP thereby activating protein kinases responsible for various biological processes. At least 11 isoenzymes of mammalian cyclic nucleotide phosphodiesterase have been identified.[2] Among them, PDE7 is a high affinity cAMP-specific PDE ($K_m = 200$ nM) with kinetic properties distinct from those of PDE4 (PDE7 K_m is about 10-fold lower than PDE4). Based on the functional role of PDE7 in T-cell activation[3] and the corresponding levels and localization of the mRNA, PDE7 inhibitors might be useful in the treatment of airway diseases,[4] T-cell related diseases,[3] CNS disorders,[5] leukaemia,[6] and fertility disorders.[7] Development of specific inhibitors[8] represents one of the approaches to uncover the physiological roles of PDE7. High throughput screening of the compound collection resulted in the identification of the 1,3,4 thiadiazole **1** (Fig. 1) as a weak PDE7 inhibitor with an IC_{50} of 1.5 μM.

This article describes our preliminary efforts towards optimizing the enzymatic inhibitory activity and the selectivity profile of these compounds versus other PDEs (especially PDE4, which is also a cAMP-specific PDE). The SAR studies on the thiadiazole template led to the



1
IC_{50} = 1500 nM

Figure 1. Schematic representation of **1**.

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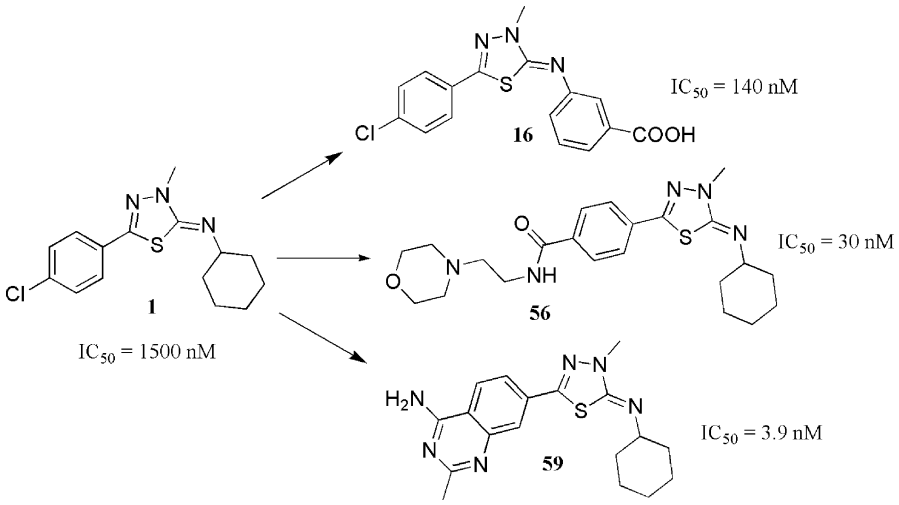


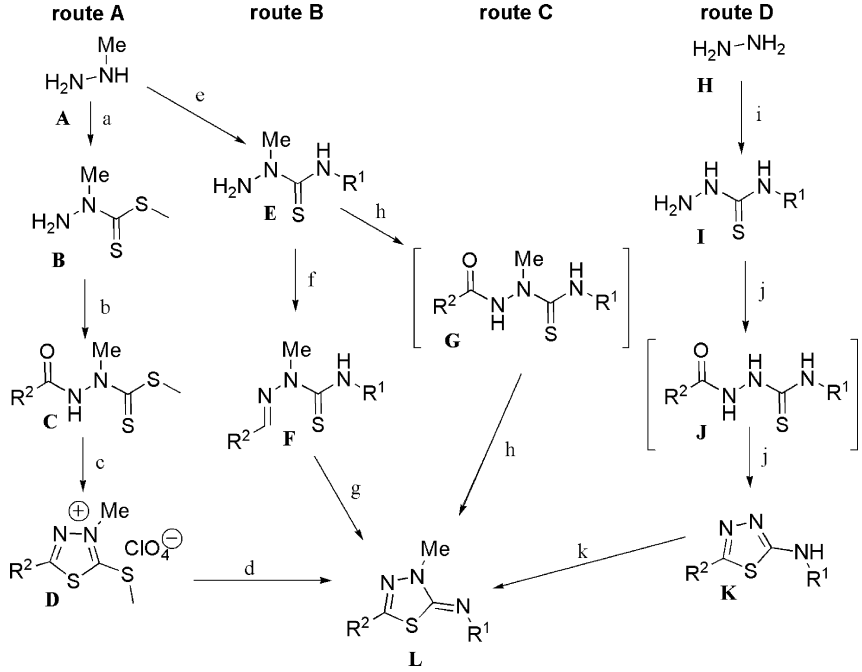
Figure 2. Schematic representation of the inhibitors designed from **1**.

