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Spiroquinazolinones as novel, potent, and selective PDE7 inhibitors. Part 1

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Abstract—The synthesis and SAR studies of spiroquinazolinones as novel PDE7 inhibitors are discussed. The best compounds from the series displayed nanomolar inhibitory affinity and were selective versus other PDE isoenzymes.

1. Introduction

Phosphodiesterase enzymes (PDEs) play an important role in various biological processes by hydrolyzing the key secondary messengers adenosine and guanosine 3′,5′-cyclic monophosphates (cAMP and cGMP) into their corresponding 5′-monophosphate nucleotides, thereby decreasing concentrations of cAMP and cGMP, respectively.[1] At least eleven isoenzymes of mammalian cyclic nucleotide phosphodiesterase have been identified on the basis of primary structure, substrate specificity, or sensitivity to cofactors or inhibitory drugs.[2] Among these, PDE7 is a high-affinity cAMP-specific PDE (K_m = 0.2 μM) and its mRNA is expressed in various cell types and tissues known to be important in the pathogenesis of several diseases such as T-cell related diseases,[3] autoimmune diseases,[3] airway diseases,[4] leukemia,[5] CNS disorders,[6] and fertility disorders.[7] In addition, a functional role of PDE7 in T-cell activation has been reported.[3]

The increasing interest in the therapeutic potential of PDE7 inhibitors is driving current medicinal chemistry efforts to identify compounds active at very low concentrations.[8] Selectivity for PDE7 over other members of the phosphodiesterase family is also important to potentially avoid secondary effects such as emesis or cardiotoxicity. In this context, compound **1** was identified by high throughput screening of the compound collection as a PDE7 inhibitor with an IC_{50} of 0.17 μM (Fig. 1).

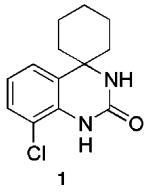

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Figure 1. Structure of the hit **1**.

This communication describes our preliminary efforts toward the identification of novel potent and selective PDE7 inhibitors by exploring the SAR around this new spiroquinazolinone hit.

2. Chemistry

The quinazolinone derivatives **1**–**21** were prepared by condensation of the readily available ureas with the desired ketones in polyphosphoric acid (PPA) at 80–100 °C (Scheme 1).[9] To the best of our knowledge, this type of intermolecular reaction between an urea and a

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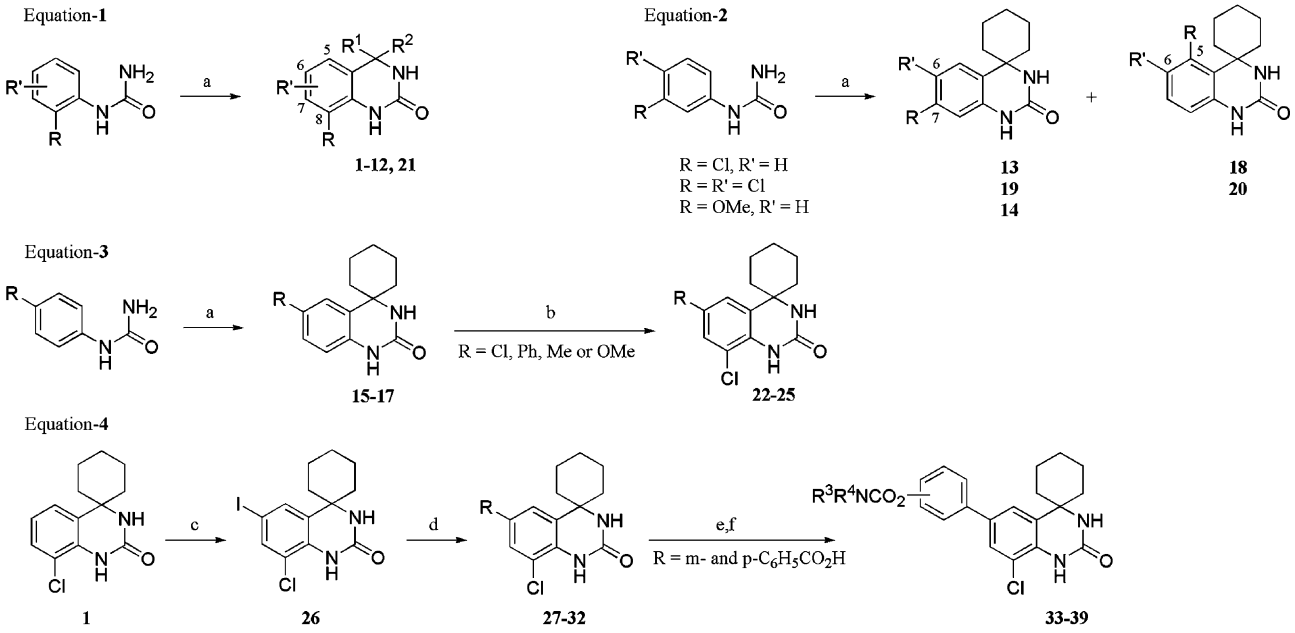


