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Spiroquinazolinones as novel, potent, and selective PDE7 inhibitors. Part 2: Optimization of 5,8-disubstituted derivatives

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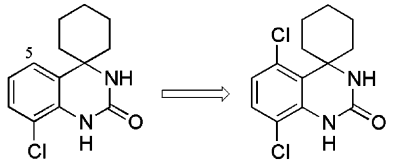
Abstract—The optimization of 5,8-disubstituted spirocyclohexane-quinazolinones into potent, selective, soluble PDE7 inhibitors with acceptable in vivo pharmacokinetic parameters is presented.

1. Introduction

Phosphodiesterases (PDE) are enzymes that degrade the key secondary intracellular messengers adenosine and guanosine 3′,5′-cyclic monophosphates (cAMP and cGMP) into their corresponding 5′-monophosphate nucleotides.[1] PDE7 is a high-affinity cAMP-specific phosphodiesterase for which two subtypes have been identified PDE7A and PDE7B. Considering the functional role of PDE7 reported in T-cells[2] as well as the distribution of its mRNA in various tissues, the use of PDE7 inhibitors may be relevant for the treatment of T-cell related diseases,[2] autoimmune diseases,[2] airway diseases,[3] leukemia,[4] CNS disorders,[5] and fertility disorders.[6] Although there have been several publications on PDE7 inhibitors,[7] there has been no report on a potent, selective, and soluble compound with a suitable in vivo pharmacokinetic profile that could be used as a tool to validate this target in animal models.

In the preceding publication,[8] we disclosed our preliminary SAR studies around the high throughput screening hit **1** (Fig. 1). Among the spiroquinazolinones exemplified, the 5,8-dichloro-spirocyclohexane-quinazolinone **2** was mentioned as a very potent PDE7 inhibitor (IC_{50} = 14 nM).[8] However, this compound is very hydrophobic (clog$P$ = 4.7).[9] In this communication, we present our preliminary results on the optimization of the substituent at position-5 in this key lead structure toward potent, selective, soluble PDE7 inhibitors with acceptable pharmacokinetic properties.



1 IC_{50} = 170 nM **2** IC_{50} = 14 nM

Figure 1. Preliminary result with a 5,8-disubstituted spiroquinazolinone.

2. Chemistry

The 5,8-disubstituted spirocyclohexane-quinazolinone derivatives **2**, **6**, and **7** were synthesized by condensation of the readily available ureas **3**, **4**, and **5** with cyclohexanone in polyphosphoric acid (PPA) at 80–100 °C (Scheme 1).[10] It is worth mentioning that the

