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SYNTHESIS AND EVALUATION OF POTENT AND SELECTIVE c-GMP PHOSPHODIESTERASE INHIBITORS

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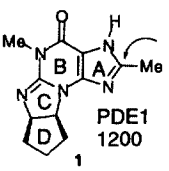
Abstract: Syntheses and structure–activity relationships (SAR) of cGMP selective phosphodiesterase inhibitors are discussed. Potent and selective inhibitors are produced when the C-2 position of tetracyclic guanine **1** is substituted with alkyl chains containing six carbon atoms.

At least seven classes of phosphodiesterases (PDEs) have been identified to date,[1] which are distinguished by their specificity for cAMP and cGMP, mechanisms of regulation, and sensitivity to various pharmacological agents. PDE1 is a calmodulin (CaM) dependent phosphodiesterase that hydrolyzes both cAMP and cGMP. PDE5 binds and selectively hydrolyzes cGMP. Both of these enzymes are found in vascular smooth muscle cells where they play a significant role in cyclic nucleotide hydrolysis.[2] Increased cGMP levels in vascular smooth muscle have been shown to demonstrate vasodilation, antiplatelet, and antiproliferative activities.[3] Recently the role of cGMP and the potential beneficial effects of inhibition of the cGMP PDE for the treatment of cardiovascular disease have been reviewed.[3] Hence, selective inhibition of enzymes involved in the hydrolysis of cGMP will be useful in modulating cardiovascular functions and treating abnormalities associated with vascular injury.

Our goal at the outset of this investigation was to synthesize potent and selective agents that inhibits c-GMP selective isozymes. We recently described[4] tetracyclic guanine derivatives represented, by structure **1**, as a new class of compounds for selective inhibition of both PDE1 and PDE5 isozymes. Our strategy was to identify optimum alkyl substituents at C-2 of **1** to improve both selectivity and potency of this class of compounds. We also planned to modify the D-region of compounds containing optimum substituents at C-2. This effort resulted in the identification of several new C-2 alkyl substituted tetracyclic guanines that exhibited superior potency and selectivity compared to **1**. In this paper, the synthesis and in vitro profiles of these compounds are described.



	IC_{50} (nM)		
	PDE1	PDE3	PDE5
1	1200	>300000	2400

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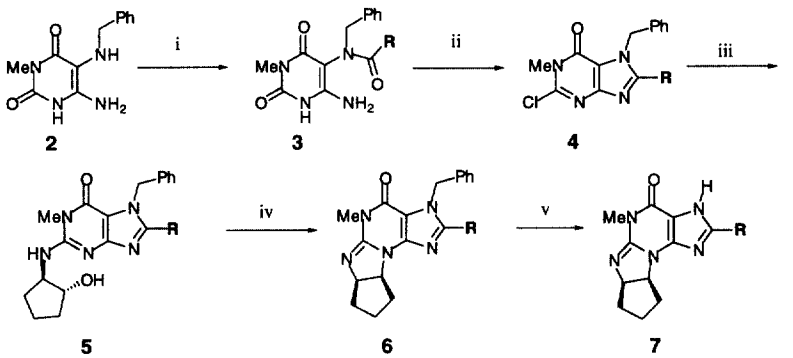
PII: S0960-894X(98)00681-7

Chemistry

Compounds described in this paper were prepared using two general methods that were recently reported[4b] from this laboratory. These methods are summarized in Scheme 1 and Scheme 2. In Scheme 1, the known compound,[4a] 6-amino-5-(benzylamino)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (**2**) was acylated with commercially available carboxylic acids to produce intermediate **3**. Treatment of intermediate **3** with excess $POCl_3$ produced chloropurine **4** in 50–70% yields. (–)-*trans*-2-Aminocyclopentanol[5] was used to displace the ring chlorine to form intermediate **5**, which upon activation with thionyl chloride, underwent stereospecific displacement to give (+)-*cis* fused tetracyclic guanine **6** with an absolute configuration of 6a*R*,9a*S*. Catalytic hydrogenolysis of the N-3-benzyl group produced desired compound **7**.

