Inhibition of Cyclic Nucleotide Phosphodiesterases from Pig Coronary Artery by Benzo-Separated Analogues of 3-Isobutyl-1-methylxanthine

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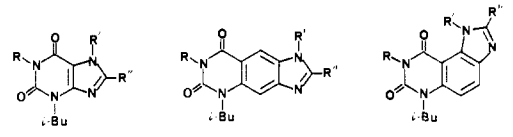
The linear and proximal benzo-separated analogues of 7-benzyl-3-isobutyl-1-methylxanthine, 3-isobutyl-1,8-dimethylxanthine, 3-isobutyl-8-*tert*-butyl-1-methylxanthine, 3-isobutyl-8-(methoxymethyl)-1-methylxanthine, and 1-isoamyl-3-isobutylxanthine have been prepared and assayed as inhibitors of the peak I and peak II forms of cyclic nucleotide phosphodiesterase from pig coronary artery. Most of the benzo analogues were less effective inhibitors of these phosphodiesterases when compared to 3-isobutyl-1-methylxanthine (IBMX) even though the active sites of both enzyme forms tolerated the stretched-out xanthines. Indeed, the linear benzo-separated analogue of 7-benzyl-IBMX was a more potent inhibitor of peak I activity than was IBMX.

It is now well established that adenosine 3′,5′-cyclic monophosphate (cAMP) plays a major regulatory role in cellular metabolism,[1] and evidence is growing that suggests a similar, less defined function may exist for guanosine 3′,5′-cyclic monophosphate (cGMP).[2] A significant component in this regulatory process is the control of the intracellular levels of cAMP and cGMP by, in part, cyclic nucleotide phosphodiesterases (PDEs) that convert each to their corresponding 5′-monophosphate. It is, therefore, not surprising that inhibition of PDEs[3] alter a number of functions that are regulated by the concentrations of the appropriate 3′,5′-cyclic nucleotide. In that regard, alkylated xanthines have been found to be potent PDE inhibitors.[3b]

Using pig coronary arteries, Wells and his co-workers[4] reported that there are two forms of phosphodiesterase present in this system: (i) a calmodulin-sensitive form (peak I) that, as a result of its greater affinity for cGMP, is considered to be the cGMP phosphodiesterase, even though it can also hydrolyze cAMP; (ii) a calmodulin-insensitive form (peak II) that is relatively specific for cAMP. Further studies[4-6] with these two enzyme forms have revealed that derivatives of 3-isobutyl-1-methylxanthine (IBMX, **1a**) possessing an alkyl or aralkyl substituent in



a series, R=Me; R′=R″=H
b series, R=Me; R′=CH_2Ph; R″=H
c series, R=R′=Me; R″=H
d series, R=Me; R′=H; R″=*t*-Bu
e series, R=Me; R′=H; R″=CH_2OMe
f series, R=*i*-Am; R′=R″=H

the 7-position (e.g., **1b**) or an alkyl group in the 8-position (e.g., **1c–e**) are potent inhibitors of peak I phosphodiesterase whereas IBMX analogues in which the 1-methyl group is replaced by a larger alkyl substituent (e.g., **1f**) show preferential inhibition of the peak II phosphodiesterase. Such data have permitted the construction of hypothetical topographical representations[4] of the peak I and peak II active sites wherein the former can tolerate steric bulk at N-7 and C-8 while the latter, with the xanthine bound in a manner rotated 180° from the binding orientation of peak I, can accommodate steric bulk at N-1. In these representations both enzyme forms require the N-3 isobutyl substituent for effective inhibition of enzyme activity.

As part of a program to develop inhibitors that not only will be selective for the coronary artery phosphodiesterases but also will show tissue selectivity for the multiple forms of PDEs,[7-9] an effort was commenced to analyze the dimensional limitations for the binding regions in pig coronary artery phosphodiesterases. As one approach to this objective, the benzo-separated[10,11] analogues **2a–f** and **3a–f** were established as target compounds. The results of this part of the study are reported here.

Chemistry. The synthesis of the linear and proximal IBMX analogues (**2a**, **3a**) has been reported elsewhere.[12]

Benzylation of **2a** gave a mixture of **2b** and a trace (by TLC) of **4**. The predominance of **2b** from this transformation was assumed to be due to the steric influence of the isobutyl moiety on N-5 on benzylation at N-3 that resulted in limiting the amount of **4**. Compounds **2b** and **4** were distinguished by an unambiguous synthesis of **4** that began by treating 1-isobutyl-7-chloro-3-methyl-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (**5a**)[12] with benzylamine to obtain **5b**. Catalytic hydrogenation of **5b** in formic acid proceeded without debenzylation to produce **4**.

Reaction of the 5-chloro isomer of **5a** (that is, **6a**)[12] with benzylamine yielded **6b**. However, catalytic hydrogenation of **6b** in formic acid gave **3a** as a result of debenzylation to relieve the steric crowding in the "bay" region of **3**. To overcome this, **6b** was subjected to reduction with iron and

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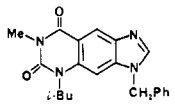

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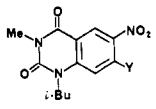
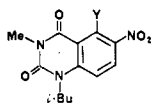
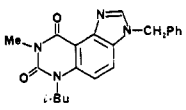


5a, Y=Cl
b, Y=NHCH$_2$Ph
c, Y=NH$_2$
d, Y=NHCOt-Bu



6a, Y=Cl
b, Y=NHCH$_2$Ph
c, Y=NH$_2$
d, Y=NHCOt-Bu



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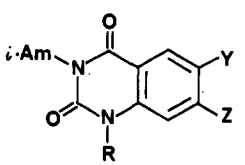
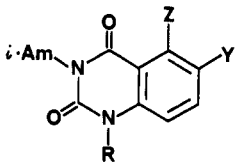
hydrochloric acid in the presence of formic acid. This pathway did yield the desired **3b**. For both synthetic and biological comparative purposes, the 3-benzyl isomer of **3b** (i.e., **7**) was prepared by benzylation of **3a**. In this synthesis, there was no indication (by TLC) of any of the more sterically hindered isomer **3b** being formed.