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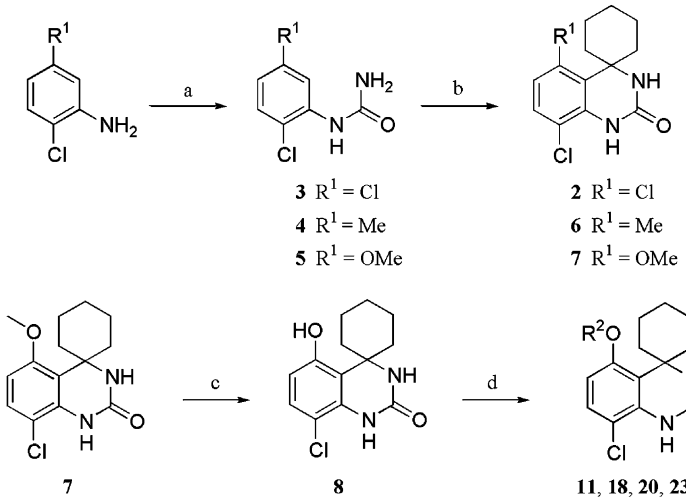
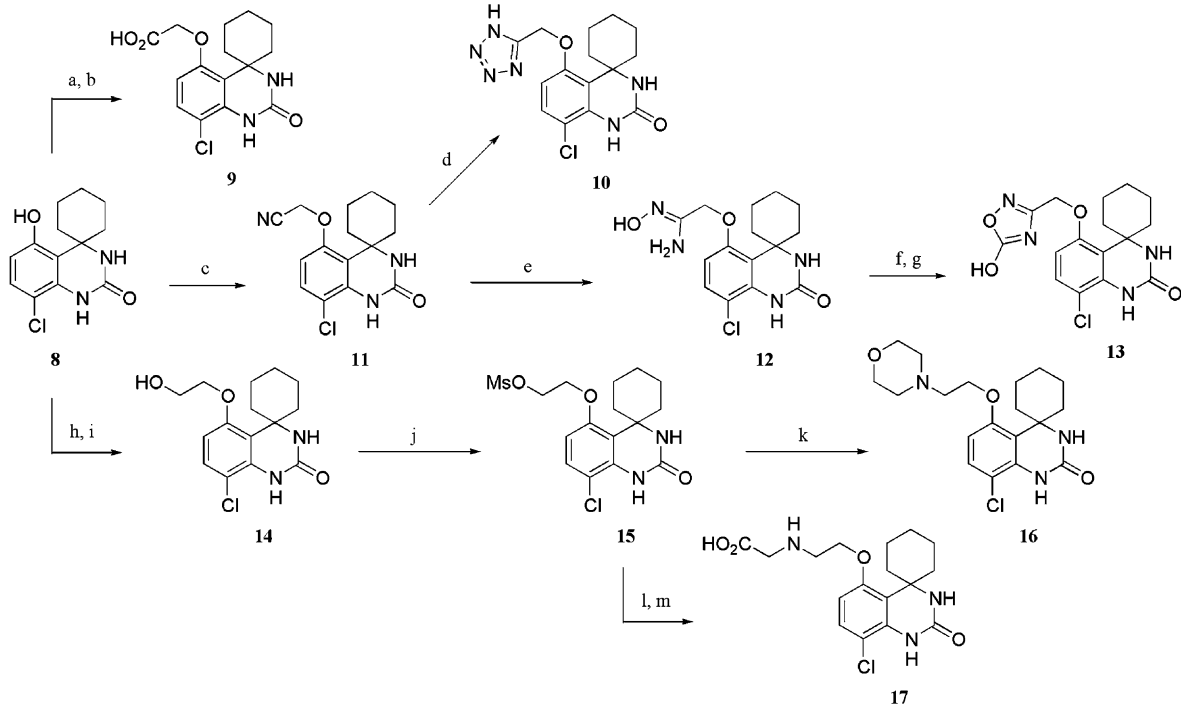


Scheme 1. Reagents and conditions: (a) KNCO, AcOH, H_2O; (b) cyclohexanone, PPA, 80–100 °C; (c) BBr_3, CH_2Cl_2, 0 °C to rt; (d) R^2Cl or R^2Br, K_2CO_3 or NaH, DMF, 100 °C.

PPA-mediated cyclization was much more efficient with the electron-rich phenyl ureas **5** and **4** (78% and 60% yield, respectively), than with **3** (7% yield). Phenol **8** was prepared by demethylation of **7** with boron tribromide. Alkylation of phenol **8** with various alkyl halides directly provided the 5-alkoxy-8-chloro-spirocyclohexane-qinazolinone derivatives **11**, **18**, **20**, **23**, and **24**.

The more functionalized 5-alkoxy-8-chloro-qinazolinone derivatives presented in this publication were prepared in several steps from phenol intermediate **8**. As shown in Scheme 2, carboxylic acid **9** was synthesized by alkylation of **8** with ethyl bromoacetate followed by ester hydrolysis. Similarly, furoic acid **19** resulted from the ethyl ester hydrolysis of **18**. Tetrazole **10** and hydroxy-oxadiazole **13** were obtained from nitrile **11** following usual protocols.[11] Alcohol **14** was derived from phenol **8** by alkylation with 2-(2-bromoethoxy)tetrahydro-2H-pyran followed by deprotection of the tetrahydropyran group under acidic conditions. The resulting alcohol was also used to prepare amines **16**,[12] **21**, and **22** by transformation into the corresponding