Scheme 1. Reagents and conditions: (a) $R^1R^2C{=}O$, PPA, 80–100 °C; (b) *N*-chlorosuccinimide, DMF, 60 °C; (c) N-iodosuccinimide, TFA, H_2SO_4, 55 °C; (d) $Pd(PPh_3)_4$, 2-pyridylZnBr in THF or $RB(OH)_2$, K_2CO_3 in DMF, reflux (where R = *p*- or *m*-C_6H_4–CO_2H, 3- or 4-pyridyl or *o*-C_6H_4CO–N-methylpiperazine); (e) $SOCl_2$, toluene, reflux; (f) R^3R^4NH, toluene (where $R^3R^4NH = H_2N$–$(CH_2)_2$–NMe_2, H_2N–$(CH_2)_3$–NMe_2, or 1-methylpiperazine).

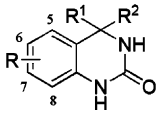
ketone has not been previously reported in the literature. The cyclization reaction performed using 2-substituted or 4-monosubstituted ureas led to a single compound as depicted in Scheme 1 (Eqs. 1 and 3). Yet when 3-chloro or 3,4-dichloro-phenylurea and cyclohexanone were used as starting materials, a mixture of regioisomers (5- and 7-chloro or 5,6- and 6,7-dichloroquinazolinones, respectively), was obtained and was separated by flash chromatography on silica gel (Eq. 2). The 6,8-disubstituted derivatives **22**–**25** were synthesized from the corresponding 6-monosubstituted intermediates by chlorination with *N*-chlorosuccinimide in DMF at 60 °C (Eq. 3). The 6-aryl substituted quinazolinone analogs **27**–**32** were obtained from **1** (Eq. 4). Iodination of **1** under acidic conditions[10] afforded iodo-intermediate **26**, which was further converted to the biaryl-derivatives via Negishi (compound **27**) or Suzuki palladium-catalyzed cross-coupling reaction (compounds **28**–**32**). Finally compounds **33**–**39** were derived from the corresponding carboxylic acids after conversion to the intermediate acid chloride and condensation with the appropriate amine.

3. Biological results and discussion

The compounds described in this paper were assessed against both PDE7A and PDE7B subtypes. Since no selectivity was observed, only the PDE7A inhibitory activities will be presented herein.