In an extension of the synthetic approach used for obtaining **3b** and **4**, the isomeric quinazolinediones **5c**[12] and **6c**[12] gave **2c** and **3c** and **2e** and **3e** upon catalytic hydrogenation in acetic acid and methoxyacetic acid, respectively. The synthesis of the 8-*tert*-butyl systems (**2d**, **3d**) required a modification of this route since no reaction occurred when the hydrogenation of **5c** and **6c** was conducted in trimethylacetic acid. Thus, reaction of **5c**[12] and **6c**[12] with trimethylacetyl chloride produced **5d** and **6d**, which underwent cyclization to **2d** and **3d** upon catalytic hydrogenation in ethanol.

The method used for obtaining **2f** and **3f** began with the reaction of methyl 2-amino-4-chlorobenzoate[13] and methyl 2-amino-6-chlorobenzoate[12] with isoamyl isocyanate. The resultant products (**8a**, **9a**) were nitrated to **8b** and **9b** followed by isobutylation to **8c** and **9c**. Amination of the latter two products (to **8d** and **9d**) with subsequent catalytic hydrogenation in formic acid gave the desired **2f** and **3f**.



8a, R=Y=H; Z=Cl
b, R=H; Y=NO$_2$; Z=Cl
c, R=i-Bu; Y=NO$_2$; Z=Cl
d, R=i-Bu; Y=NO$_2$; Z=NH$_2$



9a, R=Y=H; Z=Cl
b, R=H; Y=NO$_2$; Z=Cl
c, R=i-Bu; Y=NO$_2$; Z=Cl
d, R=i-Bu; Y=NO$_2$; Z=NH$_2$

Biological Results and Discussion. IBMX (**1a**) is one of the most potent phosphodiesterase inhibitors known, and most, but not all, of the benzo-separated analogues examined in this study were less effective inhibitors when compared to **1a** (Table I). Assuming[4] that the pyrimidine ring of the benzo-separated systems occupies the same area of the binding site as it does with the nonseparated prototypes, then the general decline in inhibitory potency observed for the **2** and **3** series may be the result of nonfavorable electronic and/or steric interactions between the imidazole portion of **2** and **3** and the binding site upon inhibitor complexation. In spite of this, the data of Table I demonstrate that the extension of the xanthine nucleus is tolerated in the active site of the enzyme forms since most of the stretched-out compounds are relatively good

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Table I. Inhibition of Peak I and Peak II Phosphodiesterases from Pig Coronary Artery by Benzo-Separated Xanthines

compd	I_{50},[a] μM peak I	I_{50},[a] μM peak II
1a	6.3 ± 0.7 (6)[b]	15 ± 2 (3)[b]
1b	4.4 ± 0.7 (7)[b]	108 ± 14 (4)[b]
1c	1.9 ± 0.1 (5)[c]	68 ± 4 (6)[c]
1d	1.8 ± 0.2 (7)[c]	58 ± 6 (6)[c]
1e	5.2 ± 1[d]	212 ± 37[d]
1f	86 ± 5 (7)[c]	17 ± 3 (6)[c]
2a	40 (2)	89 (2)
2b	1.2 ± 0.3 (4)	14 ± 1 (3)
2c	36 ± 2 (5)	>50[f]
2d	41 ± 5 (4)	>50[f]
2e	36 ± 4 (4)	>50[f]
2f	>25[e]	20 ± 2 (3)
3a	8.4 ± 0.8 (4)	18 ± 2 (3)
3b	19 ± 2 (3)	14 ± 3 (4)
3c	15 ± 1 (4)	>25[e]
3d	>50[f]	44 ± 5 (5)
3e	54 ± 3 (4)	66 ± 2 (3)
3f	>25[e]	19 ± 3 (5)
4	4.9 ± 0.1 (4)	27 ± 2 (4)
7	20 ± 1 (4)	13 ± 2 (5)

[a] Value ± SEM. Number of determinations shown in parentheses. [b] Data from ref 5. [c] Data from ref 6. [d] Data from ref 4. [e] Highest concentration used was 25 μM. At this concentration **2f** and **3f** inhibited peak I activity by 32 ± 6 and 19 ± 2%, respectively, and compound **3c** inhibited peak II activity by 45 ± 1%. [f] Highest concentration used was 50 μM. At this concentration **3d** inhibited peak I activity by 39 ± 3% and **2c–2e** inhibited peak II activity by 31 ± 1, 21 ± 1, and 27 ± 1%, respectively.

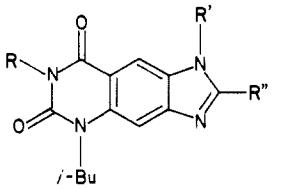
inhibitors of the phosphodiesterases. This is illustrated by (i) proximal benzo-IBMX (**3a**), whose potency is the same as that of IBMX itself, and (ii) **2b**, which is more potent in inhibiting peak I and peak II activities than the parent compound (**1b**).

In the case of the benzyl series, we previously reported[5] that the 7-benzyl substituent (**1b**) dramatically reduced the potency of IBMX (**1a**) to inhibit peak II activity but did not alter the potency for peak I inhibition. Similar trends are not observed when the data obtained from **2b** are compared to that for **2a**. In this case, the benzyl moiety induces improved potency with both enzyme forms. The reason for this difference, when compared to **1b** vs. **1a**, appears to be more complex than availing a new interactive center on the inhibitory site for the benzyl group of **2b** (which was not accessible to the benzyl of **1b**) as a result of benzo extension since molecular models indicate that the benzyl moiety of both of **1b** and **2b** could have access to the same hydrophobic sites on the enzyme. This reasoning could, however, account for the decreased inhibitory potency of **3b** (relative to **3a**) for peak I since molecular models indicate that the benzyl group of **3b** would be incapable of interacting with the same binding area as the benzyl of **1b** and **2b**. At the same time, it is interesting that **3b** is more potent than **1b** and equipotent to **2b** toward peak II.

Substitution of a benzyl group at the position corresponding to N-9 of IBMX gave **4**, which exhibited increased potency toward peaks I and II relative to **2a**, and **7**, which resulted in a reduced potency toward peak I and equipotency toward peak II when compared to **3a**. In view of the unavailability of 9-benzyl-IBMX, an analysis of these results is not currently possible.

The addition of substituents to position 8 on the imidazole ring of the parent IBMX (that is, **1c–e**) reduces the peak II inhibitory potency but has little effect on potency toward inhibition of peak I activity.[4] Within the series of extended IBMX analogues the trends are different when **2c–e** and **3c–e** are compared to **2a** and **3a**, respectively.

