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Synthesis and Structure–Activity Relationships of Guanine Analogues as Phosphodiesterase 7 (PDE7) Inhibitors

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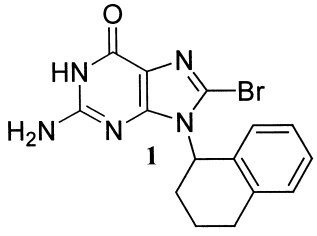
Abstract—The synthesis of a novel series of guanine analogues is reported. The compounds have been assessed in vitro and some analogues have been found to be inhibitors of phosphodiesterase type 7 (PDE7). © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

The secondary messengers cAMP and cGMP are responsible for the regulation of numerous intracellular processes. They are regulated by phosphodiesterases (PDEs) which hydrolyze them to the corresponding inactive 5′-monophosphate nucleotides. Eleven PDE gene families have been identified to date, varying in substrate specificity, inhibitor sensitivity and regulatory characteristics.[1] PDE7 is a low K_m (0.2 μM) cAMP specific enzyme which is insensitive to the standard PDE4 inhibitor, rolipram. Two splice variants, PDE7A1 and PDE7A2 were originally identified (>90% homology).[2] A further PDE7 gene has recently been identified,[3] which has been designated PDE7B as it possesses 70% homology with PDE7A in the catalytic domain. PDE7 mRNA has been found to be widely distributed, although active protein has been identified predominantly in T-cells.[4] The proposed role of PDE7 in T-cell activation[5] implies that selective inhibitors could have benefits in T-cell mediated diseases. Additionally, the presence of PDE7 in airway epithelial cells[6] suggests that inhibitors could be beneficial in diseases of the airway.

To date only two series of synthetic PDE7 inhibitors have been described,[7] generally having low micromolar IC_{50} values and no significant selectivity over the PDE4 and PDE3 isozymes. As inhibition of the other PDE isoenzymes may result in some side effects such as emesis and cardiotoxicity, selectivity for PDE7 over the other members of the phosphodiesterase family of enzymes is important. In this communication, we exemplify a series of guanine analogues which possesses PDE7 inhibitory activity in vitro and demonstrates some evidence of selectivity over the PDE4 isoenzyme.



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To find a lead, screening of internal and external databases was performed, and an initial guanine based hit (**1**) was identified.

Chemistry

Synthesis of these guanine analogues was initially attempted by alkylation of either 2-amino-6-chloropurine or 6-chloropurine (**2**). Intermediates (**3**) were generated using potassium carbonate as the base and a suitable alkylating agent. Displacement of the chlorine was then achieved using sodium hydroxide to yield the

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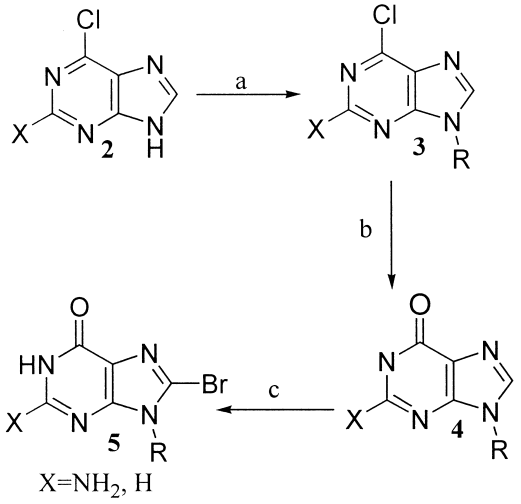
guanines (**4**).[8] Bromination[9] of the 8-position gave the desired analogues (**5**) (Scheme 1).

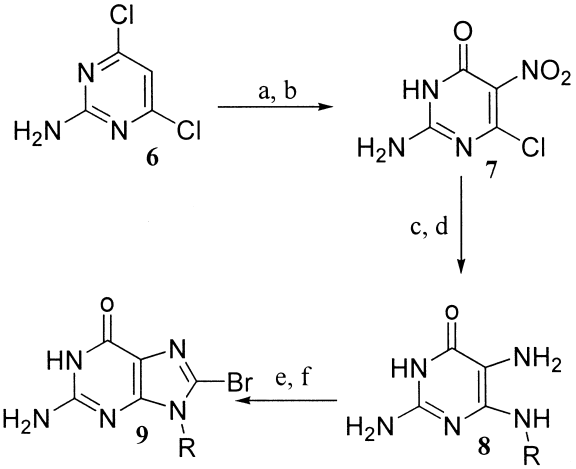
It was found that this route was not generally applicable, and so the synthesis of the guanine ring was necessary in several cases (Scheme 2).

Starting from commercially available 2-amino-4,6-dichloropyrimidine (**6**), saponification with sodium hydroxide[10] followed by nitration[11] yielded the 6-hydroxy pyrimidine (**7**). Displacement of the chlorine with a suitable amine followed by sodium dithionite reduction[12] afforded the diamines (**8**). Ring closure using triethylorthoformate was unsuccessful, due to the acid sensitivity of the diamine, however the use of formamide[13] was successful. Subsequent bromination[9] yielded the desired guanine analogues (**9**).

