5326-47-6; 6-iodoisatoic anhydride, 116027-10-2; ethyl bromoacetate, 105-36-2; *p*-iodoaniline, 540-37-4; 5-hydroxy-2-nitrobenzaldehyde, 42454-06-8; 2-chloroethyl *p*-toluenesulfonate, 80-41-1; 6-chlorohexanol acetate, 40200-18-8; 5-(benzyloxy)-2-nitrobenzoic acid, 61340-15-6; 5-(benzyloxy)-2-nitrobenzaldehyde, 58662-54-7; methyl 5-(benzyloxy)-2-nitrobenzoate, 116027-16-8; methyl 5-(benzyloxy)anthranilate, 116027-17-9; methyl 5-(benzyloxy)-2-ureidobenzoate, 116027-18-0; 6-(benzyloxy)-2,4-dichloroquinazoline, 116027-19-1; 6-(benzyloxy)quinazoline-2,4-(1*H*,3*H*)-dione, 116027-20-4; 6-(benzyloxy)-2-chloro-3,4-dihydroquinazoline, 116027-21-5; ethyl [6-(benzyloxy)-2-chloro-3,4-dihydroquinazolin-3-yl]acetate, 116027-22-6.

Inhibitors of Cyclic AMP Phosphodiesterase. 4. Synthesis and Evaluation of Potential Prodrugs of Lixazinone (*N*-Cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)-oxy]butyramide, RS-82856)[1]

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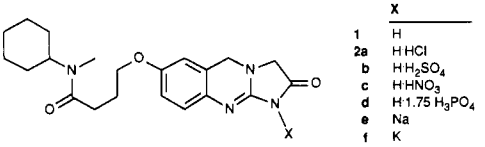
The cyclic AMP phosphodiesterase (cAMP PDE) inhibitor and cardiotonic agent lixazinone (*N*-cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide, RS-82856, 1) and its acid and base addition salts were found to be insufficiently soluble in formulations suitable for intravenous administration. These results prompted an investigation into potential prodrugs with enhanced aqueous solubility designed to deliver 1 by three distinct mechanisms: (1) decarboxylation of α-carboxamides; (2) hydrolytic loss of a solubilizing N-1-(acyloxy)methyl or (*N*,*N*-dialkylamino)methyl moiety; or (3) intramolecular closure of a guanidino ester or amide. The target compounds were evaluated as delivery systems for 1 by three criteria: (1) chemical conversion rate to 1 under physiological conditions; (2) inhibition of type IV cAMP PDE at a fixed time point; and (3) in vivo inotropic activity in anesthetized dogs by both intravenous and oral administration. Release of 1 from **4a** (series 1) was found to be too slow to be of value as a prodrug of 1, since decarboxylation could be induced only by strong acid, conditions under which hydrolytic ring opening was found to severely compete. Conversely, 1 was released too readily on exposure of (*N*,*N*-dialkylamino)methyl derivatives such as **8d** (series 2) to physiological conditions, although no large increase in aqueous solubility was realized. Finally, both the physicochemical and in vitro studies indicated that ring closure of the guanidinium esters and amides **17a–k** (series 3) to 1 was quantitative and pH- and time-dependent, suggesting the possibility of delivery of the open, water-soluble prodrug form, followed by closure to 1 in plasma. Detailed examination of these agents in vivo, however, demonstrated that only those compounds that rapidly cyclized to 1, as measured by plasma levels of 1, exhibited inotropic activity, indicating that the open prodrug form was not efficiently absorbed upon oral administration.

Selective inhibition of the high-affinity, cyclic AMP specific form (type IV) of phosphodiesterase (cAMP PDE) present in myocardial tissue is generally recognized to be the mechanism of action of a new generation of cardiotonic agents that exhibit a combination of positive inotropic, peripheral vasodilatory, and afterload reducing properties.[2-6] Recent papers have described the development of a generalized model of the active site of the type IV PDE that both accommodates this class of inhibitors and discriminates between cAMP and cGMP.[7-10] One demand of this active-site model made on potential inhibitors is the presence of an acidic hydrogen directly adjacent to a polarizable functionality, a structural requirement met by all representative members of this class. While this functional attribute is no doubt necessary for activity, it simultaneously creates a polar molecule capable of intermolecular hydrogen bonding, most often physically manifested by a high melting point and extreme insolubility, especially in physiologically relevant media.



We have previously described both the biochemical profile of one member of this class, *N*-cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]-

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(1) Contribution No. 285 from the Institute of Bio-Organic Chemistry. For part 3, see ref 21. Address inquiries to Dr. R. Alvarez.
(2) Bristol, J. A.; Chorvat, R. J. *Cardiovascular Drugs*; Bristol, J. A., Ed.; Wiley: New York, 1986; Chapter 6, p 255.
(3) Erhardt, P. W. *J. Med. Chem.* **1987**, *30*, 231.
(4) Weishaar, R. E.; Cain, M. H.; Bristol, J. A. *J. Med. Chem.* **1985**, *28*, 537.
(5) Weishaar, R. E.; Burrows, S. D.; Kobylarz, D. C.; Quade, M. M.; Evans, D. B. *Biochem Pharmacol.* **1986**, *35*, 787.
(6) Ahn, H. S.; Eardley, D.; Watkins, R.; Prioli, N. *Biochem. Pharmacol.* **1986**, *35*, 1113.
(7) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099.
(8) Rakhit, S.; Marciniak, G.; Leclerc, G.; Schwartz, J. *Eur. J. Med. Chem.-Chim. Ther.* **1986**, *21*, 511.
(9) Erhardt, P. W.; Hagedorn, A. A., III; Sabio, M. *Mol. Pharmacol.* **1988**, *33*, 1.
(10) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A.; McPhail, A. T. *J. Med. Chem.* **1987**, *30*, 1963.

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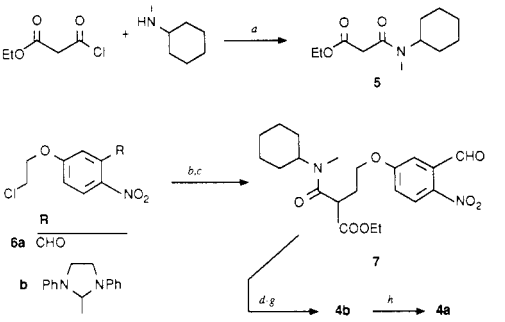
quinazolin-7-yl)oxy]butyramide (lixazinone, RS-82856, 1), and a widely varied series of analogues, as inhibitors of platelet and cardiac type IV cAMP PDE, and of ADP-induced platelet aggregation,[11,12] and the pharmacological actions of 1 as a potential cardiotonic and antithrombotic agent.[13] This compound satisfies the active-site structural requirement noted above, but displays the associated lack of solubility in aqueous media at pH values compatible with acceptable parenteral formulation.[14,15] In this paper, we describe our efforts to develop prodrug delivery systems, soluble in aqueous media, designed to provide effective plasma levels of 1 either upon intravenous administration or after gut absorption upon oral administration. Thus, in addition to both acid- and base-addition salts of 1, the compounds prepared as potential prodrugs of 1 fall into three classes: (1) α-carboxamides, designed to release 1 upon decarboxylation; (2) N-1 (*N,N*-dialkylamino)methyl or (acyloxy)methyl adducts, to provide 1 on exposure to gastric pH; and (3) ring-opened derivatives of 1, particularly guanidinium esters and amides, designed to yield 1 upon cyclization at the neutral to basic pH levels present in the gut and colon.