Table II. Alkylated Linear Benzoxanthines



compd	R	R'	R''	mp, °C	solvent[a]	yield, %	formula[b]	^{1}H NMR, δ (relative to Me_4Si)[c]
2b	Me	CH_2Ph	H	210–211	A	96[d]	$C_{21}H_{22}N_4O_2$	(Me_2SO-d_6) 0.91 (d, J = 6 Hz, 6 H, CH_3), 2.10 (m, 1 H, CH), 3.31 (s, 3 H, NCH_3), 4.00 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 5.54 (s, 2 H, CH_2 of benzyl), 7.28 (s, 5 H, C_6H_5), 7.61 (s, 1 H, H-4), 8.15 (s, 1 H, H-9), 8.61 (s, 1 H, H-2)
2c	Me	H	Me	279–282	A	82[d]	$C_{15}H_{18}N_4O_2 \cdot H_2O$	(Me_2SO-d_6) 0.91 (d, J = 6 Hz, 6 H, CH_3), 2.18 (m, 1 H, CH), 2.51 (s, 3 H, C-2 CH_3), 3.33 (s, 3 H, NCH_3), 3.95 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 7.29 (s, 1 H, H-4), 8.03 (s, 1 H, H-9), 12.4 (br s, 1 H, NH)
2d	Me	H	*t*-Bu	214–216	A	94[d]	$C_{18}H_{24}N_4O_2 \cdot H_2O$	($CDCl_3$) 0.95 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.38 (s, 9 H, $C(CH_3)_3$), 2.10 (m, 1 H, CH), 3.40 (s, 3 H, NCH_3), 3.90 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 7.62 (s, 1 H, H-4), 8.07 (s, 1 H, H-9), 8.28 (br s, 1 H, NH)
2e	Me	H	CH_2OMe	212–214	B	60[e]	$C_{16}H_{20}N_4O_3 \cdot H_2O$	(Me_2SO-d_6) 0.95 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 2.05 (m, 1 H, CH), 3.32 (s, 3 H, NCH_3), 3.40 (s, 3 H, OCH_3), 3.95 (d, J = 6 Hz, CH_2 of *i*-Bu), 4.62 (s, 2 H, CH_2 at C-2), 7.34 (s, 1 H, H-4), 8.12 (s, 1 H, H-9)
2f	*i*-Am	H	H	274–277	A	80[e]	$C_{18}H_{24}N_4O_2$	(Me_2SO-d_6) 0.92 (d, J = 6 Hz, 12 H, CH_3), 1.58 (m over m, 3 H, CH_2CH of *i*-Am), 2.15 (m, 1 H, CH of *i*-Bu), 4.02 (d over t, 4 H, CH_2 of *i*-Bu and *N*-CH_2 of *i*-Am), 7.56 (s, 1 H, H-4), 8.31 (s, 1 H, H-9), 8.42 (s, 1 H, H-2), 12.5 (br s, 1 H, NH)

[a] Recrystallization solvents: A, aqueous EtOH; B, aqueous MeOH. All compounds were obtained as either white or very light yellow crystals. [b] All of the compounds in the table gave satisfactory microanalysis for C, H, and N (±0.4%). [c] Spin multiplicities are given by the abbreviations s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). [d] Crude yield. [e] Recrystallized yield.

There is a display of equipotency vs. peak I and enhanced potency vs. peak II in the **2** series and decreased potency toward both peaks I and II in the **3** series. The reasons for this are unclear. The data do indicate, however, that the inhibitor binding sites of both enzyme forms are large enough to accommodate the extended systems possessing imidazole C substituents.

The inhibitory potency of the ring-extended analogues of the selective, non-IBMX peak II inhibitor 1-isoamyl-3-isobutylxanthine (**1f**) was determined on both forms of PDE. The preferred selectivity (that is, peak II inhibition > peak I inhibition) is lost in the benzo-separated systems. However, **2f** and **3f** are equipotent, when compared to **1f**, toward peak II, indicating that the isoamyl group at a position corresponding to N-1 of the xanthines must impart a distinctive binding ability to form II for the **f** series of inhibitors.

Conclusion. This report expands upon the structure–activity relationships previously described for the interactions of xanthine analogues with two forms of porcine coronary phosphodiesterase.[4-6,14] From this it is clear that the xanthine binding sites on the enzymes are able to accommodate the larger size of the benzo-separated xanthines, although the potencies of most analogues are decreased by the changes in structure described herein.

Experimental Section

General Methods. All melting points were obtained on a Thomas-Hoover or a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman AccuLab 3 spectrophotometer. The ^{1}H NMR spectra were determined at 60 MHz with a Varian EM-360 spectrometer and are reported in parts per million downfield from Me_4Si as an internal standard. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). The silica gel used for the column chromatographic separations was Baker, 60–200 mesh. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. The microanalyses are indicated by symbols of the elements, which indicate that the analytical results obtained for those elements were within ±0.4% of the theoretical values.

1-Benzyl-5-isobutyl-7-methylimidazo[4,5-*g*]quinazoline-6,8(5*H*,7*H*)-dione (2b). To a stirred mixture of 0.65 g (2.39 mmol) of **2a**, 0.34 g (2.46 mmol) of anhydrous K_2CO_3, and 10 mL of dry DMF was added 0.31 g (2.45 mmol) of benzyl chloride. This mixture was heated to 70 °C for 5 h. The insoluble salts were removed by filtration, and the filtrate was evaporated to dryness to give 0.83 g of crude material that contained mostly the desired product (**2b**) with only slightly detectable amounts of **4** seen on TLC analysis (AcOEt–hexane, 3:2). Compound **2b** was purified as white crystals and characterized as described in Table II.

7-(*N*-Benzylamino)-1-isobutyl-3-methyl-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (5b). A stirred suspension of **5a**[12] (1.5 g, 4.81 mmol) and benzylamine (1.5 g, 13.9 mmol) in 45 mL of 1-butanol was heated at reflux under N_2 for 24 h. The solution was then evaporated to dryness with the aid of rotary evaporator to give 1.8 g (4.7 mmol, 98%) of crude **5b**, which was recrystallized from EtOAc to give the desired product as yellow needles as described in Table IV.

