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ARTICLE TYPE

Microwave-assisted synthesis of potent PDE7 inhibitors containing a novel thienopyrimidin-4-amine scaffold

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A series of novel thienopyrimidin-4-amines have been synthesized and evaluated as phosphodiesterase (PDE) inhibitors. A rationale for the observed selectivity against PDE7 has been obtained from molecular modelling studies on the most active compounds.

"To Alan R. Katritzky, In Memoriam"

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) comprise a group of metalloisoenzymes that cleave the phosphodiester bond in the ubiquitous cyclic nucleotide second messengers cAMP (cyclic adenosine 3´,5´-monophosphate) and cGMP (cyclic guanosine 3´,5´-monophosphate) to produce 5´-AMP or 5´-GMP, respectively. Since cAMP and cGMP mediate cellular responses to a wide variety of hormones and neurotransmitters in signal transduction pathways PDEs play a pivotal role in maintaining their cellular levels.[1] To date, 11 families and 21 genes of PDEs have been reported and all members contain a conserved catalytic domain of about 300 amino acids with ~25% sequence homology. However, each PDE family recognizes a specific substrate and is selectively inhibited by different classes of small molecules. Thus, the families of PDE4, 7, and 8 prefer to hydrolyze cAMP, whereas PDE5, 6, and 9 are cGMP-specific and PDE1, 2, 3, 10, and 11 accept both cAMP and cGMP as substrates (dual specificity PDEs). The key specificity determinant for recognizing the purine moiety present in cAMP or cGMP is the so-called 'glutamine (Q) switch', which consists of an invariant glutamine residue whose side orientation is fixed differently depending on the surrounding amino acids.[2]

On the other hand, in the structure of the dual-specific PDE1B key histidine residue has been suggested to enable the invariant glutamine to toggle depending on whether it binds cAMP or cGMP.

Taking into account the importance of cAMP and cGMP concentrations in the regulation of many biochemical pathways, PDEs are presently considered to be promising targets for drug therapy. As a consequence, selective PDE inhibitors already have or promise to have considerable therapeutic utility, mainly as anti-inflammatory agents, antiasthmatics, vasodilators, smooth muscle relaxants, cardiotonic agents, antidepressants, antithrombotics and agents for improving cognitive functions such as memory etc.[3] The known heterogeneity within this family of enzymes has allowed the synthesis of several highly selective inhibitors, which have demonstrated efficacy in a variety of disorders.[4] In this context, specific inhibitors of PDE7 have been reported and these include the benzene sulphonamide BRL 50481[5] and other heterocyclic derivatives containing quinazoline and spiroquinazoline units,[6] azole compounds such as ASB 16165,[7] a thienopyrazole product and purine and pyrimidine derivatives.[8] However, there are some indications that PDE7 may be a suitable target for treating inflammation in conjunction with inhibition of PDE4, since the PDE7 inhibitor BRL 50481 enhanced the effects of rolipram, a PDE4 inhibitor, on inhibiting lymphocyte proliferation and cytokine release. This synergistic effect, which has also been observed for ASB 16165, has led to the suggestion that PDE inhibitors targeting some combinations of PDE isozymes, such as PDE7/4, PDE7/3 or PDE7/4/3, could have greater clinical efficacy than those that are highly selective for PDE7 alone.[9]

Phosphodiesterases and chemical structure for PDEs inhibitors

The compact α-helical structure of PDEs consists of three subdomains at the junction of which a deep pocket lined with highly conserved residues makes up the active site. At its wider side there is a binuclear metal ion centre ('M pocket') in which a Zn^{2+} is coordinated by two His and two Asp residues and a Mg^{2+} is coordinated by one of these aspartates. The coordination sphere of Zn^{2+} is completed by two water molecules and that of Mg^{2+} by five water molecules, one of which bridges the two metal ions. At the narrow side of the active site is the 'Q pocket', which harbours the invariant purine-selective glutamine residue that also controls the orientation of inhibitor binding, and a 'hydrophobic clamp' (also known as the 'P clamp') made up by a pair of highly conserved amino acids. A number of crystal structures of the complexes formed between the catalytic domains of PDE4B,

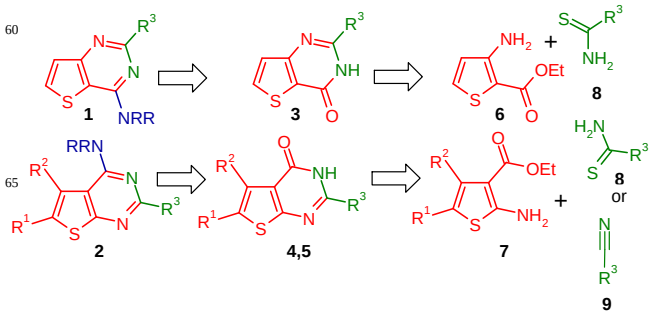
PDE4D, PDE5A and PDE7 and several inhibitors reveal different parts of these ligands sandwiched between these two hydrophobic amino acids.[10,11] A general scaffold can thus be defined for any given inhibitor based on the contacts within these two pockets plus additional interactions with a solvent-filled side pocket ('S pocket').[10] Selectivity is determined by the chemical nature of surrounding amino acids and the conformational variation allowed within each PDE active site, taking into account that the Q pocket can be further divided into two narrow but deep hydrophobic subpockets (Q_1 and Q_2) separated by a 'saddle' that is formed by the conserved glutamine and the P clamp. Given the less conserved nature of the residues in the two Q subpockets of different PDE family members, it is reasonable that these regions are effectively exploited in the search for isoform-selective PDE inhibitors. In this regard, it is worth mentioning that the pyridazinone oxygen of the PDE3- and PDE4-selective inhibitor zardaverine was found to coordinate directly with the Zn^{2+} cation in the predominant binding pose present in a complex with PDE4D2,[10] whereas in a previous cocrystal structure of the same ligand with the same PDE, the bound water molecule had not been displaced and the pyridazinone moiety did not point toward the M pocket.[12] This observation has to be taken as an indication of both the possibility of alternate binding modes for a given inhibitor, even under the same crystallization conditions, and the tightness of the water-Zn^{2+} interaction, which nevertheless allows for water-mediated hydrogen bonds with the ligands. Thus, other inhibitors, rather than coordinating directly to the Zn^{2+}, hydrogen bond to one of the water molecules in the second solvation shell of this metal ion, e.g. vardenafil, sildenafil and tadalafil in their complexes with PDE4B and PDE5A, or to a water in the coordination sphere of Mg^{2+}, e.g. the dichloropyridyl group of roflumilast, the pyridine nitrogen of piclamilast (IC_{50} values of 41 pM and 21 pM toward PDE4B and PDE4D, respectively), and the carboxylate oxygens of cilomilast.[10] Taking into account these considerations, and in line with previous work reported by Gotanda et al.,[13] Castro et al.[14] and Banerjee et al.,[15] we were particularly interested in ascertaining whether the fused aminopyrimidines thieno[3,2-*d*]pyrimidin-4-amines **1** and substituted thieno[2,3-*d*]pyrimidin-4-amines **2**, especially with a cyano substituent in R^1 (Scheme 1), could serve as scaffolds for the development of novel PDE7 inhibitors. In addition, we are keen to examine a possible secondary action of these molecules as PDE3/4 inhibitors. The work described here concerns our preliminary efforts towards achieving these goals.[16]