Chemistry

The weakly basic *N*-acylguanidine moiety of 1 (pK_a = 3.5)[15] limits the choice of acid addition salts to those formed from strong acids, such as hydrochloride (**2a**), hydrogen sulfate (**2b**), nitrate (**2c**), and hydrogen phosphate (**2d**). This same functionality is also weakly acidic (pK_a = 11.3),[15] allowing the preparation of only the sodium (**2e**) and potassium (**2f**) salts with facility. Both acid- and base-addition salts were prepared by suspension of 1 in methanol, followed by treatment with the appropriate reagent, and precipitation with ether, as precedented by the previous preparation of **2e**.[12] Preformulation studies on the parent drug (1) and its salts (**2e–e**)[14,15] were somewhat discouraging, since the weak acid–base character of 1 effectively eliminated the use of counterions, such as carboxylates or trialkylamines, which may have enhanced aqueous solubility by major disruption of crystal lattice stabilization. Because of this limitation, both the acid- and base-addition salts offered less than 1 order of manitude improvement in kinetic aqueous solubility versus the free base over a useful pH range. Even this advantage was defeated, however, since under thermodynamic conditions the free base preferentially precipitated from any such solution within days.[15]

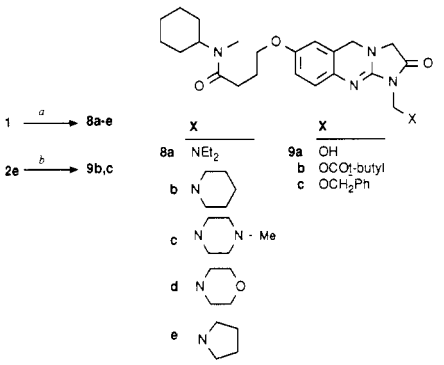
The results immediately prompted investigations into the design of chemical delivery systems for 1 possessing enhanced aqueous solubility. The structure–activity relationships derived from cAMP PDE and platelet aggregation inhibition studies[11,12] and from inotropic studies in anesthetized dogs[16] revealed little tolerance for substitution on the parent heterocycle, but a somewhat greater latitude for bulk on the oxybutyramide side chain. These restrictions left few, if any, positions for appendage of solubilizing groups permanently attached to the parent structure and eliminated the possibility of attachment of such groups to other, less active analogs of 1 potentially capable of functionalization. Instead, we first focused on approaches that would release 1 by chemical breakdown after absorption and localization in the plasma, by either decarboxylation or hydrolysis of labile adducts.

Scheme I

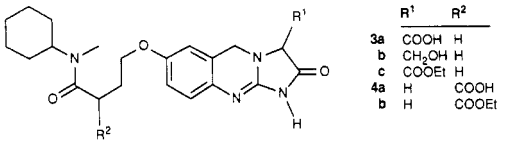


Reagents: a, Et_3N/THF; b, $PhNH(CH_2)_2NHPh$/HOAc/MeOH; c, NaH/bu_4NI/THF; d, GlyOEt·HCl/$NaBH_3CN$/NaOAc/EtOH; e, H_2/Pd-C/EtOH; f, BrCN; g, NH_4OH; h, KOH/EtOH.

Scheme II



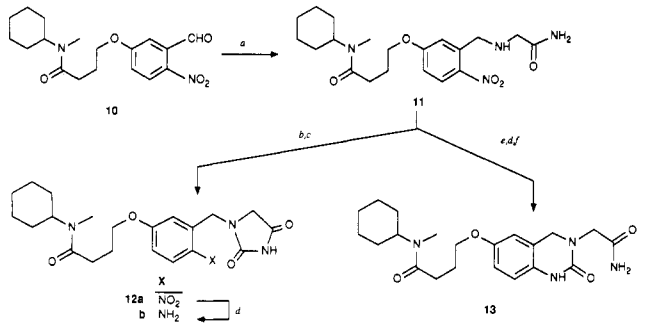
Reagents: a, dialkylamine/formalin/THF; b, $ClCH_2X$/DMF



Decarboxylation to produce 1 requires placement of the carboxyl functionality α to one of the amide carbonyls, located either at position 3 of the heterocycle (**3a**) or at C-2 of the oxybutyramide side chain (**4a**). In pursuit of the 3-carboxy analogue, we have previously reported our unsuccessful attempts to oxidize the corresponding alcohol (**3b**), derived from serine, to the desired acid (**3a**),[12] making it apparent that the required carboxyl group had to be introduced in the correct oxidation state. Attempts to incorporate either diethyl aminomalonate or differentially protected derivatives thereof into the ring-construction sequence previously used[11] consistently failed, however, to provide the corresponding ethyl ester (**3c**). The alternative target, the oxybutyramide side chain α-carboxamide (**4a**), was, however, successfully prepared from a derivative of malonic acid (Scheme I). Condensation of ethyl malonyl chloride with *N*-methylcyclohexylamine gave **5**, which was alkylated with the protected aldehyde (**6b**) derived from **6a**,[17] to give the required nitro aldehyde (**7**) after deprotection. Ring construction according to the usual

(11) Jones, G. H.; Venuti, M. C.; Alvarez, R.; Bruno, J. J.; Berks, A. H.; Prince, A. *J. Med. Chem.* **1987**, *30*, 295.
(12) Venuti, M. C.; Jones, G. H.; Alvarez, R.; Bruno, J. J. *J. Med. Chem.* **1987**, *30*, 303.
(13) Alvarez, R.; Banerjee, G. L.; Bruno, J. J.; Jones, G. H.; Liittschwager, K.; Strosberg, A. M.; Venuti, M. C. *Mol. Pharmacol.* **1986**, *29*, 554.
(14) Gu, L.; Huynh, O.; Strickley, R. G.; Lin, L.-H.; Visor, G. C. *Int. J. Pharm.*, in press.
(15) Gu, L.; Huynh, O.; Becker, A.; Peters, S.; Nguyen, H.; Chu, N. *Drug. Dev. Ind. Pharm.* **1987**, *13*, 437.
(16) Venuti, M. C.; Strosberg, A. M.; Alvarez, R.; Bruno, J. J.; Unger, S., unpublished results.
(17) Venuti, M. C.; Stephenson, R. A.; Alvarez, R.; Bruno, J. J.; Strosberg, A. M. *J. Med. Chem.*, preceding paper in this issue.

Scheme III



Reagents: a, $GlyNH_2/NaBH_3CN/NaOAc/MeOH$; b, $MeO_2CCl/Et_3N/THF$; c, NaOMe/MeOH; d, H_2/Pd-C/EtOH; e, $PhO_2CCl/Et_3N/THF$; f, i-$Pr_2NEt/DMAP/THF$.