discovery of several potent and selective PDE7 inhibitors exemplified by **16**, **56** and **59** (Fig. 2).

2. Chemistry

The thiadiazole derivatives were prepared according to well reported routes[9] as described in Scheme 1.

Four synthetic methods were explored to bring the appropriate chemical diversity at R^1, R^2 and R^3. *Route A*: the reaction conditions for this method were first reported by Molina et al.[10] Substituted hydrazine **A** was reacted with carbon disulfide and methyl iodide in presence of potassium hydroxide to form the hydrazine carbodithioate **B**. The key intermediate 1,3,4-thiadiazolium perchlorate **D** was then generated after acylation of **B** followed by intramolecular cyclization in presence of acetic anhydride and perchloric acid **D** is then reacted with a suitable amine to yield the targeted thiadiazole **L**. *Route B*: thiadiazole **L** is prepared from reaction of isothiocyanate (R^1NCS) with substituted hydrazines **A** to give **E**, followed by successive condensation of the hydrazine carbothioamide **E** with aldehyde (R^2CHO) and



Scheme 1. Reagents and conditions:[9] route A:[10] (a) CS_2, MeI, KOH, EtOH, 0–15 °C; (b) R^2COCl, toluene, reflux; (c) Ac_2O, $HClO_4$, Et_2O, −5 to 5 °C; (d) R^1NH_2, alcohol (EtOH), Et_3 N, 40–80 °C; route B:[11] (e) R^1NCS, EtOH, −5 to 15 °C; (f) R^2CHO, MeOH, 50–90 °C; (g) $FeCl_3$, alcohol (EtOH), 20–110 °C; route C:[12] (h) R^2COOH, $POCl_3$, 1,4-dioxane, reflux; route D:[12] (i) R^1NCS, EtOH, −5 to 15 °C; (j) R^2COOH, $POCl_3$, 1,4-dioxane, reflux; (k) MeX, aprotic solvent, rt to 80 °C.

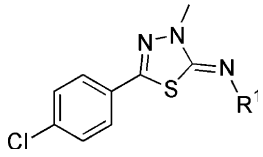
by oxidative cyclization of the resulting Schiff's base **F** in presence of iron(III) chloride according to Noto et al.[11] *Route C*: is a one pot cyclization procedure performed by reaction of substituted (R^3) hydrazine carbothioamide **E** with carboxylic acid (R^2COOH) in presence of dehydrating agents[12] (e.g., $POCl_3$). *Route D*: reaction of hydrazine **H** with isothiocyanate (R^1NCS) generated the corresponding intermediate **I**, which was reacted with carboxylic acid and in situ thermally cyclized in presence of dehydrating agent to afford **K**. The last step is a *N*-alkylation with electrophiles[12] (alkyl triflate, iodide or bromide) to give the desired thiadiazoles **L**.