Results

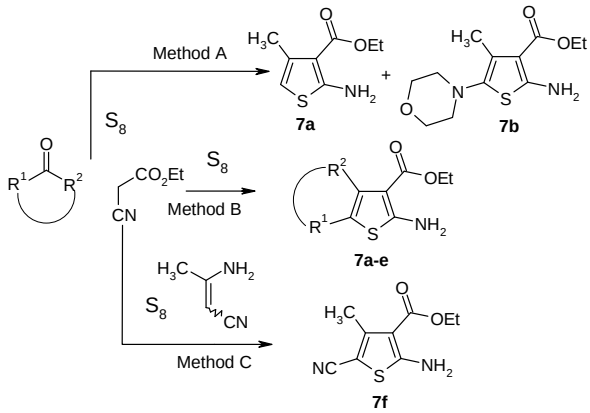
Chemistry

The general strategy for the preparation of libraries of thieno[3,2-*d*]pyrimidin-4-amines **1** and thieno[2,3-*d*]pyrimidin-4-amines **2** from the corresponding aminothiophenes **6** and **7** and thioamides **8** or substituted nitriles **9** is outlined in Scheme 1. This sequence is based on the efficient preparation of substituted thienopyrimidinones **3**–**5**, which in most cases was carried out under microwave irradiation with samples processed in a parallel manner, whereas starting materials **6**–**8** were obtained by adaptating previously reported methodologies.

Scheme 1 General strategy for the preparation of **1** and **2**



Preparation of starting thiophenes 7

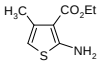
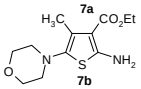
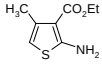
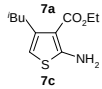
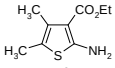
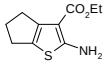
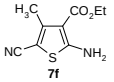


Scheme 2 Preparation of starting thiophenes **7**

Whereas ethyl 3-amino-2-thiophene-2-carboxylate **6** is a commercial product and was used as the solestarting material for the preparation of thienopyrimidinones **3**, aminothiophenes **7** were synthesized using a modified Gewald procedure.[17] Thus, a mixture of acetone (R^1, R^2 = Me, 1 equiv), ethyl cyanoacetate (1 equiv), allotropic sulfur S_8 (1−1.1 equiv) and morpholine, with ethanol as the solvent, was heated under reflux for 4 h (Method A). This reaction gave a complex mixture from which we were able to isolate the thiophene derivative **7a** (50% yield), together with a by-product identified as the 5-morpholine derivative **7b** (21% yield) (Table 1, entries 1 and 2). A related process was described previously by Pinto et al.[18] for the preparation of a series of novel serine protease inhibitors. Taking into consideration the large quantities of thiophene templates required, we focused our efforts on a search for the best conditions for this reaction in order to avoid the formation of by-products. Initial attempts to modify the number of reagent equivalents, amine base or the number of base equivalents did not affect the course of the reaction. On using the process developed by McKibben et al.,[19] in which pyridine was used as the solvent in the presence of diethylamine at room temperature, a slower reaction rate (48 h versus 4 h) was observed but the overall yield increased substantially. The reaction was also cleaner and the isolation of the pure compounds was less tedious (Method B). Several ketones were used as starting materials, including alkyl and cycloalkyl ketones (Table 1, entries 3−5 and 6, respectively). The

best results were obtained for tetra-substituted thiophenes **7d** and **7e**. On the assumption that a CN group in the R^1 position could lead to a higher affinity for the target, compound **7f** was prepared. However, in this case the best results were obtained starting from 3-aminocrotononitrile (1 equiv), rather than the corresponding ketone, along with ethyl cyanoacetate (1 equiv), allotropic sulfur S_8 (1−1.1 equiv) and piperidine. The mixture was heated under reflux for 24 h using ethanol as the solvent (Method C, Table 1, entry 8).[20] The structures of the 2-aminothiophenes **7** prepared for this project and the results obtained using these methods are shown in Scheme 2 and Table 1.

Table 1 Result in the preparation of starting thiophenes **7**

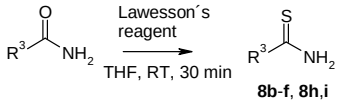
Entry	Ketone R^1	Ketone R^2	Method	**7**	Yield
1	Me	Me	A	 **7a**	50
2	Me	Me	A	 **7b**	21
3	Me	Me	B	 **7a**	65
4	Me	^{i}Bu	B	 **7c**	35
5	Et	Me	B	 **7d**	83
6	-$(CH_2)_4$-		B	 **7e**	60
8	-		C	 **7f**	70

Method A: Corresponding ketone (1 equiv), ethyl cyanoacetate (1 equiv), allotropic sulfur $\mathbf{S_8}$ (1−1.1 equiv), morpholine in EtOH, reflux 4 h; Method B: Corresponding ketone (1 equiv), ethyl cyanoacetate (1 equiv), allotropic sulfur $\mathbf{S_8}$ (1−1.1 equiv), diethylamine in pyridine, RT, 48 h; Method C: 3-Aminocrotononitrile (1 equiv), ethyl cyanoacetate (1 equiv), allotropic sulfur $\mathbf{S_8}$ (1−1.1 equiv), piperidine in EtOH, reflux 24 h.