Scheme 2. Reagents and conditions: (a) ethyl bromoacetate, K_2CO_3, DMF, 100 °C; (b) KOH, THF, H_2O; (c) bromoacetonitrile, K_2CO_3, DMF, 100 °C; (d) Me_3SnN_3, toluene, reflux; (e) $NH_2OH{\cdot}HCl$, NaOH, EtOH, reflux; (f) $ClCO_2Et$, $CHCl_3$, Et_3N; (g) DBU, CH_3CN, reflux; (h) 2-(2-bromoethoxy)tetrahydro-2H-pyran, K_2CO_3, DMF, 100 °C; (i) HCl, THF, H_2O, reflux; (j) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to rt; (k) morpholine, EtOH, 70 °C; (l) ethyl glycinate, CH_3CN, reflux; (m) HCl (6 N), 1,4-dioxane, 90 °C.

mesylate **15** followed by treatment with morpholine, ammonia, and methylamine, respectively. Similarly, compound **17** was obtained by addition of ethyl glycinate to mesylate **15** and hydrolysis of the resulting ethyl ester. Compound **25** was prepared by alkylation of phenol **8** with 2-[2-(2-chloroethoxy)ethyl]-1H-isoindole-1,3(2H)-dione and subsequent removal of the phthalamido group by treatment with hydrazine. Finally, aminoalcohol derivatives **26** and **27** were synthesized by alkylation of phenol **8** with epibromhydrin followed by condensation with methylamine and dimethylamine, respectively.

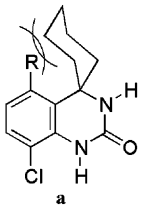
3. Biological results and discussion

The initial results in the replacement of the 5-chloro substituent in the key lead structure **2** were quite promising (Table 1). Since no selectivity for PDE7A versus PDE7B enzyme subtypes was observed, only PDE7A results are presented herein.

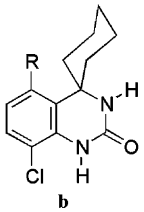
The 5-methoxy and 5-hydroxy substituted analogs **7** and **8**, respectively, were equipotent to **2** in inhibiting the PDE7 enzyme. However, the 5-methyl derivative **6** appeared to be less potent than **2**, **7**, and **8** and showed similar level of activity than **1**.

Considering the diverse nature of these substituents, neither an electronic effect on the aromatic ring (electron-donating OMe vs electron-withdrawing Cl)[13] or a lipophilic interaction (OMe vs OH) could account for these results.

The effect of the substituent at position 5 on the conformation of the spirocyclohexyl ring was evaluated. The enthalpies of formation (ΔH_f) of the two cyclohexyl chair conformers **a** and **b** for the non-substituted derivative **1** and the 5-substituted analogs were calculated with MOPAC[14] in Sybyl[15] using the semi-empiric method AM1 without minimization[16] (Fig. 2). Regardless of the nature of the substituent at position 5, the very high difference in enthalpy of formation between the two cyclohexyl conformations **a** and **b** ($\Delta(\Delta H_f) \geqslant 14.8$ kcal/mol) indicates that all the spiroquinazolinones predominantly adopt the conformation **b** in which the less hindered urea group is axially oriented. The rigid structure **b** probably represents the active conformation for all these PDE7 inhibitors in the enzyme binding site. Therefore, there is no specific influence of the substituent at position 5 on the cyclohexyl conformation that could explain the higher inhibitory activity of **2**, **7**, and **8** versus **1**.



a



b

R = H (**1**)	ΔH_f = -15.20	ΔH_f = -29.98	$\Delta\Delta H_f$ = 14.8
R = Cl (**2**)	ΔH_f = 8.92	ΔH_f = -29.80	$\Delta\Delta H_f$ = 38.7
R = Me (**6**)	ΔH_f = 37.57	ΔH_f = -29.59	$\Delta\Delta H_f$ = 67.1
R = OMe (**7**)	ΔH_f = -34.54	ΔH_f = -57.22	$\Delta\Delta H_f$ = 22.7
R = OH (**8**)	ΔH_f = -51.34	ΔH_f = -71.51	$\Delta\Delta H_f$ = 20.2

Figure 2. Structures, ΔH_f calculations (in kcal/mol), and corresponding differences in ΔH_f ($\Delta\Delta H_f$ in kcal/mol) for the chair conformers of **1**, **2**, **6**, **7**, and **8**.

There may not be a simple rationale for the observed activities and, perhaps, these compounds have different binding mode in the enzyme catalytic site. Further studies, like cocrystallization experiments, would help to rationalize these data.