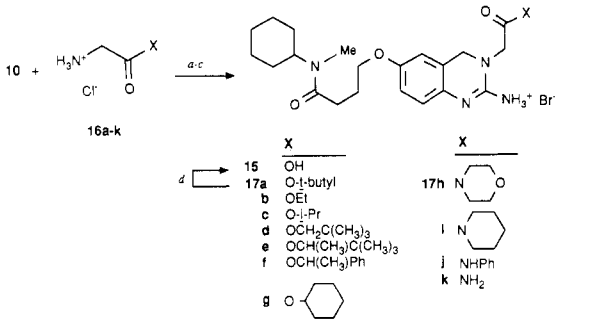
procedure[11,12] gave the ethyl ester **4b**, which was saponified quantitatively to the corresponding acid (**4a**).

Early attempts to prepare N-1 aminomethyl or hydroxymethyl adducts of **1**, as precedented by work on increasing the aqueous solubility of phenytoin,[18,19] were frustrated by the insolubility of **1** in aqueous formaldehyde. However, use of a combination of formalin, secondary amine and, uniquely, THF, a procedure developed to overcome just such insolubility problems in work with nucleosides,[20] proved useful (Scheme II) and provided a series of aminomethyl adducts (**8a–e**). Since the reaction that produces the adducts is a reversible equilibrium, only those adducts stable enough to survive the evaporative workup by crystallization were obtained. Under similar conditions, but with no amine present, there was evidence of reversible formation of the unprotected hydroxymethyl adduct (**9a**), since **1** dissolved, but only starting material was obtained on workup, an indication of the aqueous instability of this adduct. Instead, two protected hydroxymethyl adducts, (pivaloyloxy)methyl (**9b**)[21] and (benzyloxy)methyl (**9c**), were prepared by alkylation of **2e**, followed by separation of the N- and O-alkylated product isomers.[12] Attempts to remove the benzyl protection of **9c** by hydrogenolysis failed to give isolable **9a**.

Ring-opened analogues of **1** that are formally hydrolysis products of the parent drug are potentially more soluble and might be expected to cyclize to **1** under physiological conditions. Moreover, such an equilibrium between **1** and its imidazolone ring-opened product (vide infra) was observed as part of the pH-dependent hydrolysis profile of **1**.[14] To test the general viability of this approach as a delivery system for **1**, three such compound types were prepared. Reductive amination of **10**[11] with glycinamide gave **11** (Scheme III), which was sequentially treated with methyl chloroformate/triethylamine and sodium methoxide/methanol to afford the (nitrobenzyl)hydantoin **12a**. Hydrogenation of **12a** effected reduction of the nitro group to give the (aminobenzyl)hydantoin target (**12b**), the formal product of the opening of the central pyrimidine ring of **1**. Alternatively, treatment of **11** with phenyl chloroformate, followed by reduction and subsequent cyclization with DBU as base gave the 3-(carbamoylmethyl)-quinazolin-2-one **13**, a potential product of opening of the imidazole ring of **1**.

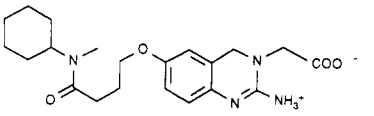
A more likely product of cleavage of the imidazole ring of **1** is, however, the guanidinium acid **14**. Indeed, this

Scheme IV



Reagents: a, $NaBH_3CN/NaOAc/EtOH$; b, H_2/Pd-C/EtOH; c, BrCN; d, HBr-HOAc/CH_2Cl_2

compound, in its zwitterionic form, is the primary hydrolysis product of **1** at pH extremes.[14] An unambiguous



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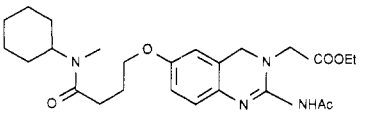
synthesis (Scheme IV) of this compound, as its hydrobromide salt (**15**), was accomplished by reductive amination of **10** with glycine *tert*-butyl ester hydrochloride (**16a**), followed by reduction of the nitro group and cyclization with BrCN to afford the guanidinium *tert*-butyl ester hydrobromide **17a**. Removal of the *t*-butyl ester with HBr in acetic acid afforded **15**, free of cyclized **1**. Other ester and amide analogues of **15** (**17b–k**), designed to vary the rate of cyclization to **1**, were most easily prepared by use of the appropriate glycine ester or amide hydrochloride (**16b–k**) in the reductive amination–cyclization sequence described above. Alternatively, HBr-catalyzed transesterification of **17a**, in a mixture of the desired alcohol, acetic acid, and alkyl acetate, could be used to obtain acid-insensitive alkyl esters of **15**. Finally, acetylation of **17a**, followed by HCl-catalyzed tranesterification, afforded the doubly blocked *N*-acetylguanidine ethyl ester **18**.



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Prodrug Evaluation

Candidate prodrugs were evaluated by three methods designed to quantify delivery of **1** in both a pharmaceutical and a pharmacological sense. First, HPLC monitoring of the incubation of compounds in pH 7.4 buffer at 37 °C was used to measure the time-dependent conversion of the prodrugs to **1**. Secondly, compounds were assayed as in vitro inhibitors of human platelet cAMP PDE, in an assay modified by the use of a 10-min preincubation period, to allow conversion of the putative prodrug to **1** and for inhibition of ADP-induced platelet aggregation. Finally, a number of the compounds were screened in the anesthetized canine inotropic model previously described,[13] to directly compare their efficacy as prodrugs of **1** in vivo. The data for these assays are presented in Table II.

Of the compounds assayed from the first two series of prodrugs, only the *N,N*-(dialkylamino)methyl adducts, represented by the N-1 morpholinylmethyl analogue **8d**, demonstrated the ability to release **1** once solubilized, but this conversion took place much too rapidly to be of any use in parenteral formulation. The ability of **8d** to provide **1** in vivo is also reflected in the inotropic assay, where

(18) Bundgaard, H.; Johansen, M. *Int. J. Pharm.* **1980**, *5*, 67.
(19) Varia, S. A.; Schuller, S.; Sloan, K. B.; Stella, V. J. *J. Pharm. Sci.* **1984**, *73*, 1068.
(20) Sloan, K. B.; Siver, K. G. *Tetrahedron* **1984**, *40*, 3977.
(21) Rasmussen, M.; Leonard, N. J. *J. Am. Chem. Soc.* **1967**, *89*, 5439.

Table I. Guanidinium Ester and Amide Analogues (**17a–k**) and Synthetic Precursors from Scheme IV

X	glycine synthon	mp, °C	anal. (C, H, N, Cl)	guanidinium analogue	mp, °C	anal. (C, H, N, Br)
$OC(CH_3)_3$	**16a**	*a*		**17a**	78–81	$C_{25}H_{39}N_4O_4Br \cdot 0.75H_2O$
OCH_2CH_3	**16b**	*a*		**17b**	91–92	$C_{23}H_{35}N_4O_4Br \cdot 1.5H_2O$
$OCH(CH_3)_2$	**16c**	98–99	$C_5H_{12}NO_2Cl$	**17c**	61–63	$C_{24}H_{37}N_4O_4Br \cdot 0.75H_2O$
$OCH_2C(CH_3)_3$	**16d**	160	$C_7H_{16}NO_2Cl$	**17d**	98–99	$C_{26}H_{41}N_4O_4Br \cdot 0.5H_2O$
$OCH(CH_3)C(CH_3)_3$	**16e**	146–147	$C_8H_{18}NO_2Cl \cdot 0.2H_2O$	**17e**	45–48	$C_{27}H_{43}N_4O_4Br \cdot 0.5H_2O$
$OCH(CH_3)C_6H_5$	**16f**	138–139	$C_{10}H_{14}NO_2Cl$	**17f**	60–62	$C_{29}H_{39}N_4O_4Br \cdot 0.5H_2O$
OC_6H_{11}	**16g**	144–145	$C_8H_{16}NO_2Cl$	**17g**	77–79	$C_{27}H_{41}N_4O_4Br \cdot 0.5H_2O$
$N(CH_2)_2O(CH_2)_2$	**16h**	221–223	$C_6H_{13}N_2O_2Cl$	**17h**	50–52	$C_{25}H_{38}N_5O_4Br \cdot 1.2H_2O$
$N(CH_2)_5$	**16i**	192–193	$C_7H_{15}N_2OCl$	**17i**	94–96	$C_{26}H_{40}N_5O_3Br \cdot 1.2H_2O$
NHC_6H_5	**16j**	208–210	$C_8H_{11}N_2OCl \cdot 0.05H_2O$	**17j**	84–86	$C_{27}H_{36}N_5O_3Br \cdot 0.5H_2O$
NH_2	**16k**	*a*		**17k**	74–75	$C_{21}H_{32}N_5O_3Br \cdot 0.5H_2O$