Preparation of starting thioamides 8

The thioamides used as starting materials for the construction of thieno[3,2-*d*]pyrimidin-4-amines **1** and thieno[2,3-*d*]pyrimidin-4-amines **2** are outlined in Scheme 3. Thioacetamide (R^3 = CH_3, **8a**) and thiobenzamide (R^3 = Ph, **8g**) are commercial products. Other thioamides (**8b**−**8f** and **8h**,**i**) can be obtained, using described methods,[21] from the corresponding amide by reaction with Lawesson´s reagent.

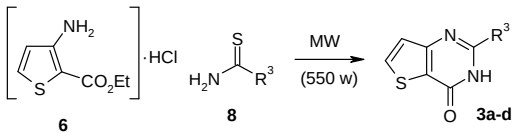
Scheme 3 Preparation of starting thioamides **8**



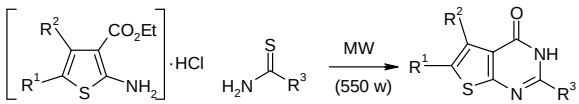
8b-f, **8h**,**i**

8a, R^3 = Me
8b, R^3 = Et **8f**, R^3 = Cyclohexyl
8c, R^3 = ^{i}Pr **8g**, R^3 = Ph
8d, R^3 = Bu **8h**, R^3 = CH_2Ph
8e, R^3 = ^{t}Bu **8i** R^3 = $(CH_2)_2$Ph

Preparation of substituted thienopyrimidinones 3a–d and 4a–q using a microwave-assisted parallel synthesis procedure



6 **8** **3a-d**



7a-e **8** **4a-q**

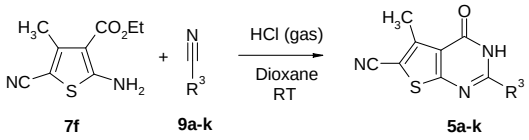
Scheme 4 and Table 2 Preparation of thienopyrimidinones **3a–d** and **4a–q** using microwave-assisted parallel synthesis

Entry	R^1	R^2	R^3	Compound	Yield
1	H	H	Me	**3a**	97
2	H	H	Et	**3b**	76
3	H	H	Bu	**3c**	64
4	H	H	$PhCH_2$-	**3d**	77
5	H	Me	Me	**4a**	48
6	H	Me	Et	**4b**	56
7	H	Me	Bu	**4c**	67
8	H	Me	Ph	**4d**	63
9	H	Me	$PhCH_2$-	**4e**	94
10	H	^{i}Bu	Cyclohexyl	**4f**	81
11	H	^{i}Bu	Ph	**4g**	98
12	H	^{i}Bu	$PhCH_2$-	**4h**	61
13	H	^{i}Bu	$Ph(CH_2)_2$-	**4i**	67
14	Me	Me	Et	**4j**	63
15	Me	Me	^{i}Pr	**4k**	37
16	Me	Me	^{t}Bu	**4l**	13
17	Me	Me	Cyclohexyl	**4m**	81
18	Me	Me	Ph	**4n**	23
19	Me	Me	$PhCH_2$-	**4o**	28
20	-$(CH_2)_4$-		Bu	**4p**	50
21	-$(CH_2)_4$-		$PhCH_2$-	**4q**	55

The distinctive properties of microwave heating offer unique opportunities for medicinal chemists to speed up lead optimization processes in early drug discovery. The technology is ideal for small-scale discovery chemistry because it allows full reaction control, short reaction times, high safety and rapid feedback.[22] Ideally, to obtain a well-defined heating pattern, a microwave apparatus with a 'single-mode' cavity is recommended as this allows uniform heating, a factor that is very important in organic chemistry to achieve higher reproducibility, predictability of results and hence optimization of yields. Although equipment costs have dropped dramatically in recent years, they have not yet fallen to a point where microwave instrumentation is viewed as a standard piece of laboratory equipment. As an inexpensive alternative,[23a] we previously described a device suitable for a domestic microwave oven that consists of a Teflon disk (5 mm thick) capable of holding up to 28 vials (20 mm diameter), which is placed in the maximum irradiation area of the oven. The calibration of the energy received by the samples was carried out as described.[23b] The device allows the rapid parallel synthesis of small chemical libraries.[24] In this context, the preparation of thienopyrimidin-4-ones, which usually requires very vigorous reaction conditions and prolonged reaction times, can be achieved starting from the corresponding thioamides **8**, amino-thiophene-carboxylates **6** and **7**, as hydrochlorides, without solvent and under microwave irradiation. In a typical general procedure, twenty 4 mL open vials containing a homogenized mixture of the corresponding thioamide **8** (3 mmol) and amino-thiophene-carboxylates **6** and **7**, as hydrochlorides (1 mmol), were inserted into a Teflon disc and irradiated at 550 Watts power for 3.5 minutes (time related to the number of samples). Under these conditions melting was observed along with the production of gas. Reactions were treated in parallel using a VacMaster Station SPE to yield the corresponding thienopyrimidin-4-ones **3** and **4**. Compounds **3** and **4** proved to be difficult to purify by chromatography due to their limited solubility and by crystallization due to co-precipitation of inorganic materials. For this reason, the subsequent reactions were successfully performed on semi-pure materials and thereafter the final products were rigorously purified. The results obtained for compounds **3** and **4** using this methodology are shown in Scheme 4 and Table 2.

Preparation of thienopyrimidin-4-ones-6-cyano substituted 5 in the presence of HCl gas

The microwave heating methodology was very efficient for the construction of thienopyrimidinones **3a–d** and **4a–q**. However, when we attempted to use this process on the aminothiophene **7f**, which bears a carbonitrile moiety on the R^1 position, only starting material was recovered. This behaviour could be attributed to the low nucleophilicity of the amino group, which in this case is conjugated with the carbonitrile function. It was then decided to use the method described by Shishoo et al.,[25] which involves bubbling dry HCl through a stirred solution of **7f** (1 equiv) and the corresponding benzonitrile **9** (1.5 equiv) in dioxane at room temperature for 2−3 hours (until saturation), and then stirring the mixture at room temperature for a further 12 h period. This procedure (Scheme 5) yielded the corresponding thienopyrimidinones **5a–k** (Table 3), which were simply isolated and purified by trituration with diethyl ether.