Initial results for the inhibition of PDE7 using our spiroquinazolinone analogs showed that the nature of the substituent in position 8 has an impact on activity (Table 1, compounds **1**–**7**). The following order of

Table 1. PDE7A1 inhibitory activity for compounds **1**–**25**



Compd	R^1–R^2	R	PDE7A1
			IC_{50}, μM[a]
1	–Spirocyclohexyl–	8-Cl	0.17
2	–Spirocyclohexyl–	8-H	6.60
3	–Spirocyclohexyl–	8-F	0.39
4	–Spirocyclohexyl–	8-Br	0.066
5	–Spirocyclohexyl–	8-I	6.85
6	–Spirocyclohexyl–	8-CH_3	0.79
7	–Spirocyclohexyl–	8-CF_3	2.96
8	–Spirocycloheptyl–	8-Br	0.068
9	–Spirocyclobutyl–	8-Br	1.40
10	–4-Methyl-spirocyclohexyl–	8-Br	0.21
11	Propyl–propyl	8-Br	2.66
12	Ethyl–ethyl	8-Br	6.54
13	–Spirocyclohexyl–	7-Cl	2.7
14	–Spirocyclohexyl–	7-OMe	9.5
15	–Spirocyclohexyl–	6-Cl	4.8
16	–Spirocyclohexyl–	6-OMe	3.2
17	–Spirocyclohexyl–	6-Ph	0.98
18	–Spirocyclohexyl–	5-Cl	0.84
19	–Spirocyclohexyl–	6,7-DiCl	3.7
20	–Spirocyclohexyl–	5,6-DiCl	0.6
21	–Spirocyclohexyl–	5,8-DiCl	0.014
22	–Spirocyclohexyl–	6,8-DiCl	0.12
23	–Spirocyclohexyl–	6-Ph,8-Cl	0.016
24	–Spirocyclohexyl–	6-Me, 8-Cl	0.16
25	–Spirocyclohexyl–	6-OMe, 8-Cl	0.16

[a] Measured against the human full length enzyme produced in bacu-

potency 8-Br > 8-Cl > 8-F > 8-CH_3 > 8-CF_3 > 8-H, 8-I cannot be easily explained considering physical parameters such as +π-effect, +σ-effect[11] or molecular volume but indicated that a size limited halogen or a methyl group were preferred.

We next explored the SAR around the cyclohexyl ring while retaining the 8-bromo substituent, which is optimal for activity. From the results of compounds **8**–**12** (Table 1), several phamacophoric features can be highlighted: preference for a cyclic ring (**4**, **8**, **9**, **10**) over two alkyl substituents (**11**, **12**) and for a six or seven membered ring (**4**, **8**) compared to the four membered ring (**9**). In addition the nonsubstituted cyclohexyl derivative **4** was 3-fold more potent than its analog with a methyl group on the cyclohexyl ring (**10**).

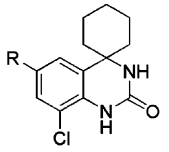
Further modifications were designed to study the influence of other substituents on the aromatic ring while keeping the spirocyclohexyl group as R^1–R^2. As illustrated by compounds **13**–**16**, the substitution of the 6- or 7-position with a chloro or a methoxy group did not lead to a significant increase in potency. However, interestingly, the 6-phenyl substituted analog **17** gave promising results with an IC_{50} of 0.98 μM. Substitution at C5 (see compound **18**) was also favorable resulting in a 7-fold increase in potency compared to the reference compound **2**. Except for **19**, a similar tendency was observed among the disubstituted derivatives synthesized (**20**–**25**). Indeed, a synergistic effect was demonstrated when combining a 5-chloro or 6-phenyl substituent with the 8-chloro substituent, leading to the most potent PDE7 inhibitors, respectively, compounds **21** and **23**.

The recognition of this effect led us to the biphenyl subseries. In this study, the 8-chloro substituent was preferred to the 8-bromo because of reduced lipophilicity and better pharmaceutical suitability. The reference compound **23** exhibited good selectivity versus PDE1, 3A, 4D, 5 with selectivity ratios greater than 85 (Table 2).