[a] Commercially available.

Table II. Evaluation of Lixazinone Prodrugs

	conversion to 1[a]		in vitro biological evaluation[d]		in vivo inotropic evaluation[e]		
no.	% 1[b]	$t_{1/2}$,[c] min	platelet PDE IC_{50}, nM	platelet aggregation IC_{50}, μM	route[f]	max efficacious dose, mg/kg	% max CF resp
1	stable		6.0	0.05	iv	0.10	71
					id	0.316	42
4a	stable[g]		5.0		iv	0.316	58
					id	1.0	20
4b	stable		6.1		iv	0.316	50
					id	1.0	50
8d	100	<10	16.0		iv	0.10	65
					id	0.316	24
					ig	0.316	25
9b	stable		150		nd		
12b	stable		3400		nd		
13	stable		18500		nd		
15	100	20 d	60		iv	inactive	
					id	inactive	
17a	<100	177	430	4.3	iv	inactive	
					id	inactive	
17b	100	2	9.3		iv	0.316	75
					id	3.16	62
17c	100	17	63	3.4	iv	0.316	40
					id	3.16	31
17d	100	14	35		nd		
17e	97	347	470	17	iv	nd	
					id	3.16	62
17f	100	1.1	8	0.15	iv	0.316	61
					id	3.16	64
17g	100	16.5	52	1.2	iv	nd	
					id	3.16	45
17h	99	8.8	52	0.7	iv	0.316	71
					id	3.16	70
17i	100	280	10		nd		
17j	99	17	100	5.4	iv	0.316	64
					id	3.16	37
17k	100	23	120	4.2	iv	0.316	37
					id	3.16	37
18	<10	355	3100	100	iv	1.0	58
					id	3.16	58

[a] Determined at pH 7.4 as described in the Experimental Section. [b] Percent drug consumed converted to **1**. [c] Half-life of drug as consumed, given in minutes unless otherwise noted. [d] Modifications to the in vitro bioassay and statistical methods used are described in the Experimental Section. [e] In vivo evaluation of inotropic activity and statistical methods are described in the Experimental Section. [f] For each compound tested, $n = 2$ by the indicated route: iv = intravenous, id = intraduodenal, ig = intragastric administration, nd = not done. [g] Decarboxylation of **4a** to **1** was observed at pH 1 after warming.

reasonable increases in cardiac contractile force were observed with submicrogram doses. However, since compounds of this class offered no solubility advantage over **1**, these results eliminated **8a–e** from further consideration.

From among the three classes of ring-cleaved analogues (**12b**, **13**, **15**, **17a–k**, **18**) of **1**, only the guanidinium esters (**17a–g**) and amides (**17h–k**) demonstrated any propensity to cyclize to **1** at physiological pH. Of particular note was that the guanidinium acid (**15**), found to be in an essentially 1:1 equilibrium with **1** at pH 1,[14] displayed a half-life of over 20 days for conversion to **1** at pH 7.4. The series of esters and amides, however, displayed a reasonably wide window of $t_{1/2}$ values (1 min to 6 h) found to correlate well with the IC_{50} values for PDE inhibition obtained in vitro (Figure 1). The single anomalous point for **17i** (X = *N*-piperidinyl) remains unexplained.

The in vivo inotropic activity of these compounds was demonstrated in the anesthetized canine model, by both intravenous (iv) and intraduodenal (id) administration routes. As expected, compounds that displayed both the ability to cyclize to **1** and to inhibit human platelet type IV cAMP PDE in vitro exhibited activity in the inotropic model, although even the most potent of the compounds (e.g., **17b**) were 3–10 times less potent than **1** by the iv and

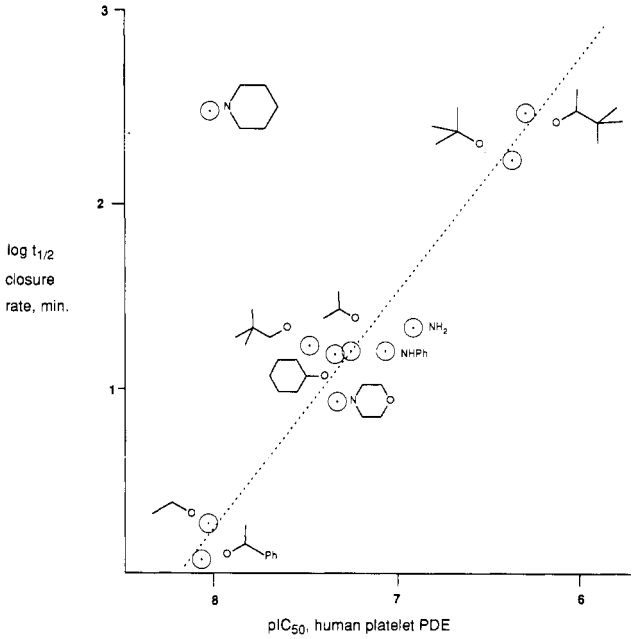

Figure 1. Cyclization rate log ($t_{1/2}$) values versus human platelet PDE pIC_{50} values for guanidinium ester (**17a–g**) and amide (**17h–k**) prodrugs of 1.

id routes, respectively. The doubly blocked *N*-acetylguanidine ester (**18**), which would require *sequentially specific* cleavage of the *N*-acetyl moiety, followed by cyclization, did not demonstrate the ability to deliver **1** in vitro, since <10% of consumed drug was converted to **1**, and PDE inhibition was greatly diminished.