5a, R^3 = Ph
5b, R^3 = 4-CH_3Ph
5c, R^3 = 4-CH_3OPh
5d, R^3 = 4-ClPh
5e, R^3 = 4-NO_2Ph
5f, R^3 = 4-CF_3Ph
5g, R^3 = 3,4-diCH_3OPh
5h, R^3 = 3,4-OCH_2OPh
5i, R^3 = 3,4,5-triCH_3OPh
5j, R^3 = 3,4-diCH_3OPhCH_2-
5k, R^3 = 3,4,5-triCH_3OPhCH_2-

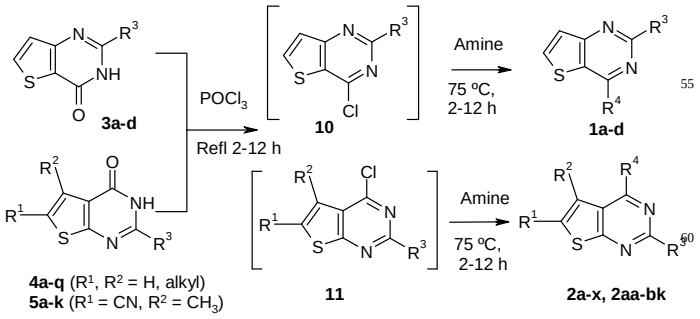
Scheme 5 and **Table 3** Preparation of thienopyrimidin-4-ones-6-cyano substituted **5** in the presence of HCl gas

Entry	R^1	R^2	R^3	5	Yield
1	CN	Me	Ph	**5a**	92
2	CN	Me	4-CH_3Ph	**5b**	72
3	CN	Me	4-CH_3OPh	**5c**	97
4	CN	Me	4-ClPh	**5d**	84
5	CN	Me	4-NO_2Ph	**5e**	47
6	CN	Me	4-CF_3Ph	**5f**	81
7	CN	Me	3,4-diCH_3OPh	**5g**	98
8	CN	Me	3,4-OCH_2OPh	**5h**	22
9	CN	Me	3,4,5-triCH_3OPh	**5i**	63
10	CN	Me	3,4-diCH_3OPhCH_2-	**5j**	47
11	CN	Me	3,4,5-triCH_3OPhCH_2-	**5k**	69

Preparation of libraries of thieno[3,2-*d*]pyrimidin-4-amines 1 and thieno[2,3-*d*]pyrimidin-4-amines 2

The general strategy for preparation of compounds **1a–d**, **2a–x** and **2aa–bk** (with a cyano substituent in R^1) from the corresponding **3–5** is outlined in Scheme 6. This sequence is based on the efficient preparation of chloro derivatives **10** and **11**, which were obtained by reaction with phosphorus oxychloride under reflux. These intermediates were not isolated and were directly reacted with the corresponding amines to furnish the final products. Thus, this stage consisted of a nucleophilic substitution of chloride by a range of cyclic and acyclic amines, proceeding in a parallel manner, by heating a solution of the corresponding chloro derivatives **10**, **11** and the amine (1.3 equiv) in ethanol and 25-mL Duran® bottles with polypropylene caps (purchased from Aldrich) inside a Heraeus T5060 oven were used to perform nucleophilic substitutions in parallel after testing that they withstood, without undergoing explosion or any liquid loss, 90 ºC for 24 h when filled with 12 mL of water. For the experiments described they were used at temperatures between 50-75 ºC,

without stirring for 12 h, and no problems were observed. The results for compounds **1** and **2** obtained using this methodology are shown in Scheme 6 and Tables 4 and 5.



Scheme 6 and **Table 4** Results from the preparation of **1a–d** and **2a–x** and *in vitro* evaluation of PDE7 inhibition

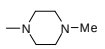
Entry	R^1	R^2	R^3	R^4	**1,2**	Yield (%)	PDE7 IC_{50} (μM) or (10)%*
1	H	H	Me		**1a**	85	200
2	H	H	Et		**1b**	84	120
3	H	H	Bu		**1c**	86	71
4	H	H	$PhCH_2$-		**1d**	9	58
5	H	Me	Me		**2a**	43	82
6	H	Me	Et		**2b**	14	78
7	H	Me	Bu		**2c**	45	67
8	H	Me	Ph		**2d**	63	22
9	H	Me	$PhCH_2$-		**2e**	82	62
10	H	Me	$PhCH_2$-		**2f**	32	(10)4.1*
11	H	Me	$PhCH_2$-		**2g**	18	150
12	H	^{i}Bu	Cyclohexyl		**2h**	45	(10)7.5*
13	H	^{i}Bu	Ph		**2i**	12	(10)12.7*
14	H	^{i}Bu	$PhCH_2$-		**2j**	63	(10)1.4*
15	H	^{i}Bu	$Ph(CH_2)_2$-		**2k**	44	(10)2.7*
16	H	^{i}Bu	Ph		**2l**	12	(10)5.1*
17	H	^{i}Bu	$PhCH_2$-		**2m**	57	(10)5.8*
18	H	^{i}Bu	$Ph(CH_2)_2$-		**2n**	54	(10)7.5*

Table 4 (cont)

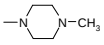
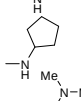
Entry	R^1	R^2	R^3	R^4	**2**	Yield (%)	PDE7 IC_{50}(μM) or (10)%*
19	Me	Me	Et		**2o**	63	19
20	Me	Me	^{i}Pr		**2p**	24	27
21	Me	Me	^{t}Bu		**2q**	26	12.9%*
22	Me	Me	Cyclohexyl		**2r**	18	29.5%*
23	Me	Me	Ph		**2s**	18	25.3%*
24	Me	Me	$PhCH_2$-		**2t**	30	22
25	Me	Me	$PhCH_2$-		**2u**	10	4.2%*
26	-$(CH_2)_4$-		Bu		**2v**	69	32
27	-$(CH_2)_4$-		Bu		**2w**	71	13.5%*
28	-$(CH_2)_4$-		$PhCH_2$-		**2x**	18	8.4