3. Biological results[13] and discussion

The different and complementary chemical approaches (Scheme 1) allowed us to undertake a rapid survey of the molecule to determine the best path for further SAR studies around the R groups. Starting from **1**, various chemical modifications of the R^3 group while maintaining R^2 as *para*-chlorophenyl group and R^1 as cyclohexyl group, did not lead to a significant improvement in terms of inhibitory activity compared to **1** (data not shown). Then, as a first approach, we decided to keep unchanged the R^3 substitution by maintaining the methyl group. We focused on determining the SAR of the R^1 and R^2 groups more thoroughly.

Using a parallel synthesis approach, a large number of different R^1 groups were introduced (route A) and evaluated. Table 1 lists the structures and the inhibitory activities of selected analogs from this study.

Table 1. In vitro data[a] for compounds **1** and **2–13**



Compd[a]	R^1	PDE7A1 IC_{50}, μM[b]	PDE4D3 IC_{50}, μM[b]
1	–Cyclohexyl	1.50	57.00
2	–Cyclobutyl	4.80	6.30
3	–Cyclopentyl	3.08	81.50
4	–Bicyclo[2.2.1]hept-2-yl	1.05	>101
5	–Cyclooctyl	1.65	>101
6	–1-Azabicyclo[2.2.1]hept-3-yl	5.90	79.00
7	–*trans*-4-Aminocyclohexyl	5.90	>101
8	–1-(Hydroxymethyl)cyclopentyl	4.00	>101
9	–1-Ethylpropyl	5.60	>101
10	–Isopropyl	3.90	17.00
11	–Propyl	7.70	26.00
12	–2-Hydroxy-1,1-dimethylethyl	1.90	37.00
13	–Phenyl	2.95	38.00

[a] All the compounds have been prepared by route A.
[b] Measured against the human full length enzyme produced in baculovirus infected sf9 cells. Values are means of three experiments.

Replacement of the cyclohexyl structural part in **1** by carbocycles (**2–5**) was devised to define the effect of the ring size and shape on activity and selectivity.

As illustrated by compounds **1**, **4** and **5**, six or eight-membered ring systems allows to maintain a micromolar activity associated with a high selectivity versus PDE4. The analogue **2** with a smaller ring was not as selective. The incorporation of some heteroatoms into or onto carbocycles (**6–8**), which could be beneficial for solubility was shown to be detrimental to the enzymatic activity. When the cyclohexyl side chain was replaced by linear (and branched) alkyl chains (**9–12**), a loss in potency was observed.

The phenyl analogue **13** showed a slight decrease of activity compared to **1**. As a final outcome of these preliminary SAR studies, these chemical modifications suggested that hydrophobic steric bulk (R^1) was favourable for activity and selectivity versus PDE4.

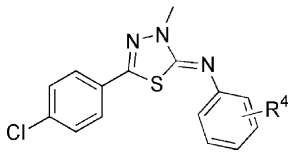
We next focused our attention on introducing ionizable and polar functions on the phenyl ring **13** with the objective to evaluate the critical determinants for activity, selectivity and solubility. Table 2 illustrates the inhibitory activity of some representative analogues prepared in this sub-series.

None of the monosubstituted neutral derivatives (**14**, **15**, **20–23**, **25**) provided any significant enhancement compared to the reference derivatives (**1** or **13**), resulting in a flat SAR. The most dramatic improvement in inhibitory activity resulted from substitution at the 3-position with a carboxylic acid type function as illustrated by **16**, **17** and **18**.

The soluble *meta*-benzoic acid derivative **16** with an IC_{50} of 140 nM exhibited a 10-fold increase in activity compared to the hit **1**. In addition, **16** was found to be more than 150-fold selective versus PDE4 and at least 50-fold versus the other PDEs. Among the two carboxylic acid bioisosteres prepared (**17** and **18**), only **18** was more potent (IC_{50} = 80 nM) compared to **16**. However, it is worth noting that both isosteric replacements were not as selective as than the corresponding carboxylic acid **16**, especially versus PDE4, PDE1 and PDE3. These results suggest that pK_a could have an impact on activity and selectivity. In addition, compound **24** (33-fold less active compared to **16**) revealed the importance of the relative position of the COOH function with respect to the phenyl ring and, as illustrated by **19**, elongation of the chain was detrimental to the inhibitory activity (over 15-fold loss in potency). Substitution of the *meta*-benzoic acid derivative **16** seemed to be tolerated at the 4-position (**29–30**) but in contrast, as illustrated by **26–28**, substitution at the 2-position decreased the inhibitory activity, confirming the results observed with **14** versus **15** and **23**.