Comparison of contractile force responses elicited by intravenous versus intraduodenal administration of individual prodrugs nearly consistently demonstrated a 10-fold difference in potency between these routes of administration. However, since intravenous administration of even the most readily cyclized prodrug did not achieve contractile force response levels attained with intravenously administered **1**, it must be assumed that competing chemical and/or metabolic consumption of the prodrug species was occurring in plasma. That none of the ester or amide prodrugs of **1**, when judged on the basis of potency on contractile force (Table II) or heart rate (not shown), were more active than **1** when administered intraduodenally to the anesthetized dog suggested that none of the prodrugs offered any bioavailability advantage over **1**. This conclusion was confirmed in a comparative oral bioavailability study of **2b** and **17b**, at equivalent doses of **1**, carried out with conscious dogs (n = 6). This study showed that **17b**, which was found to rapidly cyclize to **1** ($t_{1/2}$ = 2 min) and to be nearly equipotent with **1** as a time-dependent PDE inhibitor (IC_{50} = 9.3 versus 6 nM), was nearly identical with **1** in overall bioavailability, as measured by mean plasma concentrations (Figure 2). Measurement of various pharmacokinetic parameters (Table III) also tended to support this conclusion, even though the large animal-to-animal variability observed (Table III) for **2b** was somewhat reduced with **17b** in this study. These results make it apparent that, upon id administration, cyclization was occurring before absorption and that the observed potency decrease between **1** and its prodrugs was due to incomplete time-dependent closure upon either iv or id delivery.

Conclusion

The investigation into the in vivo delivery of a suitable chemical precursor (prodrug) of **1** yielded a series of compounds (**17a–k**), formally ring-cleaved precursors of **1**,

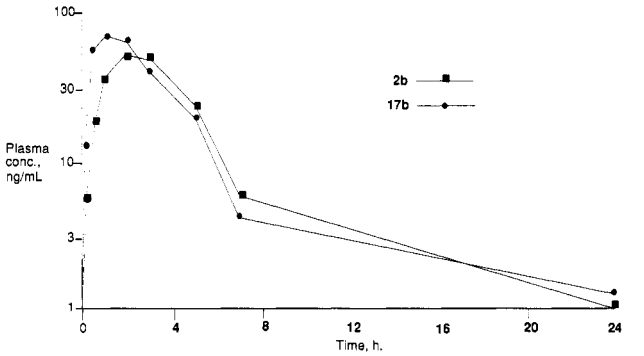

Figure 2. Mean plasma concentrations of **1** in conscious female dogs (n = 6) after oral administration of **2b** or **17b** at doses equivalent to 15 mg of **1**. Standard deviations for derived pharmacokinetic parameters are given in Table III.

Table III. Bioavailability of **1** in Conscious Dogs following Oral Administration of **2b** or **17b**[a]

	pharmacokinetic parameters:[b] mean ± SD[c]			
compd	C_{max}, ng/mL	T_{max}, h	$T_{1/2}$, h	AUC, ng/mL h
2b	60.5 ± 52.9	1.33 ± 0.98	3.19 ± 1.87[d]	256.7 ± 277.4
17b	72.0 ± 23.0	1.08 ± 0.49	3.00 ± 2.77	268 ± 127.3

[a] Single doses of **2b** or of **17b** administered at a dose equivalent to 15 mg of **1**. [b] Definitions: C_{max} = maximum plasma concentration; T_{max} = elapsed time to C_{max}; $T_{1/2}$ = elapsed time to one-half C_{max}; AUC = area under the curve, i.e., the cumulated plasma concentration levels, as measured from 0–24 h. [c] Data reported as the mean ± standard deviation for n = six dogs, except as noted. [d] Mean ± SD for n = 5.

which displayed improved solubility, potentially suitable for parenteral formulation, but which offered no major advantage in oral bioavailability. Although compounds from this study were unsuitable for parenteral formulation, investigation into complex formation between **2b** and various solubilizing ligands, especially ascorbic acid, has provided a formulated product of enhanced solubility and suitable chemical and physical stability for clinical use.[22-24] These formulations have allowed the continued evaluation of **1** in clinical trials as one of a number of phosphodiesterase inhibitors targeted as potential agents for the treatment of congestive heart failure.[25,26]

Experimental Section

Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were obtained on either an EM-390 (90 MHz) or a Bruker WM 300 (300 MHz) instrument. Infrared spectra were recorded as KBr pellets with a Perkin-Elmer 237 grating spectrometer. All compounds exhibited NMR and IR spectral data consistent with the proposed structures. Elemental analyses were performed either by Atlantic Microlabs, Atlanta, GA, or by the Analytical and Environmental Research Department, Syntex Research, on samples dried 24 h at ambient temperature and high vacuum. Results were within 0.4% of theoretical values, unless otherwise stated. All organic extracts were dried over sodium sulfate prior to evaporation.

Acid-Addition Salts of 1 (2a–d). A suspension of **1** (3.0 g) in methanol (100 mL) was brought to reflux and treated with the

(22) Visor, G. C.; Lin, L.-H.; Kenley, R. A.; Venuti, M. C.; Alvarez, R. A. *Drug. Des. Delivery* **1987**, *2*, 121.
(23) Benjamin, E. J.; Visor, G. C. Eur. Pat. Appl. EP 212647, 4 March 1987; *Chem. Abstr.* **1987**, *107*, 46312*j*.
(24) Visor, G. C.; Lin, L.-H.; Benjamin, E.; Strickley, R. G.; Gu, L. *J. Parenter. Sci. Technol.* **1987**, *41*, 120.
(25) Taylor, M. D.; Sircar, I.; Steffen, R. P. *Annu. Rep. Med. Chem.* **1987**, *22*, 85.
(26) Jafri, S. M.; Goldstein, S. *Hosp. Ther.* **1987**, 31.

a 3–5-fold excess of the desired acid dissolved in water (10 mL). The mixture was stirred until clear and then was cooled and poured into diethyl ether (500 mL). The resulting precipitate was collected by filtration, washed with ether, and dried over P_2O_5 at high vacuum.

Hydrochloride 2a: mp 199–201 °C. Anal. ($C_{21}H_{28}N_4O_3$·HCl·$2H_2O$) C, H, N, Cl.

Hydrogen sulfate 2b: mp 204–205 °C. Anal. ($C_{21}H_{28}N_4O_3$·H_2SO_4·H_2O) C, H, N, S.

Hydrogen phosphate 2c: mp 199–201 °C. Anal. ($C_{21}H_{28}N_4O_3$·$1.75H_3PO_4$$0.5H_2O$) C, H, N, P.

Nitrate 2d: mp 129–131 °C. Anal. ($C_{21}H_{28}N_4O_3$·HNO_3·H_2O) C, H, N.

Base-Addition Salts of 1 (2e,f). The potassium salt of **1** (**2f**) was prepared by the method previously reported for the sodium salt (**2e**).[12]

Potassium salt 2f: mp 189–192 °C. Anal. ($C_{21}H_{27}N_4O_3K$·H_2O) C, H, N.

***N*-Cyclohexyl-*N*-methylmalonamide Monoethyl Ester (5).** A solution of ethyl malonyl chloride (Lancaster Synthesis; 50 g, 332 mmol) in THF (100 mL) was added dropwise to a chilled solution of *N*-methylcyclohexylamine (48 mL, 365 mmol) and triethylamine (58 mL, 415 mmol) in THF (500 mL). After stirring 30 min at ambient temperature, the mixture was filtered and evaporated. The residue was dissolved in diethyl ether (500 mL) and washed with 1 M HCl (3 × 200 mL), saturated $NaHCO_3$ (3 × 200 mL), and brine (2 × 200 mL) and then was dried, filtered, and evaporated. Kugelrohr distillation afforded **5** as a clear oil (37 g, 163 mmol, 49%), bp 160 °C (0.1 mm). Anal. ($C_{12}H_{21}NO_3$·$0.25H_2O$) C, H; N: calcd, 6.04; found, 5.48.