3-Benzyl-5-isobutyl-7-methylimidazo[4,5-*g*]quinazoline-6,8(5*H*,7*H*)-dione (4). A mixture of 1.3 g (3.4 mmol) of **5b**, 150 mL of absolute EtOH, and a catalytic amount of 5% Pd/C was shaken under 52 psi of H_2 for 3 h. After removal of the catalyst by filtration, the filtrate was evaporated to dryness and to the residue was added 100 mL of 97% formic acid. After this mixture was refluxed under N_2 for 2 h, it was treated with decolorizing charcoal, filtered, and evaporated to dryness in vacuo. The resultant oil solidified upon the addition of H_2O and neutralization with solid $NaHCO_3$. The precipitate was then obtained by filtration, washed with H_2O, and dried to afford 0.7 g (1.96 mmol, 58%) of crude **4**, which was recrystallized from aqueous EtOH as white needles: mp 152–154 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.85 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.95 (m, 1 H, CH of *i*-Bu), 3.30 (s, 3 H, NCH_3), 3.96 (*d*, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 5.58 (s, 2

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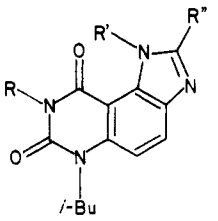
Table III. Alkylated Proximal Benzoxanthines



compd	R	R′	R″	mp, °C	solvent[a]	yield, %	formula[b]	^{1}H NMR, δ (relative to Me_4Si)[c]
3b	Me	CH_2Ph	H	134–135	A	58[d]	$C_{21}H_{22}N_4O_2 \cdot 1^1/_4H_2O$	(Me_2SO-d_6) 1.05 (d, J = 6 Hz, 6 H, 2 CH_3), 1.62 (m, 1 H, CH), 3.48 (s, 3 H, NCH_3), 4.12 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 5.90 (s, 2 H, CH_2 of benzyl) 7.10–7.40 (m, 7 H, C_6H_5, H-2 and H-5), 8.15 (d, J = 8 Hz, 1 H, H-4)
3c	Me	H	Me	173–175	A	79[e]	$C_{15}H_{18}N_4O_2$	(Me_2SO-d_6) 0.95 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 2.10 (m, 1 H, CH), 2.55 (s, C-2 CH_3), 3.36 (s, 3 H, NCH_3), 4.01 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 7.19 (d, J = 8 Hz, 1 H, H-5), 8.82 (d, J = 8 Hz, 1 H, H-4), 11.2 (br s, 1 H, NH)
3d	Me	H	*t*-Bu	214–215	A	61[e]	$C_{18}H_{24}N_4O_2$	($CDCl_3$) 0.98 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.52 (s, 9 H, $C(CH_3)_3$), 2.15 (m, 1 H, CH), 3.50 (s, 3 H, NCH_3), 4.03 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 6.98 (d, J = 8 Hz, 1 H, H-4), 7.95 (d, J = 8 Hz, 1 H, H-4), 10.52 (br s, 1 H, NH)
3e	Me	H	CH_2OMe	250–252	A	54[e]	$C_{16}H_{20}N_4O_3$	(Me_2SO-d_6) 0.93 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 2.09 (m, 1 H, CH), 3.34 (s, 3 H, NCH_3), 3.39 (s, 3 H, OCH_3), 4.05 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 4.64 (s, 2 H, CH_2 at C-2), 7.31 (d, J = 8 Hz, 1 H, H-5), 8.00 (d, J = 8 Hz, 1 H, H-4), 11.1 (br s, 1 H, NH)
3f	*i*-Am	H	H	211–212	A	60[d]	$C_{18}H_{24}N_4O_2$	(Me_2SO-d_6) 0.90 (d, J = 6 Hz, 12 H, CH_3), 1.40 (m over m, 4 H, CH of *i*-Bu and CH_2CH of *i*-Am), 3.90–4.20 (d over t, 4 H, CH_2 of *i*-Bu and *N*-CH_2 of *i*-Am), 7.35 (d, J = 8 Hz, 1 H, H-5), 8.05 (d, J = 8 Hz, 1 H, H-4), 8.18 (s, 1 H, H-2)

[a] Recrystallization solvent: A, aqueous EtOH. All compounds were obtained as either white or very light yellow crystals. [b] All compounds in this table gave satisfactory microanalysis for C, H, and N (±0.4%). [c] Spin multiplicities are given by the abbreviations s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). [d] Recrystallized yield. [e] Crude yield.

H, CH_2 of benzyl), 7.32 (s, 6 H, C_6H_5 and H-4), 8.29 (s, 1 H, H-9), 8.56 (s, 1 H, H-2). Anal. ($C_{21}H_{22}N_4O_2$) C, H, N.

5-(*N*-Benzylamino)-1-isobutyl-3-methyl-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (6b). A stirred suspension of **6a**[12] (2.2 g, 7.05 mmol) and benzylamine (2.2 g, 20.5 mmol) in 1-butanol (66 mL) was heated at reflux under N_2 for 24 h. The solution was then evaporated to dryness with the aid of a rotary evaporator, and to the residue was added petroleum ether (60–110 °C), which resulted in a gum. The mixture was evaporated again to dryness and the residue subjected to column chromatographic purification using toluene–AcOEt (9:1). The fractions containing the major band were combined and evaporated to dryness to give a yellow residue that was triturated with petroleum ether (60–110 °C). The resultant material was isolated by filtration and dried to give **6b** as described in Table IV.

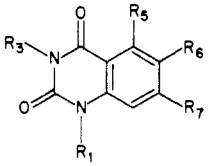
1-Benzyl-6-isobutyl-8-methylimidazo[4,5-*f*]quinazoline-7,9(6*H*,8*H*)-dione (3b). A heterogeneous mixture of 1 g (2.62 mmol) of **6b**, 0.88 g of Fe powder, 10 mL of concentrated hydrochloric acid, and 10 mL of 97% formic acid was refluxed for 2 h. At the completion of the reflux period, the clear solution was evaporated to dryness in vacuo and the residue neutralized with saturated Na_2CO_3 solution. The precipitate that formed was isolated by filtration, dried, and subjected to column chromatography using toluene–AcOEt (1:1) as the eluting mixture. By this means **3b** was obtained and further purified and characterized as presented in Table III.