Although compounds **7** and **8** are very potent PDE7 inhibitors, their aqueous solubility (<4 μg/mL in pH 7.4 buffer solution for both) and metabolic stability in human microsomes ($t_{1/2}$ = 28.4 and 68.4 min for **7** and **8**, respectively), can be improved.

In order to improve drug-like characteristics, we explored substitution of the 5-OH group with various side chains containing polar substituents thereby reducing the overall hydrophobicity of the spirocyclohexane-quinazolinone core.

Neutral, acidic, and basic side chains with various lengths between the spiroquinazolinone core and the polar or ionizable group as well as different lipophilicity and pK_a for the ionizable group were selected (Table 2). These three classes of compounds with diverse physicochemical properties were designed in an attempt to obtain tools with varied tissue distribution profiles that could be used in the appropriate animal disease model depending on the targeted location of the PDE7 enzyme. Beside their effect on solubility, the influence of the ionizable side chains on potency and selectivity was investigated.

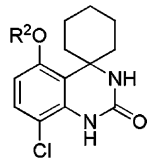
Among the neutral derivatives **11**, **14**, and **18**, alcohol **14** shows the best balance between potency, selectivity, and solubility. Although compound **14** (IC_{50} = 55 nM) is a less potent PDE7 inhibitor than **7** (IC_{50} = 14 nM), it was selected for further in vivo evaluation due to its improved solubility (18 μg/mL in pH 7.4 buffer solution for **14** vs <4 μg/mL for **7**) and metabolic stability in human microsomes ($t_{1/2}$ = 220.7 min for **14** vs 28.4 min for **7**) probably resulting from its lower hydrophobicity (clog P = 3.04 and 3.91 for **14** and **7**, respectively).

Table 1. PDE7A1 inhibitory activity for compounds **1**, **2**, and **6**–**8**

Compd	R^1	PDE7A1 IC_{50} (μM)[a]
1	H	0.17
2	Cl	0.014
6	Me	0.1
7	OMe	0.014
8	OH	0.013

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

Table 2. In vitro potency, selectivity, and solubility for neutral compounds **11**, **14**, **18**; acids **9**, **10**, **13**, **19**, **20**; bases **16**, **21**–**27**, and amino acid **17**



Compd	R^2	PDE7A1 IC_{50} (μM)[a]	PDE1 IC_{50} (μM)[b]	PDE3A3 IC_{50} (μM)[a]	PDE4D3 IC_{50} (μM)[a]	PDE5 IC_{50} (μM)[c]	Solubility pH 7.4 (μg/mL)[d]	Solubility pH 1 (μg/mL)[e]
11	$-CH_2CN$	0.028	5.86	94	1.7	>101	4	4
14	$-CH_2CH_2OH$	0.055	10.5	>73	12.2	>91	18	11
18	$-CH_2-$(5-CO_2Et–Furan-2-yl)	0.011	2.98	78	1.02	80		
9	$-CH_2CO_2H$	0.046	4.76	>68	15	>101	>500	1
10	$-CH_2-$(1H-Tetrazol-5-yl)	0.004	0.32[f]	11.8	0.328	>88	345	<1
13	$-CH_2-$(5-OH-[1,2,4]Oxadiazol-3-yl)	0.0035	0.611	21.9	0.823	>101	113	<1
19	$-CH_2-$(5-CO_2H–Furan-2-yl)	0.016	1.6	10.8	1.13	>101	100	<1
20	$-CH_2CH_2CH_2SO_3H$	0.015	0.65	12.7	2.41	60	>500	>500
16	$-CH_2CH_2-$(4-Morpholino)	0.038	29	>101	7.35	>101	4	>500
21	$-CH_2CH_2NH_2$	1.04	33	>101	10.5	73.2	190	>500
22	$-CH_2CH_2NHMe$	0.967	72.4	>101	53.2	>101	>500	>500
23	$-CH_2CH_2NMe_2$	0.157	19	>101	3.28	74	195	>500
24	$-CH_2CH_2CH_2NMe_2$	1.24	>101	>101	23.7	98		
25	$-CH_2CH_2OCH_2CH_2NH_2$	1.62	>101	>101	99	>101	>500	>500
26	$-CH_2CH-(OH)-CH_2NHMe$	0.213	>101	>101	12	>101	>500	>500
27	$-CH_2CH-(OH)-CH_2NMe_2$	0.4	>101	>101	55	>101	137	>500
17	$-CH_2CH_2NHCH_2CO_2H$	0.042	29.7	>101	14.7	>101	230	>500