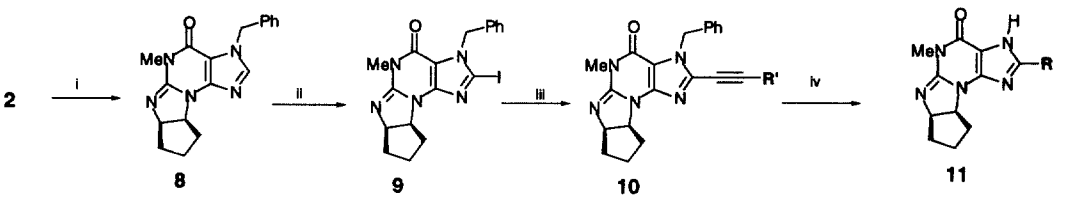
Scheme 1[a]



[a]*Reagents*: (i) RCOOH, EDCI, DMAP, DMF; (ii) $POCl_3$, Δ; (iii) (–)-*trans*-2-aminocyclopentanol[5], *i*PrNEt, NMP; (iv) $SOCl_2$, CH_2Cl_2; (v) 10%$Pd(OH)_2$, NH_4CO_2H, MeOH, Δ.

The procedure described in Scheme 2 was used for the preparation of compounds **11a** and **11b** for which carboxylic acids were not available commercially. The advanced stage intermediate **8** was prepared from compound **2** using our published procedure.[4b] As illustrated in Scheme 2, compound **8** was iodinated using lithium diisopropylamide and 1-chloro-2-iodoethane in a 60% yield. The alkylacetylene compound **10** was prepared by palladium-catalyzed coupling of the iodo intermediate **9** with an appropriate terminal acetylene using copper iodide as a co-catalyst. Treatment of **10** with ammonium formate in refluxing methanol in the presence of Pearlman's catalyst produced desired compound **11**. Compounds with D-region modifications (**12–16**) were prepared from intermediate **4** and corresponding commercially available amino alcohols using the sequence in Scheme 1.

Scheme 2[a]



[a]*Reagents*: (i) Ref 4; (ii) LDA/THF, $ClCH_2CH_2I$; (iii) HCCR′, $Pd(PPh_3)_4$, Et_3N, CuI, DMF; (iv) 10%$Pd(OH)_2$, NH_4CO_2H, MeOH, Δ.

Results and Discussion

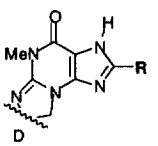
Table 1 lists the structures and PDE isozyme inhibitory activities[6] of C-2 alkyl substituted tetracyclic guanines. In general these compounds are much more effective inhibitors of PDE1 and PDE5 than PDE3. These compounds exhibit a trend for increasing potency with increasing carbon count at C-2. This trend is observed for all three isozymes. The optimum inhibitory activity and selectivity is observed when the carbon count is six for both straight and branched chain compounds (**7e** and **7g**).

Table 1. Phosphodiesterase inhibitory activity of C-2 alkyl derivatives of tetracyclic guanine **7**

Compounds	R	IC_{50}(nM) PDE1	PDE3	PDE5
1	$-CH_3$	1250	>300000	2400
7a	$-CH_2CH_3$	295	150000	1200
7b	$-(CH_2)_2CH_3$	350	80000	800
7c	$-(CH_2)_3CH_3$	110	38000	140
7d	$-(CH_2)_4CH_3$	31	20000	40
7e	**$-(CH_2)_5CH_3$**	**36**	**12000**	**10**
11a	**$-(CH_2)_6CH_3$**	**33**	**10000**	**14**
11b	$-(CH_2)_7CH_3$	160	7000	30
7f	**$-(CH_2)_2CH(CH_3)_2$**	**14**	**15000**	**50**
7g	**$-(CH_2)_3CH(CH_3)_2$**	**6**	**35000**	**10**
7h	$-CH(CH_2CH_3)_2$	150	150000	1400
7i	$-CH[(CH_2)_2CH_3]_2$	60	80000	150

Structure–activity relationships were also investigated via modifications of the D-region. The in vitro profiles of selected compounds from D-region variations are presented in Table 2. These results indicate that the modification of the D-region of compound **7e** does not produce significant change in its PDE inhibitory activity (compounds **12–14**). Where as in the branched chain series (compounds **7f** and **7g**), although the inhibitory activity for PDE1 and PDE5 remains similar, a twofold loss in inhibitory activity for PDE3 was observed when the D-region was replaced with an isopropyl moiety (compounds **15** and **16**).