3-Benzyl-6-isobutyl-8-methylimidazo[4,5-*f*]quinazoline-7,9(6*H*,8*H*)-dione (7). Following a procedure similar to that used in preparing **2b**, 0.48 g (3.79 mmol) of benzyl chloride was added to a stirred mixture of 1 g (3.67 mmol) of **3a**[12] and 0.51 g (3.53 mmol) of anhydrous K_2CO_3 in 15 mL of dry DMF. This mixture was then heated at 70 °C for 15 h. The insoluble salts that resulted were removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue was triturated with H_2O, isolated by filtration, and recrystallized from EtOH–H_2O to give 1.28 g (3.53 mmol, 96%) of **7** as white crystals: mp 130–132 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.9 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.55 (m, 1 H, CH of *i*-Bu), 3.35 (s, 3 H, NCH_3), 4.05 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 6.19 (s, 2 H, CH_2 of benzyl), 6.85–7.2 (m, 5 H, C_6H_5), 7.3 (d, J = 6 Hz, 1 H, H-5), 8.05 (d, J = 6 Hz, 1 H, H-4), 8.35 (s, 1 H, H-2). Anal. ($C_{21}H_{22}N_4O_2 \cdot {}^3/_4H_2O$) C, H, N.

5-Isobutyl-2,7-dimethylimidazo[4,5-*g*]quinazoline-6,8-(5*H*,7*H*)-dione (2c), 5-Isobutyl-2-(methoxymethyl)-7-methylimidazo[4,5-*g*]quinazoline-6,8(5*H*,7*H*)-dione (2e), 6-Isobutyl-2,8-dimethylimidazo[4,5-*f*]quinazoline-7,9-(6*H*,8*H*)-dione (3c), and 6-Isobutyl-2-(methoxymethyl)-8-methylimidazo[4,5-*f*]quinazoline-7,9(6*H*,8*H*)-dione (3e). A mixture of 1 g (3.42 mmol) of **5c**[12] (for **2c** and **2e**) or **6c**[12] (for **3c** and **3e**) in 45–50 mL of AcOH (for **2c** and **3c**) or methoxyacetic acid (for **2e** and **3e**) and a catalytic amount of 10% Pd/C was shaken under 52 psi of H_2 for 4–7 h. After removal of the catalyst by filtration, the filtrate was refluxed for 6–12 h under N_2 and then treated, if necessary, with decolorizing charcoal. After filtration, the filtrate was evaporated to dryness with the aid of a rotary evaporator and to the residue was added 10 mL of H_2O. Neutralization of this mixture with solid Na_2CO_3 resulted in a precipitate that was isolated by filtration, washed with H_2O, and purified and characterized as **2c**, **2e**, **3c**, and **3e** as described in Tables II and III.

1-Isobutyl-3-methyl-7-(trimethylacetamido)-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (5d). A mixture of 1 g (3.42 mmol) of **5c**[12] in 25 mL of trimethylacetyl chloride was refluxed for 3 h under the exclusion of moisture and then evaporated to dryness under reduced pressure. The residue was suspended in petroleum ether (60–110 °C) and filtered to afford 1.2 g (3.18 mmol, 93%) of crude **5d**, which was recrystallized from EtOH–petroleum ether (60–110 °C) as yellow crystals: mp 169–170 °C; IR (KBr) 1650 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.32 (s, 9 H, $C(CH_3)_3$), 2.18 (m, 1 H, CH of *i*-Bu), 3.39 (s, 3 H, NCH_3), 3.90 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu),

Table IV. Substituted Quinazoline-2,4(1*H*,3*H*)-diones



compd	R_1	R_3	R_5	R_6	R_7	mp, °C	solvent[a]	yield, %	formula[b]	^{1}H NMR, δ (relative to Me_4Si)[c]
5b	*i*-Bu	Me	H	NO_2	$NHCH_2Ph$	158–160	C	98[d]	$C_{20}H_{22}N_4O_4$	(Me_2SO-d_6) 0.67 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.38 (m, 1 H, CH), 3.19 (s, 3 H, NCH_3), 3.66 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 4.69 (d, J = 6 Hz, 2 H, CH_2 of benzyl), 6.15 (s, 1 H, H-8), 7.30 (m, 5 H, C_6H_5), 8.61 (s, 1 H, H-5), 9.02 (br s, 1 H, NH)
6b	*i*-Bu	Me	$NHCH_2Ph$	NO_2	H	141–142	E	49[e]	$C_{20}H_{22}N_4O_4$	($CDCl_3$) 1.00 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 2.19 (m, 1 H, CH), 3.40 (s, 3 H, NCH_3), 3.92–4.19 (d over d, 4 H, CH_2 of *i*-Bu and benzyl), 6.40 (d, J = 8 Hz, 1 H, H-8), 7.28 (m, 5 H, C_6H_5), 8.02 (d, J = 8 Hz, 1 H, H-7), 10.56 (br s, 1 H, NH)
8a	H	*i*-Am	H	H	Cl	262–265	D	65[d]	$C_{13}H_{15}ClN_2O_2$	(Me_2SO-d_6) 0.89 (d, J = 6 Hz, 6 H, CH_3), 1.30–1.60 (m over m, 3 H, CH_2CH), 3.81 (t, J = 6 Hz, NCH_2), 7.10–7.17 (d over d, 2 H, H-6 and H-8), 7.8 (d, J = 8 Hz, 1 H, H-5)
8b	H	*i*-Am	H	NO_2	Cl	204–206	C	73[d]	$C_{13}H_{14}ClN_3O_4$	(Me_2SO-d_6) 0.91 (d, J = 6 Hz, 6 H, CH_3), 1.28–1.74 (m over m, 3 H, CH_2CH), 3.83 (t, J = 6 Hz, 2 H, NCH_2), 7.26 (s, 1 H, H-8), 8.42 (s, 1 H, H-5), 11.91 (br s, 1 H, NH)
8c	*i*-Bu	*i*-Am	H	NO_2	Cl	110–111	C	44[e]	$C_{17}H_{22}ClN_3O_4 \cdot {}^1/_2H_2O$	($CDCl_3$) 0.88–1.05 (d over d, 12 H, CH_3), 1.25–1.84 (m over m, 3 H, CH_2CH of *i*-Am), 2.04 (m, 1 H, CH of *i*-Bu), 3.88–4.16 (t over d, 4 H, NCH_2 of *i*-Am and *i*-Bu), 7.21 (s, 1 H, H-8), 8.75 (s, 1 H, H-5)
8d	*i*-Bu	*i*-Am	H	NO_2	NH_2	144–146	E	74[d]	$C_{17}H_{24}N_4O_4$	($CDCl_3$) 0.92–1.02 (d over d, 12 H, CH_3), 1.30–1.75 (m over m, 3 H, CH_2CH of *i*-Am), 2.10 (m, 1 H, CH of *i*-Bu), 3.74–4.12 (t over d, 4 H, NCH_2 of *i*-Am and *i*-Bu), 6.28 (s, 1 H, H-8), 6.43 (br s, 2 H, NH_2), 9.01 (s, 1 H, H-5)
9a	H	*i*-Am	Cl	H	H	194–195	D	54[e]	$C_{13}H_{15}ClN_2O_2$	(Me_2SO-d_6) 0.90 (d, J = 6 Hz, CH_3), 1.32–1.80 (m over m, 3 H, CH_2CH of *i*-Am), 3.82 (t, J = 6 Hz, NCH_2 of *i*-Am), 7.02–7.40 (d over t, 2 H, H-7 and H-8), 7.62 (d, J = 8 Hz, 1 H, H-6)
9b	H	*i*-Am	Cl	NO_2	H	218–221	A	48[e]	$C_{13}H_{14}ClN_3O_4$	($CDCl_3$) 0.90 (d, J = 6 Hz, CH_3), 1.40–1.80 (m over m, 3 H, CH_2CH of *i*-Am), 3.90 (t, J = 6 Hz, 2 H, NCH_2 of *i*-Am), 7.20 (d, J = 8 Hz, 1 H, H-8), 7.80 (d, J = 8 Hz, 1 H, H-7)
9c	*i*-Bu	*i*-Am	Cl	NO_2	H	100–101	A	52[e]	$C_{17}H_{22}ClN_3O_4$	($CDCl_3$) 0.90–1.02 (d over d, 12 H, CH_3), 1.40–2.10 (m over m, 4 H, CH_2CH of *i*-Am and CH of *i*-Bu), 3.80–4.20 (t over d, 4 H, NCH_2 of *i*-Am and *i*-Bu), 7.15 (d, J = 8 Hz, 1 H, H-8), 7.88 (d, J = 8 Hz, 1 H, H-7)
9d	*i*-Bu	*i*-Am	NH_2	NO_2	H	165–166	*f*	84[e]	$C_{17}H_{24}N_4O_4$	($CDCl_3$) 0.90–1.02 (d over d, 12 H, CH_3), 1.40–2.10 (m over m, 4 H, CH_2CH of *i*-Am and CH of *i*-Bu), 3.80–4.20 (t over d, 4 H, NCH_2 of *i*-Am and *i*-Bu), 6.40 (d, J = 8 Hz, 1 H, H-8), 8.45 (d, J = 8 Hz, 1 H, H-7), 8.8 (br s, 1 H, NH_2)