*% inhibition at 10 μM

Table 5 Results from the preparation of **2aa–bq** and *in vitro* evaluation of PDE7 inhibition

Entry	R^3	R^4	**2**	Yield (%)	PDE7 IC_{50} (μM) or (10)%*
1	Ph		**2aa**	58	1.8
2	Ph		**2ab**	52	2.9
3	Ph		**2ac**	40	9.0%*
4	Ph		**2ad**	33	5.9%*
5	Ph		**2ae**	52	0.42
6	4-CH_3Ph		**2af**	92	1.1
7	4-CH_3Ph		**2ag**	32	0.49
8	4-CH_3OPh		**2ah**	55	0.31
9	4-CH_3OPh		**2ai**	46	6.9
10	4-CH_3OPh		**2aj**	40	20.4%*
11	4-CH_3OPh		**2ak**	23	1
12	4-CH_3OPh		**2al**	44	0.24

Table 5 (cont)

Entry	R^3	R^4	2	Yield (%)	PDE7 IC_{50} (μM) or (10)%*
13	4-CH_3OPh		**2am**	23	0.26
14	4-CH_3OPh		**2an**	43	0.65
15	4-ClPh		**2ao**	69	3.3
16	4-ClPh		**2ap**	45	1.2
17	4-NO_2Ph		**2aq**	24	12.7%*
18	4-NO_2Ph		**2ar**	57	0.34
19	4-NO_2Ph		**2as**	44	6.1
20	4-CF_3Ph		**2at**	35	38.7%*
21	4-CF_3Ph		**2au**	33	1.3
22	3,4-diCH_3OPh		**2av**	47	0.037
23	3,4-diCH_3OPh		**2aw**	22	0.028
24	3,4-OCH_2OPh		**2ax**	57	0.33
25	3,4-OCH_2OPh		**2ay**	39	0.17
26	3,4,5-triCH_3OPh		**2az**	95	0.0046
27	3,4,5-triCH_3OPh		**2ba**	63	0.002
28	3,4,5-triCH_3OPh		**2bb**	36	0.18
29	3,4,5-triCH_3OPh		**2bc**	44	0.0017
30	3,4,5-triCH_3OPh		**2bd**	33	0.13
31	3,4,5-triCH_3OPh		**2be**	26 (40)**	0.0051
32	3,4-diCH_3OPhCH_2-		**2bf**	60	1.3
33	3,4-diCH_3OPhCH_2-		**2bg**	80	0.0046
34	3,4-diCH_3OPhCH_2-		**2bh**	39	0.039
35	3,4,5-triCH_3OPhCH_2-		**2bi**	82	0.0018
36	3,4,5-triCH_3OPhCH_2-		**2bj**	80	0.009
37	3,4,5-triCH_3OPhCH_2-		**2bk**	82	0.0062

*%inhibition at 10 μM

4-aminopyridine, NaH, and **11 in dry DMF, rt for 3 h.

Under optimal conditions, substituted thienopyrimidinones **3a–c** reacted in the first instance in the presence of phosphorus oxychloride and later in the presence of morpholine (used as a model amine) to give thieno[3,2-*d*]pyrimidin-4-amines **1a–c** in excellent yields (entries 1–3, Table 4). However, the application of these conditions to **3d** (entry 4, Table 3) gave 1d in only 9% yield. This process was applied to thienopyrimidinones **4a–q** with variable results. In general, yields appear to be better in the presence of morpholine but the nature of substituent in R^1–R^3 must be taken into account. Variable results were also obtained with thienopyrimidinones **5a**–**k**. These compounds bear a cyano substituent in R^1 and this could favour the nucleophilic substitution of chloride by the corresponding amine (2nd stage, Scheme 6). However, this favourable situation could be counteracted by a slight tendency to form the halogenated derivative **11** in the presence of phosphorus oxychloride. In general, better results were obtained in the reaction with primary amines (for example, entries 26, 27, 32, 33 and 35−37, Table 5) whereas the poor results obtained on starting from 4-aminopyridine (entry 31, Table 5) can be attributed to the lower nucleophilicity of the exocyclic nitrogen in this kind of heterocyclic derivative. For **2be**, better results were obtained by reaction of 4-aminopyridine in the presence of NaH in DMF and halogenated derivative **11** for 2 h, at room temperature (entry 31, Table 5 and ESI).

Biological and molecular modelling results

The results of the *in vitro* evaluation of the synthesized compounds **1** and **2** as PDE7 inhibitors are given in Tables 4 and 5. For the most active compounds the IC_{50} in μM is given whereas for derivatives with low activity only the % inhibition at 10 μM is displayed.

Thieno[3,2-*d*]pyrimidin-4-morpholine derivatives **1a**–**1d**, with R^1, R^2 = H and R^4 = morpholine, were relatively poor PDE7 inhibitors with IC_{50} values in the range 58−200 μM (entries 1−4, Table 4) and with the best value obtained for **1d**, with R^3 = benzyl. Similar unsatisfactory results were obtained for thieno[2,3-*d*]pyrimidin-4-amines **2a**–**x**. The presence of bulkier substituents at R^2, e.g. isobutyl, is associated with a decrease in activity and thus this moiety limits the activity in the set **2h**–**n** (entries 12−18, Table 4). The most active compounds in this series are **2d**, **2o** and **2t** (entries 8, 19 and 24, respectively), all of them morpholine or methylpiperazine derivatives with R^1 and R^2 = H and/or Me and R^3 = Ph, Et or $PhCH_2$, respectively. But since other compounds, such as **2p** (entry 20, Table 4), again a morpholine derivative, with R^1 and R^2 = Me and R^3 = isopropyl, also displays modest values as a PDE7 inhibitor, no significant differences can be attributed to the substitution pattern in these positions. Likewise, the presence of a fused ring on R^1–R^2 does not seem to lead to enhanced inhibition (cf. **2v** and **2w**; entries 26 and 27) whereas an improvement is detected for **2x**, which is endowed with a fused structure and a methylpiperazine in R^4.

On the other hand, much better PDE7 inhibition results were obtained for the series **2aa**–**2bk**, with R^1 = CN and R^2 = Me. Molecular modelling studies were then carried out to examine the molecular basis of PDE7 inhibitory activity for these compounds.