Table 2. Phosphodiesterase inhibitory activity of D-region modified derivatives of tetracyclic guanine **7**.



Compounds	D-region	R	IC_{50}(nM) PDE I	PDE III	PDE V
12		$-(CH_2)_5CH_3$	14	15000	11
13		$-(CH_2)_5CH_3$	25	28000	8
14		$-(CH_2)_5CH_3$	25	18000	5
15		**$-(CH_2)_2CH(CH_3)_2$**	**15**	**40000**	**22**
16		**$-(CH_2)_3CH(CH_3)_2$**	**12**	**65000**	**6**

In summary, our current work[7] demonstrates that a six carbon chain or branched alkyl substituent is optimum for the C-2 position of tetracyclic guanine **1**. The D-region modifications improve the selectivity profile of branched chain derivatives only. Compound **16** is identified to be most potent and selective inhibitor in the C-2 alkyl series of tetracyclic guanine derivatives.

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References and Notes

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6. PDE inhibitory activity was measured based on a radioenzymatic assay as previously described.[8] Bovine aorta and bovine lung were the enzyme sources for isozymes PDE1 and PDE5, respectively. PDE3 activities were measured using recombinant bovine adrenal PDE, bovine heart and dog lung PDE isozymes as enzyme sources.

7. All compounds were characterized by ^{1}H NMR and MS. Data of selected compounds are reported below: **7a** ^{1}H NMR ($CDCl_3$) δ 1.6–2.25 (m, 6H), 2.55 (s, 3H), 3.44 (s, 3H, NCH_3), 4.81 (t, J =7.1 Hz, 1H), 4.92 (t, J = 7.1 Hz, 1H); MS (CI) *m/z* 246 (MH^+); **7b** ^{1}H NMR ($CDCl_3$) δ 1.00 (t, J =7.5 Hz, 3H, CH_3), 1.6 - 2.0 (m, 7H), 2.28 (dd, J = 7.5, 12.5 Hz, 1H), 2.80 (t, J = 7.5 Hz, 2H), 3.30 (s, 3H, NCH_3), 4.72 (t, J = 7.0 Hz, 1H), 4.90 (t, J = 7.0 Hz, 1H); MS (FAB) 274 (MH^+); **7c** ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 7 Hz, 3H), 1.35–2.30 (m, 10H), 2.82 (t, J = 8Hz, 2H), 3.40 (s, 3H), 4.74 (t, J = 7 Hz, 1H), 4.88 (t, J = 7H, 1H); MS *m/z* 288 (MH^+); **7e** ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 6.4 Hz, CH_3), 1.34 (br, 6H, $(CH_2)_3$), 1.55 (m, 1H), 1.7–1.88 (m, 5H), 1.94 (m, 1H, CH), 2.23 (dd, 1H, J =13.2, 5.5 Hz, CH),