2-[5-(2-Chloroethoxy)-2-nitrophenyl]-1,4-diphenyl-1,2,4,5-tetrahydroimidazole (6b). 1,2-Dianilinoethane (Aldrich; 70.8 g, 333 mmol) was added to a solution of **6a**[17] (63.8 g, 278 mmol) in methanol (500 mL) containing acetic acid (1 mL). After stirring overnight at room temperature, the mixture was stored in the freezer 72 h to yield a precipitate, which was collected by filtration and washed sparingly with cold aqueous methanol to afford **6b** (100 g, 236 mmol, 85%), mp 78–79 °C. Anal. ($C_{23}H_{22}N_3O_3Cl$·$0.25H_2O$) C, H, N; Cl: calcd, 8.28; found, 8.76.

***N*-Cyclohexyl-*N*-methyl-2-(ethoxycarbonyl)-4-(3-formyl-4-nitrophenoxy)butyramide (7).** A solution of **5** (28.4 g, 125 mmol) in THF (100 mL) was added to a dispersion of washed NaH (3.0 g, 125 mmol) in THF (250 mL). After the mixture was stirred for 1 h at ambient temperature, a solution of **6b** (58 g, 137.5 mmol) and tetra-*n*-butylammonium iodide (4.62 g, 12.5 mmol) in THF (250 mL) was added, and the resulting mixture was maintained at reflux for 5 days. The cooled mixture was evaporated, and the residue was dissolved in ethyl acetate (500 mL). The organic layer was washed with water (2 × 300 mL) and then vigorously stirred with 3 M HCl (300 mL) for 2 h. The layers were separated, and the organic solution was washed with 1 M HCl (2 × 200 mL) and brine (2 × 200 mL) and then was dried, filtered, and evaporated. The residue was chromatographed over silica gel (0–10% ethyl acetate in dichloromethane as eluant) to afford **7** (31.5 g, 78 mmol, 62%) as a syrup. Anal. ($C_{21}H_{28}N_2O_7$·H_2O) C, H, N.

***N*-Cyclohexyl-*N*-methyl-2-(ethoxycarbonyl)-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (4b).** Standard ring construction[11,12] using **7** (30 g, 75 mmol) afforded a crude product after treatment with ammonium hydroxide. Chromatography over silica gel (0–10% ethanol in dichloromethane) and combination of the desired fractions yielded **4b** (16.5 g, 36 mmol, 48%) on evaporation, mp 185–186 °C after recrystallization from ethanol–diethyl ether. Anal. ($C_{24}H_{32}N_4O_5$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-2-carboxy-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (4a).** A suspension of **4b** (4.0 g, 8.75 mmol) in ethanol (50 mL) was treated with a solution of KOH (2.9 g, 43.8 mmol) in water (10 mL) at room temperature. After the solution was stirred for 1 h at room temperature, the pH was adjusted to 7.5 with 6 M HCl. Evaporation of the ethanol afforded a precipitate of **4a** (3.2 g, 7.5 mmol, 86%) mp 210–212 °C. Anal. ($C_{22}H_{28}N_4O_5$·$1.75H_2O$) C, H, N.

(*N*,*N*-Dialkylamino)methyl Adducts of 1 (8a–e). A mixture of **1** (3.85 g, 10 mmol), the desired secondary amine (260 mmol), and 37% aqueous formaldehyde (18 mL) in THF (100 mL) was left to stand for 3–10 days at room temperature. The mixture was then evaporated at high vacuum and crystallized from diethyl ether–THF to yield the adducts **8a–e**. This procedure failed to give isolable products with dimethylamine and bis(hydroxyethyl)amine in single attempts.

(*N*,*N*-Diethylamino)methyl adduct 8a: yield 26%; mp 105–106 °C. Anal. ($C_{26}H_{39}N_5O_3$·$0.25H_2O$) C, H, N.

(1-Piperidinyl)methyl adduct 8b: yield 36%; mp 162–163 °C. Anal. ($C_{27}H_{39}N_5O_3$·$1.5H_2O$) C, H, N.

(4-Methyl-1-piperazinyl)methyl adduct 8c: yield 52%; mp 155–157 °C. Anal. ($C_{27}H_{40}N_6O_3$) C, H, N.

(4-Morpholinyl)methyl adduct 8d: yield 78%; mp 142–143 °C. Anal. ($C_{26}H_{37}N_5O_4$) C, H, N.

(1-Pyrrolidinyl)methyl adduct 8e: yield 83%; mp 121–123 °C. Anal. ($C_{26}H_{37}N_5O_3$) C, H, N.

O-Protected hydroxymethyl adducts 9b,c were prepared by alkylation of **2e** with the appropriate chloromethyl reagent, followed by chromatography to separate the O- and N-alkylation products.[12]

(Pivaloyloxy)methyl adduct 9b: yield 16% as an amorphous foam. Anal. ($C_{27}H_{38}N_4O_5$·H_2O) C, H, N.

(Benzyloxy)methyl adduct 9c: yield 28%, mp 105–106 °C from ether–petroleum ether. Anal. ($C_{29}H_{36}N_4O_4$·$0.5H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[3-[[(carbamoylmethyl)amino]methyl]-4-nitrophenoxy]butyramide (11).** Anhydrous sodium acetate (16.4 g, 200 mmol) was added to a refluxing solution of glycinamide hydrochloride (26.5 g, 240 mmol) in methanol (500 mL). After stirring overnight at ambient temperature, the mixture was filtered, and to the filtrate was added **10**[11] (34.8 g, 100 mmol), followed by sodium cyanoborohydride (3.78 g, 60 mmol) in 30 min. After stirring 3 h at room temperature, the mixture was evaporated and partitioned between ethyl acetate and saturated sodium bicarbonate (500 mL each). The organic extract was washed with additional saturated sodium bicarbonate (3 × 300 mL) and brine (2 × 300 mL) and then was dried, filtered, and evaporated. Reevaporation from dichloromethane afforded **11** (36.5 g, 90 mmol, 90%) as an amorphous, hygroscopic foam. Anal. ($C_{20}H_{30}N_4O_5$·$0.5H_2O$) C, H, N.

1-[5-[3-(*N*-Cyclohexyl-*N*-methylcarbamoyl)propoxy]-2-nitrobenzyl]hydantoin (12a). A solution of methyl chloroformate (2.41 mL, 30 mmol) in THF (25 mL) was added dropwise to a chilled solution of **11** (10.2 g, 25 mmol) and triethylamine (4.88 mL, 35 mmol) in THF (200 mL). After stirring overnight at ambient temperature, the mixture was filtered, and the filtrate was concentrated and dissolved in ethyl acetate (200 mL). The organic solution was washed with 1 M HCl (2 × 100 mL) and brine (2 × 200 mL) and then was dried, filtered, and evaporated to give a crisp beige foam (10.8 g).

A solution of the above carbamate (9.3 g, 20 mmol) in methanol (150 mL) was treated with sodium methoxide (2.70 g, 50 mmol), and the resulting mixture was heated at reflux for 18 h. The reaction was quenched by the addition of 1 M HCl and then evaporated. The residue was dissolved in ethyl acetate (250 mL), and the solution was washed with water (2 × 200 mL) and brine (2 × 200 mL) and then was dried, filtered, and evaporated. Chromatography of the residue on silica gel (0–5% methanol in dichloromethane as eluant) and evaporation of the appropriate fractions afforded **12a** (5.20 g, 12 mmol, 60%) as a crisp, tan foam. Anal. ($C_{21}H_{28}N_4O_6$) C, H, N.