In our models, the thienopyrimidine scaffold is placed in the Q pocket of PDE7 with the cyano group at position R^1 pointing to the M pocket, where it is able to interact directly with the Zn^{2+} cation by displacing a coordinating water molecule. This proposal is consistent with the considerable boost in inhibitory potency in this series brought about by incorporation of this moiety (Table 5), which we identified as the most important activity cliff in this structure-activity landscape (see below).[26] In this orientation, the methyl group at position R^2 is located in the Q_1 subpocket and the substituent at position R^4 is clamped by the side chains of Val380 and Phe416 inside the large Q_2 subpocket, which allows some of these compounds, e.g. **2be** and **2bc** (Table 5, entries 31 and 29, respectively), to establish direct hydrogen bonding interactions with the carboxamide of Gln413 (the 'Q switch') and lower their IC_{50} values. Finally, the substituents at position R^3 extend out of the Q pocket into the solvent so that the phenyl ring makes van der Waals contact with the side chain of Leu401 whereas the more favourable benzyl moiety, as in **2bk**, has the phenyl ring stacked on the side chain of Leu420 and the *meta*-methoxy substituent interacting with Leu401 (Figure 1).

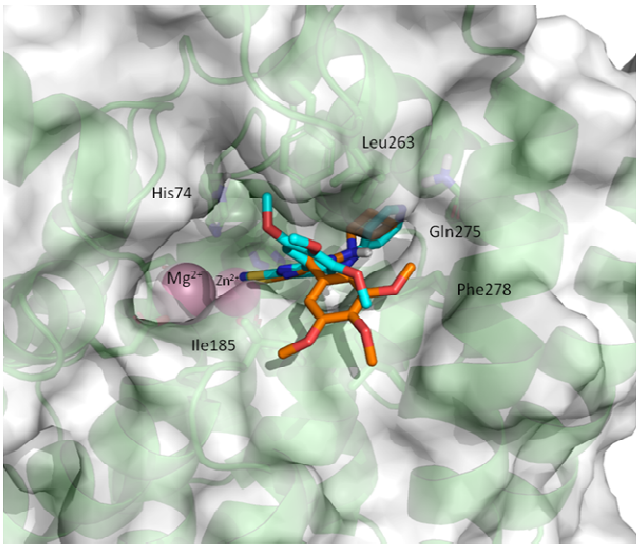
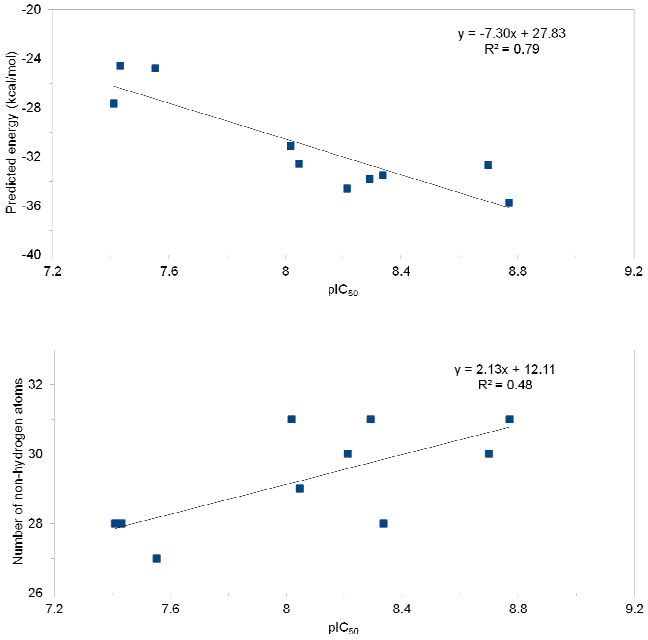


Figure 1. Detail of the binding mode proposed for **2be** (represented as sticks with C atoms coloured in cyan) and **2bk** (represented as sticks with C atoms coloured in orange) in the active site of hPDE7 (green ribbon and lines). Metal ions are shown as spheres and water molecules are not displayed for the sake of clarity.

Structure-activity relationships

To validate the proposed binding mode we used the ChemScore function,[27] as implemented in the CRDOCK suite,[28] to calculate the binding affinities in a reduced set of modelled complexes containing the most potent inhibitors. When we compared these scores with the experimental pIC_{50} values (Figure 2) the Pearson correlation coefficient (r^2 = 0.79) was significantly better than when only the number of non-hydrogen atoms per ligand was used (r^2 = 0.48).

Figure 2 ChemScore



The structure-activity relationship (SAR) space was also explored within the SAR Index (SARI) framework.[29] When systematic topological comparisons using MACCS fingerprints were applied to SMILES string representations of the whole series of compounds and the potencies were expressed as pIC_{50} values, a SARI of 0.39 was obtained with continuity and discontinuity scores of 0.42 and 0.64, respectively. These numerical parameters reveal a SAR landscape with activity cliffs[26] and provide a quantitative estimate for the observations reported above. In fact, Figure 3 confirms that very similar compounds (Tanimoto scores $\geq$ 0.9) can have differences in inhibitory activities of up to 2 log units. These cliffs are mostly due to the dramatic increase in affinity brought about by the cyano moiety at position R^1, which is proposed to coordinate directly to Zn^{2+}, and to the beneficial effect of incorporating the three methoxy substituents in the phenyl ring at position R^3.