[a] Recrystallization solvent: A, aqueous EtOH; C, AcOEt; D, aqueous DMF; E, EtOH. All compounds were obtained as either white or light yellow crystals. [b] All compounds in this table gave satisfactory microanalysis for C, H, and N (±0.4%). [c] Spin multiplicities are given by the abbreviations s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). [d] Crude yield. [e] Recrystallized yield. [f] Compound obtained analytically pure from the reaction.

8.86 (s, 1 H, H-8), 9.00 (s, 1 H, H-5), 11.1 (br s, 1 H, NH). Anal. ($C_{18}H_{24}N_4O_5$) C, H, N.

1-Isobutyl-3-methyl-5-(trimethylacetamido)-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (6d). By using a procedure similar to that employed for preparing **5d**, a mixture of 0.85 g (2.9 mmol) of **6c**[12] and 50 mL of trimethylacetyl chloride gave 0.75 g (2.02 mmol, 69%) of **6d** as light yellow needles after recrystallization from aqueous EtOH: mp 132–133 °C; IR (KBr) 1650 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.86 (d, J = 6 Hz, 6 H, CH_3 of *i*-Bu), 1.24 (s, 9 H, $C(CH_3)_3$), 1.90 (m, 1 H, CH of *i*-Bu), 3.28 (s, 3 H, NCH_3), 3.95 (d, J = 6 Hz, 2 H, CH_2 of *i*-Bu), 7.29 (d, J = 8 Hz, 1 H, H-8), 8.10 (d, J = 8 Hz, 1 H, H-7), 12.0 (br s, 1 H, NH). This compound was not subjected to microanalysis but used directly for the synthesis of **3d**, which did give a satisfactory analysis.

5-Isobutyl-2-*tert*-butyl-7-methylimidazo[4,5-*g*]quinazoline-6,8(5*H*,7*H*)-dione (2d). A mixture of 1.7 g (4.51 mmol) of **5d** in 100 mL of absolute EtOH containing a catalytic amount of 10% Pd/C was shaken under 52 psi of H_2 for 6 h. The catalyst was removed by filtration and the filtrate refluxed for 4 h under N_2. The solution was then evaporated to dryness to give **2d**, which was purified and characterized as described in Table II.

6-Isobutyl-2-*tert*-butyl-8-methylimidazo[4,5-*f*]quinazoline-7,9(6*H*,8*H*)-dione (3d). A mixture of 0.75 g (1.99 mmol) of **6d** and a catalytic amount of 10% Pd/C in 100 mL of absolute EtOH was shaken under 52 psi of H_2 for 12 h. The mixture was filtered to remove the catalyst, and the filtrate was saturated with anhydrous HCl. The mixture was refluxed for 4 h and then evaporated to dryness with the aid of a rotary evaporator. The resulting oil was neutralized with saturated $NaHCO_3$ solution to give a light yellow precipitate that was isolated by filtration, washed with H_2O, and dried to give **3d** as a light yellow solid (see Table III).

3-Isoamyl-7-chloroquinazoline-2,4(1*H*,3*H*)-dione (8a). The isoamyl isocyanate used in this synthesis was prepared by refluxing a mixture of 25 mL of 4-methylvaleryl chloride,[15] 12 g (184.5 mmol) of NaN_3, and 200 mL of dry C_6H_6 for 36 h with the exclusion of moisture. After cooling, the salts were removed by filtration and the filtrate was evaporated in vacuo to leave a liquid residue that was added to a mixture containing 7.5 g (40.4 mmol) of methyl 2-amino-4-chlorobenzoate,[13] 50 mL of dry toluene, and 2 mL of Et_3N. The new mixture was refluxed in an oil bath at 110–120 °C for 24 h with the exclusion of moisture. After cooling to room temperature, the white precipitate was isolated by filtration, washed with Et_2O, and dried to give crude **8a** (Table IV). It was of sufficient purity for use in the synthesis of **8b**.