1-[5-[3-(*N*-Cyclohexyl-*N*-methylcarbamoyl)propoxy]-2-aminobenzyl]hydantoin (12b). A solution of **12a** (4.3 g, 10 mmol) in ethanol (300 mL) and concentrated HCl (3 mL) was hydrogenated at 60 psi over 10% Pd–C (1.0 g). After 1 h, the mixture was filtered and evaporated and then reevaporated three times from absolute ethanol. The resulting foam was triturated with diethyl ether–acetone, and the precipitate was collected by filtration to yield **16b** (2.85 g, 6.5 mmol, 65%), mp 138–140 °C. The product exhibited satisfactory spectral data, but was too hygroscopic to obtain a proper elemental analysis.

***N*-Cyclohexyl-*N*-methyl-4-[[3-(carbamoylmethyl)-1,2,3,4-tetrahydro-2-oxoquinazolin-6-yl]oxy]butyramide (13).** A solution of phenyl chloroformate (2.4 mL, 19.25 mmol) in THF (25 mL) was added to a cold solution of **11** (7.1 g, 17.5 mmol) and triethylamine (3.0 mL, 21.8 mmol) in THF (100 mL). After

stirring at ambient temperature for 1 h, the mixture was evaporated, and the residue was dissolved in ethyl acetate (200 mL). The solution was washed with 1 M HCl (3 × 200 mL) and brine (2 × 200 mL) and then was dried, filtered, and evaporated to yield an amorphous foam.

A solution of the above product (1.24 g, 2.5 mmol) in DMF (30 mL) was hydrogenated at 60 psi over 10% Pd–C overnight. The mixture was filtered and evaporated to give a thick syrup, which was dissolved in THF (25 mL) and treated with *N,N*-diisopropylethylamine (0.44 mL, 2.5 mmol) and DMAP (20 mg). After stirring overnight at ambient temperature, the reaction mixtue was evaporated, and the residue was dissolved in ethyl acetate (50 mL). The solution was washed with 1 M HCl (2 × 25 mL) and brine (2 × 25 mL) and then was dried, filtered, and evaporated. Chromatography over silica gel (0–10% methanol in dichloromethane as eluant) afforded **13** (240 mg, 0.6 mmol, 24%), mp 148–150 °C. Anal. ($C_{21}H_{30}N_4O_4 \cdot 0.25H_2O$) C, H, N.

Method A. Glycine Ester Hydrochlorides[27] 16c–g. The general procedure for preparation of **16c–g** is illustrated by the following example.

Glycine Cyclohexyl Ester Hydrochloride (16g). Chloroacetyl chloride (32 mL, 400 mmol) was added dropwise to a well-stirred solution of cyclohexanol (42 mL, 400 mmol) and triethylamine (62 mL, 440 mmol) in diethyl ether (400 mL) efficiently cooled to 0 °C. After stirring 1 h at ambient temperature, the mixture was filtered to remove triethylamine hydrochloride, and the filtrate was sequentially washed with 1 M HCl (3 × 200 mL), saturated sodium bicarbonate (2 × 200 mL), and brine (2 × 200 mL). The organic layer was dried, filtered, and evaporated, and the residue was Kugelrohr distilled (100 °C, 5 Torr) to afford cyclohexyl chloroacetate as a clear, colorless oil (58 g, 346 mmol, 87%).

The chloroacetate was added dropwise to a suspension of sodium azide (28 g, 433 mmol) in DMF (50 mL) cooled to 0–10 °C. After stirring at room temperature overnight, the mixture was poured into water (200 mL) and extracted with diethyl ether (3 × 200 mL). The organic extract was washed with brine (2 × 200 mL) and then was dried, filtered, and evaporated to give cyclohexyl azidoacetate (58 g, 333 mmol, 96%).

The azidoacetate was added to a mixture of 10% Pd–C (3.0 g) and ethanol (300 mL) in a three-neck Morton flask equipped with a gas outlet connected to a nitrogen bubbler system, a thermometer, and a fritted gas inlet tube. Hydrogen was introduced via the fritted inlet tube in a slow stream over 3 h. The temperature increased to 45 °C and then returned to room temperature over the course of the reduction. The catalyst was removed by filtration, and the filtrate was evaporated. The resulting oil was dissolved in diethyl ether (500 mL) and treated with saturated HCl–ethanol until just acidic. The solution was evaporated, and the residue was triturated with diethyl ether and chilled overnight to give **16g** (63 g, 325 mmol, 98%).

Substitution of other alcohols for cyclohexanol afforded glycine ester hydrochlorides **16c–f** in yields of 85–98% (**16a,b** commercially available). Physical data for the complete series are listed in Table I.

Method B. Glycine amide hydrochlorides 16h–j were prepared by the general procedure illustrated by the preparation of **16h**.

BOC-glycine Morpholinylamide. A solution of BOC-glycine (Bachem; 44 g, 250 mmol) in ethyl acetate (1 L) cooled to 0 °C was treated with DCC (54 g, 263 mmol). After 10 min, morpholine (26 mL, 300 mmol) was added dropwise, and the mixture was stirred at room temperature overnight. The precipitate was removed by filtration, and the filtrate was washed with 1 M sodium bisulfate (3 × 250 mL), saturated sodium bicarbonate (3 × 250 mL), and brine (2 × 300 mL). The organic extract was dried, filtered, and evaporated, and the residue was crystallized from ethyl acetate–diethyl ether to give BOC-glycine morpholinylamide (44.6 g, 183 mmol, 73%), mp 123–124 °C. Anal. ($C_{11}H_{20}N_2O_4$) C, H, N.

Similarly prepared were the following.

BOC-glycine piperidinylamide: yield 98%; mp 43–45 °C (lit.[28] mp 42–44 °C).

BOC-glycine anilide: yield 85%; mp 142–143 °C. Anal. ($C_{13}H_{18}N_2O_3$) C, H, N.

Glycine Morpholinylamide Hydrochloride (16h). A solution of BOC-glycine morpholinylamide (36.6 g, 150 mmol) in ethyl acetate (500 mL) was treated with saturated HCl–ethyl acetate (200 mL). After stirring overnight at room temperature, the mixture was diluted with diethyl ether to 2-L total volume, and the precipitate was collected by filtration to yield **16h** (25.5 g, 141 mmol, 94%).

Deprotection of other BOC-glycine amides by this procedure afforded **16i–j** in comparable yields (**16k** commercially available). Physical data are listed in Table I.