In conclusion to these studies around R^1, the cyclohexyl and the *meta*-benzoic group were the preferred structural features identified to bring some inhibitory activity.

Table 2. In vitro data for compounds **14**–**30**



Compds[a]	R^4	PDE7A1 IC_{50}, μM[b]	PDE4D3 IC_{50}, μM[b]	PDE1 IC_{50}, μM[c]	PDE3A3 IC_{50}, μM[b]	PDE5 IC_{50}, μM[d]
14	2-OH	5.3	>101	>101	>101	>101
15	3-OH	1.8	>101	9.81	6.13	10.94
16	3-COOH	0.14	21.33	16.38	7.47	7.27
17	3-[5-Hydroxy-1,2,4-oxadiazol-3-yl]	0.44	5.63	3.56	1.89	7.56
18	3-[2H-tetrazol-5-yl]	0.080	4.26	3.34	1.71	7.00
19	3-CH_2COOH	2.13	23.00	40.34	8.57	20.16
20	3-CN	5.7	36.33	>101	47.21	>101
21	3-$CONH_2$	1.63	44.50	7.83	2.65	>101
22	3-F	6.6	>101	>101	>101	>101
23	4-OH	1.7	13	>101	101	>101
24	4-COOH	4.60	>101	28.93	30.68	40.35
25	4-F	2.2	>101	85.68	>101	101
26	2-OH, 5-COOH	1.31	58.25	28.84	49.42	18.77
27	2-Me, 5-COOH	1.37	20.5	14.06	20.54	23.60
28	2-OH, 3-COOH	0.26	14.2	9.28	9.83	5.18
29	3-COOH, 4-F	0.16	39.66	21.58	16.98	17.88
30	3-COOH, 4-OH	0.15	7.05	5.23	5.58	3.97

[a] Compounds **3** and **29**–**44** prepared by route A.
[b] Measured against the human full length enzyme produced in baculovirus infected sf9 cells. Values are means of three experiments.
[c] Measured against the human full length enzyme partially purified from THP-1 cell pellets. Values are means of three experiments.
[d] Measured against the human full length enzyme partially purified from MCF-7 cell pellets. Values are means of three experiments.

We next examined the SAR of the R^2 chemical part. Table 3 summarizes a broad survey around the impact of phenyl substitution (R^5) on activity and selectivity versus PDE4. Preliminary modification of the relative position of the OH, COOMe or Cl group from *ortho* to *meta* or *para* position resulted in a clear increase in inhibitory activity as exemplified by comparison of **31**–**33** with **1**, **34**–**36** or **41**–**42**. Consequently, the 3 and 4-position were targeted for more detailed chemical investigations. Interestingly, a more pronounced effect was demonstrated with neutral hydrogen bond donor (HBD) or hydrogen bond acceptor (HBA) groups as illustrated by **34**–**35** compared to **36** or by **41**–**42** compared to **1**, **45** and **47**.

Consequently, we undertook an analysis of the importance of these properties on activity and selectivity by the preparation of some compounds combining both HBD and HBA properties (Table 3).