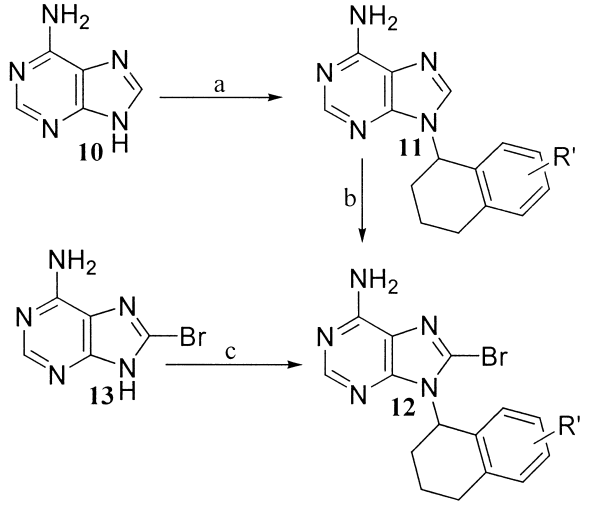
Synthesis of adenine analogues was performed by reacting adenine (**10**) (Scheme 3) with a series of commercially available alcohols under Mitsunobu[14] conditions producing the alkylated compounds (**11**). The 8-position was then brominated[15] with bromine in a methanol/acetate buffer to yield the bromides (**12**). In cases where access to the desired alcohol was not possible it was found that the substituent could be introduced via alkylation of 8-bromoadenine (**13**) using the same alkylation conditions which had been employed for the guanine series of analogues.



Scheme 1. (a) K_2CO_3, RX, (b) aq NaOH, (c) Br_2, H_2O.



Scheme 2. (a) aq NaOH, (b) H_2SO_4, HNO_3, (c) Et_3N, RNH_2, DCM, (d) $Na_2S_2O_4$, DMF, H_2O, (e) $HCONH_2$, (f) Br_2, H_2O.



Scheme 3. (a) PPh_3, DEAD, ROH, (b) Br_2, MeOH, NaOAc/AcOH, (c) K_2CO_3, RX.

Structure–Activity Relationships

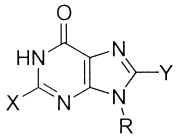
Initial results for the inhibiton of PDE7 using our guanine analogues (Table 1) showed removal of the bromine reduced activity (compare **1** and **5a**) as did the removal of the amino group (**5a** and **5b**).

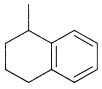
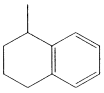
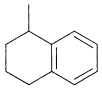
Several analogues were synthesised starting with the replacement of the saturated six membered ring with a five membered (**5c**) and a seven membered ring (**9a**) (Table 2). Neither of these alterations was found to enhance either the activity or the selectivity. However when the tetralin ring was substituted with bromine (**9b**), methoxy (**9d**) or a nitro group (**9c**) the results were far more encouraging, and bromine substitution (**9b**) was found to provide the most potent compound. The most active analogues were also screened against the other PDE isoenzymes present in T-cells, PDE3 and PDE4, and found to have good selectivity for PDE7 over these enzymes.

The natural ligand for PDE7 is cAMP, which contains an adenine base. By replacing the guanine moeity with an adenine in our compounds, a better overlay with cAMP would be predicted. Potentially this would improve inhibitor potency. This hypothesis was investigated by the synthesis of a series of analogues (**12**) based around the initial guanine lead (**1**).

The results for the inhibition of PDE7 using the adenine analogues were surprisingly poor. None of the analogues

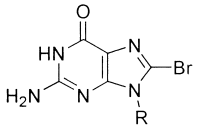
Table 1. In vitro data for guanine analogues[a]

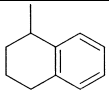
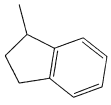
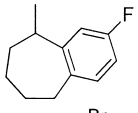
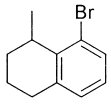
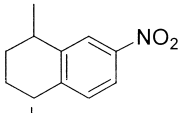
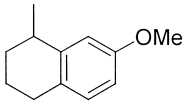


Compd	R	X	Y	PDE7[16]	PDE4[17]
1		NH_2	Br	4.88	15%
5a		NH_2	H	4%	7%
5b		H	H	IA[b]	IA[b]

[a]Compound results are IC_{50} (μM) or when quoted as a '%' refer to percentage inhibition at 10 μM.
[b]Compound results denoted by 'IA' were considered inactive at 10 μM.

Table 2. In vitro data for tetralin replacements[a]



Compd	R	PDE7[16]	PDE4[17]	PDE3[18]
1		4.88	15%	30%
5c		22%	27%	NT[b]
9a		10%	13%	NT[b]
9b		1.31	10%	14%
9c		2.31	42%	19%
9d		4.22	20%	NT[b]

[a]Compound results are IC_{50} (μM) or when quoted as a '%' refer to percentage inhibition at 10 μM.
[b]Compound results denoted by 'NT' were not tested in the listed assay.

synthesised were found to offer any advantage over the guanine series. The adenine amino group was also replaced with both a methoxy group and a chloro substituent, but in each case no improvement of activity or selectivity was observed.

In conclusion a series of analogues based around our original hit has been successfully synthesised. Our original guanine lead (**1**) was found to have low micromolar activity against PDE7 with good selectivity over both PDE4 and PDE3. This lead has been enhanced to improve the activity, whilst retaining its selectivity over the other key PDE isoenzymes. The 8-bromo-9-substituted series are the most potent and selective compounds published to date. Efforts are ongoing in the PDE7 area of research at Celltech R & D and the results of further studies will be published in due course.

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