3-Isoamyl-7-chloro-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (8b). A mixture of 7 g (26.24 mmol) of **8a** in 26 mL of concentrated H_2SO_4 was cooled to −10 °C with mechanical stirring. To the mixture was added, dropwise, 1.03 mL of fuming HNO_3 at such a rate that the temperature of the mixture did not rise above −10 °C. When the addition was completed, the mixture was allowed to warm to room temperature and then heated on a steam bath for 10 min. The mixture was then poured onto ice and neutralized with solid Na_2CO_3–H_2O. The resulting precipitate was isolated by filtration and dried to give **8b** as described in Table IV.

3-Isoamyl-1-isobutyl-7-chloro-6-nitroquinazoline-2,4-(1*H*,3*H*)-dione (8c). To a stirred mixture of 3.2 g (10.26 mmol) of **8b** and 1.45 g (10.49 mmol) of anhydrous K_2CO_3 in 30 mL of dry DMF was added 2 g (10.9 mmol) of 1-iodo-2-methylpropane,[16] and the mixture was heated at 80 °C for 20 h. The resultant insoluble salts were removed by filtration, and the filtrate was evaporated to dryness. The residue thus obtained was chromatographed on a silica gel column using a hexane–AcOEt (9:1) solvent system. The first band was collected, the solvent removed, and the residue purified and characterized as light yellow needles of **8c** (see Table IV).

7-Amino-3-isoamyl-1-isobutyl-6-nitroquinazoline-2,4-(1*H*,3*H*)-dione (8d) and 5-Amino-3-isoamyl-1-isobutyl-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (9d). A mixture of 1 g (2.72 mmol) of **8c** or **9c** in 10 mL of EtOH saturated with NH_3 was heated in a sealed, stainless-steel reaction vessel at 130 °C for 24 h. After the vessel was cooled in a freezer for 1 h, the mixture was filtered and the solid thus obtained was washed with petroleum ether (60–110 °C) and purified and characterized as **8d** and **9d** (see Table IV).

7-Isoamyl-5-isobutylimidazo[4,5-*g*]quinazoline-6,8-(5*H*,7*H*)-dione (2f) and 8-Isoamyl-6-isobutylimidazo[4,5-*f*]quinazoline-7,9(6*H*,8*H*)-dione (3f). A mixture of 0.94 g (2.7 mmol) of **8d** or **9d** and 50 mL of 97% formic acid, to which a catalytic amount of 10% Pd/C had been added under N_2, was shaken under 52 psi of H_2 for 18 h. The catalyst was removed by filtration and the filtrate refluxed for 2 h under N_2. The excess formic acid was removed in vacuo, and 50 mL of toluene was added to the residue. The new mixture was refluxed for 30 min under N_2 and the solvent removed in vacuo to give crude **2f** or **3f**, which was purified and characterized as described in Tables II and III, respectively.

3-Isoamyl-5-chloroquinazoline-2,4(1*H*,3*H*)-dione (9a). A solution of 10.2 g (44.8 mmol) of methyl 2-acetamido-6-chlorobenzoate[12] in 380 mL of MeOH, which had been previously saturated with anhydrous HCl, was refluxed for 1 h. The white residue, which resulted after the solvents were removed in vacuo, was suspended in Et_2O, and to this was added saturated Na_2CO_3 solution. The aqueous layer was obtained and extracted again with Et_2O. The Et_2O solutions were combined, dried over anhydrous $CaCl_2$, and filtered, and the filtrate was evaporated to dryness to give an oily residue. To this residue was added 30 mL of dry toluene, 1 mL of Et_3N, and isoamyl isocyanate obtained from 25 mL of 4-methylvaleryl chloride (see the preparation of **8a**). This mixture was refluxed for 3 days, and the solvents were removed in vacuo to give crude **9a**, which was purified and characterized as described in Table IV.

3-Isoamyl-5-chloro-6-nitroquinazoline-2,4(1*H*,3*H*)-dione (9b). A mixture of 5.95 g (22.3 mmol) of **9a** in 22 mL of concentrated H_2SO_4 was cooled to −10 °C with mechanical stirring. To the mixture was added, dropwise, 0.88 mL of fuming HNO_3 at such a rate that the temperature of the mixture did not rise above −10 °C. When the addition was completed, the mixture was allowed to warm to room temperature and then heated on a steam bath for 10 min. The mixture was poured onto ice and the resulting precipitate isolated by filtration and resuspended in H_2O. The suspension was neutralized with solid $NaHCO_3$. The solid was again obtained by filtration, washed with H_2O, and dried to give crude **9b**, which was purified and characterized as described in Table IV.

3-Isoamyl-1-isobutyl-5-chloro-6-nitroquinazoline-2,4-(1*H*,3*H*)-dione (9c). By using the same procedure as described for preparing **8c**, treating 5.21 g (16.7 mmol) of **9b** and 2.36 g (17.08 mmol) of anhydrous K_2CO_3 in 49 mL of dry DMF with 3.26 g (17.7 mmol) of 1-iodo-2-methylpropane gave **9c** (see Table IV) as the third band following chromatography.

Phosphodiesterase Assays. The two major forms of phosphodiesterases (peaks I and II) were isolated from porcine coronary arteries and assayed as described previously.[5,6,17] Briefly, the media plus intima layers of porcine coronary arteries were obtained as previously described[14] and homogenized in 4 mL/g (wet weight) of a solution containing 20 mM Tris-HCl (pH 7.5), 2 mM $Mg(OAc)_2$, and 1 mM dithiothreitol at 4 °C. The homogenate was centrifuged for 20 min at 48000*g* at 4 °C. DEAE-cellulose chromatography of the soluble fraction was conducted as described previously.[17] Peak fractions (peaks I and II) were pooled and dialyzed against 20 mM Tris-HCl (pH 7.5) containing 2 mM $Mg(OAc)_2$ and 1 mM dithiothreitol. These preparations were then stored at −70 °C in small aliquots. Assays were performed with 1 μM substrate (cGMP for peak I and cAMP for peak II) at 30 °C for 30 min at enzyme dilutions that gave 10–20% hydrolysis. Peak I activity was assayed in the presence of calcium + calmodulin. Xanthine analogues (2.5 mM) were dissolved in 75% Me_2SO, and the final concentration of Me_2SO in the assay was 3%. This level of Me_2SO does not affect inhibition of the two phosphodiesterase forms by IBMX or papaverine.[5] Values for

(15) Available from Fisher Scientific.
(16) Available from Aldrich Chemical Co.
(17) Keravis, T. M.; Wells, J. N.; Hardman, J. G. *Biochim. Biophys. Acta.* **1980**, *613*, 116.