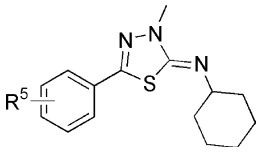
As expected, substitutions with some neutral HBD/HBA groups such as amide (**38**, **44**, **48**) or a combination of OH and OMe functions (**40**) caused a dramatic improvement in activity with compounds displaying IC_{50} values in the 60 nM range. The primary sulfonamides (**37**, **43**) were found less potent compared to the corresponding amide analogues (**38**, **44**). Based on the fact that sulfonamides and amides allow wide chemical variations, several analogues were prepared to improve drug-like properties. The sulfonamide **37** was optimized by introduction of a chloro-substituent at the 4-position (**53**) and by a *N*-substitution of the sulfonamide function with an ethyl chain (**54**). These modifications resulted in nearly 70-fold increase in activity compared to **1**, with more than 260-fold selectivity versus PDE4.

The best balance between activity and solubility within the amide series was found with compounds **56** (IC_{50} = 30 nM, solubility = 17 μg/mL at pH 7.4) and **57** (IC_{50} = 65 nM, solubility = 83 μg/mL at pH 7.4) prepared by a coupling reaction between **45** and the corresponding primary amines.

The fact that the secondary amide **57** is more potent than the corresponding *N*-methylated product **55** supports the hypothesis of the important role of HBD functions for activity. Focusing on the 4-$CONH_2$ derivatives, we varied the nature of functional groups at the 3-position as shown in Table 3 by compounds **49**–**52**. Incorporation of a methyl or a methoxy group at the 3-position (**49** and **50**, respectively) did not allow to improve activity compared to the nonsubstituted compound **44**. In contrast, introduction of the 3-OH (**51**) or 3-NH_2 (**52**) maintained the level of activity in the same range as for the reference derivative **44**.

These latter results suggest a nearly planar ring system as the favourable conformation for PDE7 affinity, due to an intramolecular hydrogen bond interaction between the lone electron pair of the oxygen atom from the carbonyl function (4-position) and the available hydrogen atom of the donor group at the 3-position.

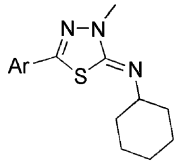
Table 3. In vitro data for compounds **31**–**57**

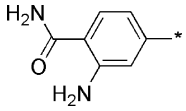
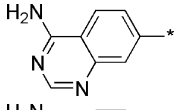
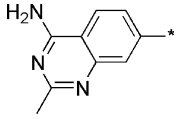


Compds	Route	R_5	PDE7A1 IC_{50}, μM[a]	PDE4D3 IC_{50}, μM[a]
31	D	2-OH	6.40	71.00
32	D	2-COOMe	7.50	30.00
33	B	2-Cl	1.30	40.00
34	D	3-OH	0.29	22.17
35	D	3-COOMe	0.14	19.00
36	B	3-Cl	0.70	>101
37	C	3-SO_2 NH_2	0.20	15.05
38	D	3-$CONH_2$	0.09	19.25
39	D	3-COOH	0.87	22.25
40	D	3-OH, 4-OMe, 5-OH	0.061	11.88
41	D	4-OH	0.24	12.23
42	D	4-COOMe	0.23	95.5
1	D	4-Cl	1.5	57.00
43	D	4-SO_2NH_2	0.15	10.97
44	D	4-$CONH_2$	0.061	15.3
45	D	4-COOH	0.55	32.25
46	B	4-SO_2Me	0.13	25
47	C	4-NH_2	0.56	25.75
48	C	4-$NHCOCH_3$	0.068	34.25
49	D	3-Me, 4-$CONH_2$	0.93	44.66
50	D	3-OMe, 4-$CONH_2$	0.47	>101
51	D	3-OH, 4-$CONH_2$	0.12	19.33
52	D	3-NH_2, 4-$CONH_2$	0.083	26.00
53	D	3-SO_2NH_2, 4-Cl	0.069	5.90
54	D	3-SO_2NHEt, 4-Cl	0.022	5.84
55	D	4-CON(Me)$CH_2CH_2N(CH_3)_2$	0.47	>101
56	D	4-$CONHCH_2CH_2$-*N*-morpholinyl	0.030	8.77
57	D	4-$CONHCH_2CH_2N(CH_3)_2$	0.065	33.33

[a] Measured against the human full length enzyme produced in baculovirus infected sf9 cells. Values are means of three experiments.