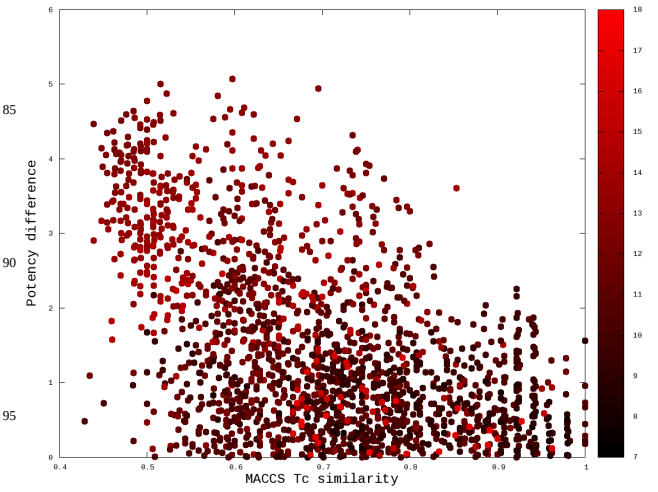


Figure 3

2a (Table 4, entry 5) vs. **2af** (Table 5, entry 6): CN + change at R^3
2f (Table 4, entry 10) vs. **2g** (Table 4, entry 11): NH → NCH_3 at R^4
2a (Table 4, entry 5) vs. **2at** (Table 5, entry 20): CN + change at R^3
2at (Table 5, entry 20) vs. 2av (Table 5, entry 22): CF_3 at R^3 → $diOCH_3$

Structural basis for the selectivity

The results of the in vitro evaluation as PDE3 and PDE4 inhibitors for the most potent compounds as PDE7 inhibitors (**2az**, **2ba**, **2bc**, **2be** and **2bg**–**bk**) are given in Table 6. For clarity, Table 6 displays in the first column the values for PDE7 inhibition. Once again, for the most active compounds the IC_{50} in µM is given whereas for derivatives with low activity only the % inhibition at 10 µM is displayed.

According to the proposed model, the selectivity of these compounds for PDE7 over PDE4 and PDE3 can be ascribed to the groups at positions R^3 and R^4. Amino acid substitutions in the residues lining the Q pocket and the hydrophobic clamp are common in all members of the PDE family except for the strictly conserved Phe residue (Phe416 in PDE7). Despite the fact that all of these residues are hydrophobic in nature, their varying sizes modify both the shapes and the ceilings of the Q_2 subpocket, where the R^3 and R^4 substituents are proposed to be located.

To illustrate this, we rationalized the inhibitory profiles of **2bh** and **2be**. The former compound contains a propargyl substituent on the amine at position R^4 and inhibits PDE4 poorly even though it is a nanomolar inhibitor for PDE3 and PDE7 (Table 6, entry 6). The rigidity imposed by the triple bond forces this moiety to be accommodated directly perpendicular to the Q_2 subpocket. In the case of PDE7A and PDE3, the wall of the cavity is made up by the side chains of Ile412 and Leu987, respectively, while in PDE4 the positionally equivalent residue is Ser368. We suggest that it is the decrease in hydrophobicity brought about by the hydroxyl group present in the side-chain of this amino acid that is detrimental for the affinity towards PDE4 because of the high desolvation penalty that accompanies complex formation. Compound **2bh** thus appears to be a candidate for a dual PDE7/3 inhibitor, which may be suitable when inhibition of these two isoforms is needed to achieve therapeutic benefit.[9] In the case of **2be**, which inhibits PDE7 at far lower concentrations than those necessary for equivalent inhibition of PDE3 and PDE4 (Table 6, entry 4), we note that the side-chain carboxylate of Glu407 is hydrogen bonded to the peptide backbone NH of Leu401, the side chain of which then packs against that of Ile412 and interacts closely with the trimethoxyphenyl substituent at position R^3. In PDE3 and PDE4 the position of this isoleucine is occupied by either Phe976 or Met357, respectively, and no glutamate (replaced by either Pro982 or Ala363) stabilizes the conformation of this loop so that the interaction of the R^3 substituent with this residue is most likely lost.

Table 6 *In vitro* evaluation of selected PDE7 inhibitors over PDE3 and PDE4

Entry	**2**	PDE7 IC_{50} (µM) or (10)%*	PDE3 IC_{50} (µM) or (10)%*	PDE4 IC_{50} (µM) or (10)%*
1	**2az**	0.0046	10.1%*	20.0%*
2	**2ba**	0.002	20.0%*	7.0%*
3	**2bc**	0.0017	2.8	26.1%*
4	**2be**	0.0051	31.4%*	32.4%*
5	**2bg**	0.0046	31.4	54.3%*
6	**2bh**	0.039	0.032	14
7	**2bi**	0.0018	10	6.7
8	**2bj**	0.009	9.6	44.6%*
9	**2bk**	0.0062	16	9.5

*% inhibition at 10 µM

Similar considerations can be formulated for **2az** and **2ba** (Table 6, with values for *in vitro* evaluation of the IC_{50} in µM as PDE7 inhibitors of 0.0046 and 0.002, entries 1 and 2, respectively), all of which have a trimethoxyphenyl substituent at position R^3 and very low activity for the inhibition of PDE3 and 4. This selectivity profile makes these compounds the molecules of choice for further development as selective PDE7 inhibitors.

Conclusions

A novel series of thieno[3,2-*d*]pyrimidin-4-amines and thieno[2,3-*d*]pyrimidin-4-amines have been synthesized from thienopyrimidinones. The target compounds were prepared in most cases under microwave irradiation and processed in a parallel manner. These compounds have been tested *in vitro* as PDE7 inhibitors. For the most active compounds, corresponding to 4-aminothieno[2,3-*d*]pyrimidine-6-carbonitrile derivatives, molecular modelling studies provided a rationale for the improved PDE7 inhibitory activity and their selectivity over PDE3 and PDE4 isoforms. The selectivity profile of **2az**, **2ba** and **2be**, all of which have a trimethoxyphenyl substituent on the pyrimidine ring, makes these compounds the molecules of choice for further developments as selective PDE7 inhibitors. On the other hand, **2bh** containing a propargyl substituent on the amine position and a dimethoxybenzyl group on the pyrimidine position is a nanomolar inhibitor of PDE7 and PDE3 but inhibits PDE4 poorly. Therefore this compound stands out as a good candidate for a dual PDE7/3 inhibitor, which may be suitable when inhibition of these two isoforms is needed to achieve a full therapeutic effect.