[a] Measured against the human full length enzyme produced in baculovirus infected sf9 cells. Values are means of three experiments.
[b] Measured against the human full length enzyme partially purified from THP-1 cell pellets. Values are means of three experiments.
[c] Measured against the human full length enzyme partially purified from MCF-7 cell pellets. Values are means of three experiments.
[d] Determined by stirring in pH 7.4 Na_2HPO_4/NaH_2PO_4 buffer for 24 h.
[e] Determined by stirring in pH 1 HCl/KCl buffer for 24 h.
[f] Measured against the human full length PDE1A enzyme produced in baculovirus infected sf9 cells.

As one could expect, the acidic derivatives **9**, **10**, **13**, **19**, and **20** were more soluble than the neutral derivatives at pH 7.4 but not at pH 1, except for sulfonic acid **20**. Interestingly, the bioisosteric substitution of the carboxylic group in **9** (IC_{50} = 46 nM) for a presumably less acidic tetrazole (**10**) or hydroxy-oxadiazole (**13**) led to more potent PDE7 inhibitors (IC_{50} = 4 and 3.5 nM, respectively). However, the latter were less selective versus PDE1 and PDE4 than **9**. In addition, since carboxylic acid **9** was found to be more selective versus other PDEs than **19** and sulfonic acid **20**, it was chosen as a representative of the class of acids for further in vivo evaluation.

The basic spiroquinazolinones **16** and **21**–**27** were found to be generally less potent than the neutral or acidic derivatives but more selective versus other PDEs. Despite its lower solubility in pH 7.4 buffer solution, the least basic morpholine **16** was found to be soluble at pH 1 and potent enough (IC_{50} = 38 nM) to be selected for further evaluation.

In order to combine the advantages of acidic derivatives in terms of potency and solubility at pH 7.4 with the advantages of basic compounds in terms of selectivity and solubility at pH 1, the amino acid analog **17** was tested. As expected, it was demonstrated to be more potent (IC_{50} = 42 nM) than most basic compounds, more selective than the acids and to have good solubility in both pH buffers.

The rat in vivo pharmacokinetic profiles were determined for the selected compounds **9**, **14**, **16**, and **17** (Table 3).

The acid **9** and the neutral derivative **14** displayed more favorable pharmacokinetic parameters ($t_{1/2}$ = 1.4 h, F = 21%, and $t_{1/2}$ = 1.5 h, F = 27%, respectively), than **16** and **17**. The longer half-life for **9** and **14** compared to **16** resulted mainly from a lower clearance. Besides, oral bioavailabilities for **9** and **14** were superior to those obtained for **16** and **17**. Regarding **17**, since its clearance is acceptable, this probably reflects insufficient intestinal absorption, which could be explained by its zwitterionic nature. It is worth noting that, although **9** is acidic and

Table 3. Rat pharmacokinetic profile for compounds **9**, **14**, **16**, and **17**

Compd	Cl (mL/min/kg)[a]	V_d (L/kg)[a]	$t_{1/2}$ (h)[b]	F (%)[b]
9	39.4	1.04	1.4	21
14	33.6	1.41	1.5	27
16	62.8	1.02	0.32	3.7
17	39.9	0.47	2.7	6.5

[a] Dose 0.5 mg/kg iv.
[b] Dose 2.5 mg/kg po.

14 neutral, both compounds turn out to have similar volumes of distribution.

4. Conclusion

A novel series of 5-substituted 8-chloro-spirocyclohexane-quinazolinones have been developed as potent, selective, and soluble PDE7 inhibitors. The neutral, basic, and acidic compounds **14**, **16**, and **9**, respectively, are useful inhibitors to reveal the functional role of the enzyme PDE7 in vitro. More importantly, derivatives **9** and **14** are the first reported selective PDE7 inhibitors that display in vivo pharmacokinetic parameters suitable to validate this target in rat models.

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