I_{50}, defined here as the concentration of drug that inhibits hydrolysis of 1 μM substrate by 50%, were determined by using the xanthine analogues at concentrations between 1 and 100 μM. Due to limited solubility or limited degree of inhibition, I_{50} values could not be established for some analogues, and the levels of inhibition at 25 or 50 μM were determined. The presence or absence of calcium + calmodulin did not affect the level of inhibition of the peak I enzyme by the xanthine analogues. None of the analogues affected the 5'-nucleotidase or subsequent steps of the assay. IBMX inhibited the activity of peaks I and II with I_{50} values of 6.6 and 13.3 μM, respectively, which are similar to values reported previously.[4]

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Registry No. **2a**, 101031-51-0; **2b**, 101031-52-1; **2c**, 101031-53-2; **2d**, 101031-54-3; **2e**, 101031-55-4; **2f**, 101031-56-5; **3a**, 101031-57-6; **3b**, 101031-58-7; **3c**, 101031-59-8; **3d**, 101031-60-1; **3e**, 101031-61-2; **3f**, 101031-62-3; **4**, 101031-63-4; **5a**, 101031-64-5; **5b**, 101031-65-6; **5c**, 101031-66-7; **5d**, 101031-67-8; **6a**, 101031-68-9; **6b**, 101031-69-0; **6c**, 101031-70-3; **6d**, 101031-71-4; **7**, 101031-72-5; **8a**, 101031-73-6; **8b**, 101031-74-7; **8c**, 101031-75-8; **8d**, 101031-76-9; **9a**, 101031-77-0; **9b**, 101031-78-1; **9c**, 101031-79-2; **9d**, 101031-80-5; trimethylacetyl chloride, 3282-30-2; isoamyl isocyanate, 1611-65-0; 4-methylvaleryl chloride, 38136-29-7; methyl 2-amino-4-chlorobenzoate, 5900-58-3; 1-iodo-2-methyl propane, 513-38-2; methyl 2-acetamido-5-chlorobenzoate, 70625-65-9.

Synthesis and Antimitogenic Activities of Four Analogues of Cyclosporin A Modified in the 1-Position

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Cyclosporin A (CSA, 1), an immunosuppressive cyclic undecapeptide, contains a unique N-methylated amino acid, (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(*N*-methylamino)-6-octenoic acid, called both C-9-ene and MeBmt [(4*R*)-*N*-methyl-4-butenyl-4-methylthreonine] that may be essential for the biological activity of CSA. In order to determine the minimal portion of MeBmt needed for antimitogenic activity, four analogues of CSA specifically modified in the 1-position have been synthesized. These are (MeThr^1)CSA (**4**), (MeAbu^1)CSA (**5**), (MeAbu^1,Sar^{10})CSA (**6**), and [(MeLeu(3-OH)^1)]CSA (**7**). The synthesis of analogues was carried out by forming a linear undecapeptide that was cyclized at the two non-N-methylated amino acids. The structure of cyclic analogues **4**–**7** and their corresponding precursors were established unequivocally by ^{1}H NMR, FAB mass spectrometry, elemental analysis, and HPLC. The inhibition of Con A stimulated thymocytes by CSA (1), DH-CSA (**2**), **7**, **4**, **5**, and **6** gave IC_{50}'s (nM) of 4, 10, 600, 8×10^3, 15×10^3, and 40×10^3, respectively. The increase in IC_{50} by modification of the side chain in MeBmt suggested the importance of this amino acid in the 1-position of CSA for full antimitogenic activity.

Cyclosporin A (CSA) 1,[1] a unique and unusually effective immunosuppressive (IS) agent[2] isolated from the fungal species *Tolypocladium inflatum Gams*,[3] is a neutral, homodetic, hydrophobic cyclic peptide. The structure of CSA, established by chemical degradation[4] as well as by X-ray crystallographic analysis of an iodo derivative,[5] contains 11 amino acids, seven of which are N-methylated (Figure 1a). All amino acids have the 2*S* configuration except for the D-Ala at position 8 which has the *R* configuration. All cyclosporins isolated to date contain a unique N-methylated amino acid at position 1, (2*S*,3*R*,4*R*,6*E*)-3-hydroxy-4-methyl-2-(*N*-methylamino)-6-octenoic acid, initially called C-9-ene and more recently MeBmt [(4*R*)-*N*-methyl-4-butenyl-4-methyl-L-threonine]. X-ray crystallographic analysis of an iodo derivative of CSA revealed that a major portion of the molecule (residues 1–6) adopts an antiparallel β-pleated sheet conformation that contains three transannular hydrogen bonds; these include the NH of Abu hydrogen bonded to the C=O of Val, the NH of Val to the C=O of Abu, and the NH of Ala-7 to the C=O of MeVal. A fourth hydrogen bond is found as a γ-turn between the NH of D-Ala-8 to the C=O of MeLeu-6. Residues 7–11 form an open-loop featuring a cis amide bond between the MeLeu residues 9 and 10.[5a] The structural features are also found in the solution and X-ray structures of CSA.[5b]

Wenger has reported detailed synthetic procedures for the syntheses of MeBmt[6] and CSA[7] and biological data for several analogues of CSA in which positions 1, 2, 3, and 11 are modified (see Figure 1b).[8] Of particular interest were the effects on biological activity of modifying the MeBmt residue. While dihydro CSA (**2**, DH-CSA), formed by hydrogenation of the double bond, retains high biological activity, the deshydroxy analogue **3** and the (MeThr^1)CSA **4** analogues do not, so that a major portion of the unusual amino acid MeBmt appears to be essential for high biological activity. In order to further evaluate

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