2.79 (t, 2H, J =7.7 Hz, CH_2), 3.37 (s, 3H, NCH_3), 4.72 (t, 1H, J =7.3 Hz), CH), 4.87 (t, 1H, J =7.5 Hz, CH); MS (CI) *m/z* 316 (M+); **7f** ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 6H, J = 6.4 Hz, $CH(CH_3)_2$), 1.53–2.28 (m, 9H, $NCH(CH_2)_3CHN$=, $CH_2CH_2CH(CH_3)_2$), 2.82 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 3.44 (s, 3H, CH_3N), 4.76 (t, 1H, J = 7.1 Hz, $NCH(CH_2)_3CHN$=), 4.92 (t, 1H, J = 6.9 Hz, $NCH(CH_2)_3CHN$=); **7g** ^{1}H NMR ($CDCl_3$) δ 0.88 (d, 6H, J = 6.6 Hz, $CH(CH_3)_2$), 1.26 (m, 2H, $(CH_2)_2CH_2CH(CH_3)_2$), 1.55–2.27 (m, 9H, $NCH(CH_2)_3CHN$=, $CH_2CH_2CH_2CH(CH_3)_2$), 2.81 (t, 2H, J = 7.7 Hz, $CH_2(CH_2)_2CH(CH_3)_2$), 3.43 (s, 3H, CH_3N), 4.77 (t, 1H, J = 7.4 Hz, $NCH(CH_2)_3CHN$=), 4.92 (t, 1H, J = 7.1 Hz, $NCH(CH_2)_3CHN$=); **12** ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, CH_3), 1.2–1.4 (m, br, 6H, $(CH_2)_3$), 1.6–1.7 (m, 4H), 1.75 (m, 2H, CH_2), 1.8–2.0 (br, 4H), 2.8 (t, 2H, CH_2), 3.4 (s, 3H, NCH_3), 3.9 (s, 2H); Ms FAB 330.2 (M + 1); **13** ^{1}H NMR ($CDCl_3$) δ 0.8 (t, 3H, CH_3), 1.2-1.35 (m, 6H, $(CH_2)_3$), 1.4 (s, 6H, $(CH_3)_2$), 1.75 (m, 2H, CH_2), 2.8 (m, 2H, CH_2), 3.4 (s, 3H, NCH_3), 3.82 (s, 2H), CH_2); MS (CI) *m/z* 304 (MH^+); **14** ^{1}H NMR ($CDCl_3$) δ 0.83 (t, J = 6.8 Hz, 3H, CH_3), 0.90 (d, J = 6.7 Hz, 3H, CH_3), 1.0 (d, J = 6.7 Hz, 3H, CH_3), 1.1–1.4 (b, 6H, $(CH_2)_3$), 1.75 (m, 2H, CH_2), 1.85 (m, 1H, CH), 2.79 (t, J = 7.7 Hz, 2H, CH_2), 3.4 (s, 3H, NCH_3), 3.85 (t, 1H), 4.06 (m, 1H), 4.09 (m, 1H); MS (CI) *m/z* 318 (MH^+); **15** ^{1}H NMR ($CDCl_3$) δ 0.95 (m, 9H, $CH_2CH_2CH(CH_3)_2$, $CHCH(CH_3)_2$), 1.04 (d, 3H, J = 6.7 Hz, $CHCH(CH_3)_2$), 1.64 (m, 3H, $CH_2CH_2CH(CH_3)_2$), 2.05 (m, 1H, $CHCH(CH_3)_2$), 2.85 (t, 2H, J = 7.9 Hz, $CH_2CH_2CH(CH_3)_2$), 3.53 (s, 3H, CH_3N), 3.96 (m, 1H, $CHCH(CH_3)_2$), 4.20 (m, 2H, $=NCHCH_2N$); **16** ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 6H, J = 6.6 Hz, $CH_2CH_2CH_2CH(CH_3)_2$), 0.92 (d, 3H, J = 6.7 Hz, $CHCH(CH_3)_2$), 1.02 (d, 3H, J = 6.8 Hz, $CHCH(CH_3)_2$), 1.23 (m, 2H, $(CH_2)_2CH_2CH(CH_3)_2$), 1.56 (m, 1H, $CH_2CH_2CH_2CH(CH_3)_2$), 1.78 (m, 2H, CH_2CH_2 $CH_2CH(CH_3)_2$), 1.95 (m, 1H, $CHCH(CH_3)_2$), 2.79 (t, 2H, J = 7.7 Hz, $CH_2CH_2CH_2CH(CH_3)_2$), 3.45 (s, 3H, CH_3N), 3.88 (m, 1H, $CHCH(CH_3)_2$), 4.14 (m, 2H, $=NCHCH_2N$).

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