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Notes and references

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1 M. Conti and J. Beavo, *Annu. Rev. Biochem.*, 2007, **76**, 481.
2 K. Y. Zhang, G. L. Card, Y. Suzuki, D. R. Artis, D. Fong, S. Gillette, D. Hsieh, J. Neiman, B. L. West, C. Zhang, M. V. Milburn, S. H. Kim, J. Schlessinger and G. Bollag, *Mol. Cell.*, 2004, **15**, 279.
3 C. Lugnier, *Pharmacol. Ther.*, 2006, **109**, 366.
4 M. Redondo, J. G. Zarruk, P. Ceballos, D. I. Perez, C. Perez, A. Perez-Castillo, M. A. Moro, J. Brea, C. Val, M. I. Cadavid, M. I. Loza, N. E. Campillo, A. Martínez and C. Gil, *Eur. J. Med. Chem.*, 2012, **47**, 175.
5 S. J. Smith, L. B. Cieslinski, R. Newton, L. E. Donnelly, P. S. Fenwick, A. G. Nicholson, P. J. Barnes, M. S. Barnette and M. A. Giembycz, *Mol. Pharmacol.*, 2004, **66**, 1679.
6 A. I. Sanchez, V. Martinez-Barrasa, C. Burgos, J. J. Vaquero, J. Alvarez-Builla, E. Terricabras and V. Segarra, *Bioorg. Med. Chem.*, 2013, **21**, 2370; E. Lorthiois, P. Bernardelli, F. Vergne, C. Oliveira, A.-K. Mafroud, E. Proust, L. Heuze, F. Moreau, M. Idrissi, A. Tertre, B. Bertin, M. Coupe, R. Wrigglesworth, A. Descours, P. Soulard and P. Berna, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 4623.
7 K. Kadoshima-Yamaoka, M. Murakawa, M. Goto, Y. Tanaka, H. Inoue, H. Murafuji, A. Nagahira, Y. Hayashi, K. Nagahira, K. Miura, T. Nakatsuka, K. Chamoto, Y. Fukuda and T. Nishimura, *Immunol. Lett.*, 2009, **122**, 193.
8 C. Gil, N. E. Campillo, D. I. Perez and A. Martinez, *Expert Opin. Ther. Patents*, 2008, **18**, 1127.
9 H. Dong, C. Zitt, C. Auriga, A. Hatzelmann and P. M. Epstein, *Biochem. Pharmacol.*, 2010, **79**, 321.
10 G. L. Card, B. P. England, Y. Suzuki, D. Fong, B. Powell, B. Lee, C. Luu, M. Tabrizizad, S. Gillette, P. N. Ibrahim, D. R. Artis, G. Bollag, M. V. Milburn, S. H. Kim, J. Schlessinger and K. Y. Zhang, *Structure*, 2004, **12**, 2233.
11 T. Castano, H. Wang, N. E. Campillo, S. Ballester, C. Gonzalez-Garcia, J. Hernandez, C. Perez, J. Cuenca, A. Perez-Castillo, A. Martinez, O. Huertas, J. L. Gelpi, F. J. Luque, H. Ke and C. Gil, *ChemMedChem*, 2009, **4**, 866.
12 M. E. Lee, J. Markowitz, J. O. Lee and H. Lee, *FEBS Lett.*, 2002, **530**, 53.
13 K. Gotanda, A. Shinbo, Y. Nakano, H. Kobayashi, M. Okada and A. Asagarasu, *PCT Int. Appl.*WO 2006135080 A1 2006.
14 A. Castro, M. J. Jerez, C. Gil, F. Calderon, T. Domenech, A. Nueda and A. Martinez, *Eur. J. Med. Chem.*, 2008, **43**, 1349.
15 M. Safavi, M. Baeeri and M. Abdollahi, *Expert Opin.Drug Discov.* 2013, **8**, 733; A. Banerjee, P. S. Yadav, M. Bajpai, R. R. Sangana, S. Gullapalli, G. S. Gudi and L. A. Gharat, *Bioorg. Med. Chem.Lett.*, 2012, **22**, 3223.
16 A part of this work was previously described in E. Terricabras, V. M. Segarra Matamoros, J. Alvarez-Builla, J. J. Vaquero and J. M. Minguez, *PCT Int. Appl.*, WO 2004065391 A1, 2004.
17 K. Gewald, E. Schinke and H. Boettcher, *Chem. Ber.*, 1966, **99**, 94; K. Gewald, *Chem. Ber.*, 1965, **98**, 3571.
18 I. L. Pinto, R. L. Jarvest and H. T. Serafinowska, *Tetrahedron Lett.*, 2000, **41**, 1597.
19 B. P. McKibben, C. H. Cartwright and A. L. Castelhano, *Tetrahedron Lett.*, 1999, **40**, 5471.
20 M. H. Elnagdi and A. W. Erian, *Liebigs Ann. Chem.*, 1990, 1215.
21 O. E. Jensen, S. O. Lawesson, R. Bardi, A. M. Piazzesi and C. Toniolo, *Tetrahedron*, 1985, **41**, 5595; M. P. Cava and M. I. Levison, *Tetrahedron* 1985, **41**, 5061; B. Yde, N. M. Yousif, U. Pedersen, I. Thomsen and S. O. Lawesson, *Tetrahedron*, 1984, **40**, 2047.
22 J. Gising, L. R. Odell and M. Larhed, *Org. Biomol. Chem.*, 2012, **10**, 2713.
23 a) B. M. Barchin, A. M. Cuadro and J. Alvarez-Builla, *Synlett*, 2002, 343. b) K. W. Watkins, *J. Chem. Ed.*, 1983, **60**, 1043.
24 Parallel synthesis in microwave ovens has been described to prepare thioamide and pyridine libraries: R. Olsson, H. C. Hansen and C. M. Andersson, *Tetrahedron Lett.*, 2000, **41**, 7947.
25 K. Dave, C. J. Shishoo, M. B. Devani, R. Kalyanaraman, S. Ananthan, G. V. Ullas and V. S. Bhadti, *J. Heterocycl. Chem.*, 1980, **17**, 1497.
26 R. Guha and J. H. Van Drie, *J. Chem. Inf. Model*, 2008, **48**, 646.
27 M. D. Eldridge, C. W. Murray, T. R. Auton, G. V. Paolini and R. P. Mee, *J. Comput. Aided Mol. Des.*, 1997, **11**, 425.
28 A. Cortes Cabrera, J. Klett, H. G. Dos Santos, A. Perona, R. Gil-Redondo, S. M. Francis, E. M. Priego, F. Gago and A. Morreale, *J Chem. Inf. Model*, 2012, **52**, 2300.
29 L. Peltason and J. Bajorath, *J. Med. Chem.*, 2007, **50**, 5571.