Table 4. In vitro data for compounds **52** and **58**–**59**



Compds	Ar	PDE7A1 IC_{50}, μM[a]	PDE4D3 IC_{50}, μM[a]
52		0.083	26.00
58		0.027	>101
59		0.0039	92.33

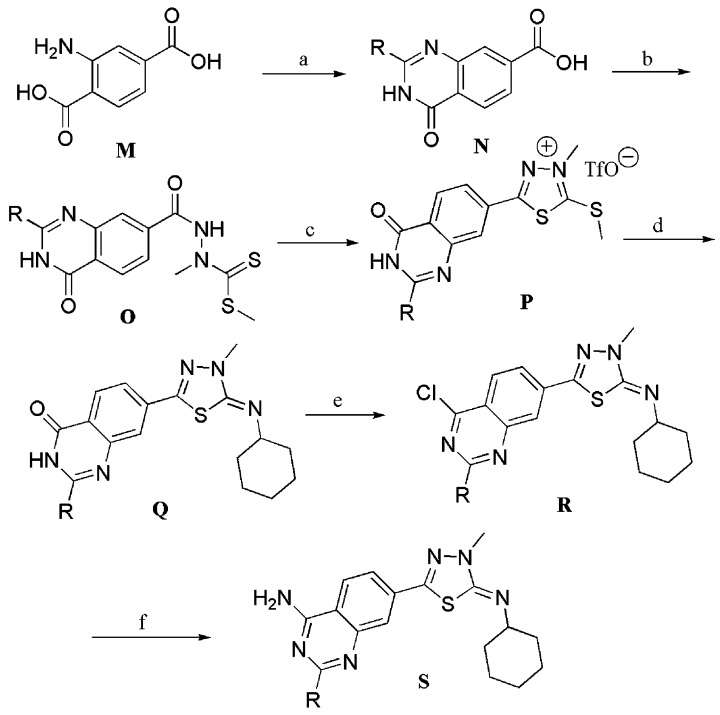
[a] Measured against the human full length enzyme produced in baculovirus infected sf9 cells. Values are means of three experiments.

Based on this hypothesis and in order to improve activity, further changes were devised aimed at designing a rigid bioisostere of the amino-amide system able to mimic the same type of HBD/HBA properties. The most evident option was to prepare 4-aminoquinazoline derivatives, which are, interestingly, structural analogues of the adenine part of cAMP. As expected, two of these analogues presented in Table 4 led to highly potent inhibitors illustrated by compounds **58** and **59**. The 4-amino-2-methylquinazoline derivative **59** displayed an IC_{50} of 3.9 nM on the PDE7 enzyme and exhibited a very high selectivity versus PDE4.

The quinazoline derivatives **58** and **59** have been prepared by a modified route shown in Scheme 2.

4. Conclusion

In conclusion, a dramatic improvement in activity and selectivity was achieved from the micromolar hit compound **1** by modification of R^1 and R^2 chemical parts. We have highlighted a structurally novel series of PDE7 inhibitors (Fig. 2) displaying low nanomolar



Scheme 2. Reagents and conditions: (a) $RCONH_2$, (R=Me or H), 1-methyl-2-pyrrolidinone, 200 °C; (b) methyl-1-methylhydrazine carbodithioate, DMF, TOTU, 0 °C; (c) TMSOTf, toluene, <40 °C; (d) cyclohexylamine, EtOH, reflux; (e) $SOCl_2$, toluene; (f) NH_3, 1,4-dioxane, Δ.

inhibitory activity and exhibiting high selectivity over PDE4. The findings from this study will be useful for further optimization of these newly discovered chemical leads, which will be the subject of additional reports in due course.

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13. All the compounds illustrated in this report were not selective for either enzyme subtype PDE7A or PDE7B. Consequently, only PDE7A inhibitory activities are presented.