Replacement of the phenyl group in position 6 by other heterocycles showed that the unsubstituted 2-, 3-, or 4-pyridyl derivatives (**27**–**29**) were slightly less potent than the reference compound **23**. In order to improve drug-like characteristics, especially solubility, we further explored the substitution of the aromatic ring by introducing ionizable functions. Introduction of a carboxy group in *para* (**30**) or *meta* (**31**) position led to almost equipotent compounds but with lower selectivity versus the others tested PDEs. Similar results were obtained when an amide group was introduced in *para*-position as illustrated by compound **33**. Interestingly, substituting the amide function in **33** with an alkylamine group (**34**–**37**) resulted in compounds with good selectivity and greater solubility at pH 7.4 (30–70 μg/mL in pH 7.4 buffer solution[12]). It is worth noting that introduction on the phenyl ring of functionalities with different physicochemical properties (from carboxylic acid to basic amines) led only to little variation in PDE7 inhibitory activity.

Further studies around the N-substitution of the amide showed that the length of the linker could be extended from 2 to 3 carbon atoms and that both *meta*- or *para*-substitutions were tolerated as demonstrated by

Table 2. In vitro data for biphenyl-compounds **23** and **27**–**39**



Compd	R	PDE7A1	PDE1	PDE3A3	PDE4D3	PDE5
		IC_{50}, μM[a]	IC_{50}, μM[b]	IC_{50}, μM[a]	IC_{50}, μM[a]	IC_{50}, μM[c]
23	–Phenyl	0.016	3.26	>101	1.42	69.8
27	–2-Pyridyl	0.033	8.30	45.1	1.8	68.4
28	–3-Pyridyl	0.051	2.86	14.8	1.55	19.3
29	–4-Pyridyl	0.031	3.01	11.2	1.01	9.47
30	–*p*-C_6H_4–CO_2H	0.013	0.89	21.3	0.47	8.50
31	–*m*-C_6H_4–CO_2H	0.025	0.71	16.7	0.74	12.6
33	–*p*-C_6H_4–$CONH_2$	0.020	1.28	0.28	0.69	11.6
34	–*p*-C_6H_4–CONH–$(CH_2)_2$–NMe_2	0.021	6.29	42.2	1.63	>67.3
35	–*m*-C_6H_4–CONH–$(CH_2)_2$–NMe_2	0.026	11.6	>79.9	1.59	>101
36	–*p*-C_6H_4–CONH–$(CH_2)_3$–NMe_2	0.024	6.47	44.5	1.50	>50.7
37	–*m*-C_6H_4–CONH–$(CH_2)_3$–NMe_2	0.019	5.16	78.1	1.46	>84.9
38	–*p*-C_6H_4–CO–N-Methylpiperazine	0.014	7.01	29.3	1.70	29.3
39	–*m*-C_6H_4–CO–N-Methylpiperazine	0.044	6.39	31.6	1.31	52.4
32	–*o*-C_6H_4–CO-N-Methylpiperazine	1.52	20.1	>92	15.7	>101

[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.

compounds **34**–**37**. Conformational restriction of the basic side chain via incorporation into a piperazine (compounds **38** and **39**) maintained a similar activity level however the solubility decreased (<5 μg/mL in pH 7.4 buffer solution[12]), which most likely results from a lower pK_a. The point of attachment for the piperazine carboxy side chain was also examined and a significative reduction in potency was observed as the point of attachment was moved from *para* or *meta* to *ortho* (see compounds **38**, **39**, and **32**).

The most active, selective, and soluble compounds **34**–**37** were considered for further in vitro and in vivo evaluation.

4. Conclusion

The spiroquinazolinone derivatives represent a new class of PDE7 inhibitors. Preliminary SAR around this series led to the discovery of a promising biphenyl subseries. In the course of this study, introduction of ionizable functions onto the aromatic ring, aimed at improving solubility in this series, resulted in the identification of soluble and highly potent PDE7 inhibitors. The best compounds tested **34**–**37** also showed good selectivity versus PDE1, 3A, 4D, and 5.

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