***N*-Cyclohexyl-*N*-methyl-4-[[2-amino-3-[(*tert*-butyloxycarbonyl)methyl]-3,4-dihydroquinazolin-6-yl]oxy]butyramide Hydrobromide (17a).** A solution of glycine *tert*-butyl ester hydrochloride (**16a**; Bachem; 10.0 g, 60 mmol) in ethanol (200 mL) was treated with anhydrous sodium acetate (4.10 g, 50 mmol). After stirring overnight, the mixture was evaporated, and to the filtrate was added **10** (8.75 g, 25 mmol), followed by sodium cyanoborohydride (0.98 g, 15 mmol) in 30 min. After 3 h at ambient temperature, the mixture was evaporated, and the residue was dissolved in ethyl acetate (200 mL). The solution was washed with saturated sodium bicarbonate (3 × 200 mL) and brine (2 × 200 mL) and then was dried, filtered, and evaporated. The residue was dissolved in ethanol (300 mL) and hydrogenated at 60 psi over 10% Pd–C overnight. The catalyst was removed by filtration, and the filtrate was treated dropwise with a solution of cyanogen bromide (2.92 g, 27.5 mmol) in ethanol (50 mL). After the mixture was stirred overnight at room temperature, the solution was thoroughly evaporated, and the resulting foam was dried at high vacuum to yield **17a** (5.50 g, 10 mmol, 40%).

Substitution of glycine ester (**16b–g**) or amide (**16h–k**) hydrochlorides into the above procedure afforded the corresponding guanidinium hydrobromides (**17b–k**) in 35–60% yields. Physical data for this series are listed in Table I.

***N*-Cyclohexyl-*N*-methyl-4-[[2-amino-3-(carboxymethyl)-3,4-dihydroquinazolin-6-yl]oxy]butyramide Hydrobromide (15).** A solution of **17a** (5.50 g, 10 mmol) in dichloromethane (50 mL) was treated with 30% HBr in acetic acid (Aldrich, 5 mL). After stirring overnight, the reaction mixture was concentrated, and the residue was triturated with ether to afford **15** (5.9 g) as an amorphous solid. Anal. ($C_{21}H_{30}N_4O_4 \cdot 2HBr \cdot 1.75H_2O$) C, H, N, Br.

***N*-Cyclohexyl-*N*-methyl-4-[[2-acetamido-3-[(ethoxycarbonyl)methyl]-3,4-dihydroquinazolin-6-yl]oxy]butyramide (18).** A solution of **17a** (5.50 g, 10 mmol) in dichloromethane (50 mL) and acetic anhydride (10 mL) was treated with triethylamine (10 mL) dropwise. After stirring at room temperature overnight, the reaction mixture was evaporated to a brown oil, which was then dissolved in ethyl acetate (100 mL). The organic solution was washed with water (3 × 50 mL), saturated sodium bicarbonate (2 × 50 mL), and brine (2 × 50 mL) and then was dried, filtered, and evaporated to give an amorphous foam at high vacuum. The foam was dissolved in ethyl acetate (25 mL) and treated with saturated HCl in ethanol (20 mL). After stirring 48 h at ambient temperature, the mixture was evaporated, and the residue was dissolved in ethyl acetate (100 mL) and washed with half-saturated sodium bicarbonate (3 × 50 mL), water (2 × 50 mL), and brine (2 × 50 mL). The organic extract was filtered to remove insoluble **1** (0.8 g) and then was dried, filtered, and evaporated. Chromatography of the residue over silica gel (0.5% ethanol–30% ethyl acetate in dichloromethane) and evaporation of the appropriate fractions afforded an amorphous foam which crystallized from cold diethyl ether to yield **18** (1.30 g, 2.75 mmol, 27.5%), mp 96–97 °C. Anal. ($C_{25}H_{36}N_4O_5$) C, H, N.

Determination of Conversion Rate. Typically, 100 μL of a 1.0 mg/mL stock methanol solution of the putative prodrug was added to 10 mL of 0.05 M phosphate buffer adjusted to pH 7.4 and to 0.5 M ionic strength at 37 °C. Aliquots (0.5 mL) of this

(27) Based on the general method described in: Moore, A. T.; Rydon, H. N. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 586.

(28) Clausen, K.; Thorsen, M.; Lawesson, S.-O.; Spatola, A. F. *J. Chem. Soc., Perkin Trans. 1* **1984**, 785.

solution were removed to predetermined time intervals and were quenched with 100 μL of acetic acid to quench the reaction. The concentrations of the test compound and **1** were determined by HPLC, using a Spectra Physics Model 8700 pump, Kratos 757 variable-wavelength detector, Perkin-Elmer ISS-100 autosampler, and Spectra Physics 4100 computing of a mixture of methanol/water (0.01 M KH_2PO_4–0.01 M heptanesulfonic acid)/THF, varied in ratio of 40–60/50–30/10, depending on the polarity of the test compound. The flow rate was 1.0 mL/min, and the detection wavelength was 254 nm. In all cases studied, pseudo-first-order kinetics for the disappearance of the test compound were observed. In addition, both compounds **1** and **17a–k** were found to be stable to the conditions of this analysis. The time to half-consumption of the test compounds ($t_{1/2}$) and the percent of that converted to **1** are given in Table II.

Cyclic AMP Phosphodiesterase Assay. Human platelet cyclic AMP PDE was prepared as previously described.[13] The phosphodiesterase incubation medium contained 10 mM Tris·HCl buffer, pH 7.7, 10 mM $MgSO_4$, and 1 μM [^{3}H]AMP (0.2 μCi) in a total volume of 1.0 mL. Test compounds were dissolved in dimethylsulfoxide immediately prior to addition to the incubation medium, and the resulting mixture was allowed to stand for 10 min prior to the addition of the enzyme. After addition of the enzyme, the contents were mixed and incubated for 10 min at 30 °C. Assays were performed in triplicate at five different inhibitor concentrations, the mean of the determinations (n = 3) at each concentration was plotted, and the IC_{50} values reported in Tables I and III were determined graphically. Standard deviations from mean values in each experiment were generally less than ±5%. IC_{50} values presented are from representative experiments, were highly reproducible, and varied by less than a factor of 0.5–2 times of the initial determination.

Inotropic Studies. Cardiovascular evaluation of test compounds was carried out in instrumented, anesthetized dogs according to methods described by usin ref 13. Dosing was carried out in half-log steps (i.e., 0.1, 0.316, 1.0, 3.16, 10, 31.6, 100 mg/kg), with sufficient time between doses for the leveling of effects to occur before the next higher dose was administered. On average, the compounds of this study produced maximal effects within 10 min on iv dosing and within 15–20 min on id administration. The maximum efficacious dose was determined graphically from plots of the mean of the determinations (n = 2) at each dose level administered for cardiac force, heart rate, and blood pressure response curves. This dosing regimen produces a standard error of ±0.5 log order of magnitude for the maximum efficacious dose reported. Standard deviation from mean values determined in each experiment were generally ±25%, an indication of the considerable intersubject variability encountered in the measurement of, in particular, cardiac contractile force changes.

Comparative Bioavailability Studies. Compounds **2b** or **17b**, in amounts equivalent to 15 mg of **1**, were formulated in hard gelatin capsules with Dipac (50/50, w/w) and were analyzed for drug content by HPLC methods.[14,15,26] Six female dogs weighing 9.4–10.5 kg (10.1 ± 0.2 kg, mean ± standard error) were fasted from 18 h prior to dosing to 4 h after dosing. Water was allowed ad libitum. Dogs received, with 1 week between doses, single doses of formulated **2b** and **17b**. A small volume of water (20 mL) followed each dose. Samples of blood were collected from a jugular vein from each animal at preset intervals (0, 0.25, 0.5, 1, 2, 3, 5, 7, and 24 h) after oral administration of each dose. Plasma was separated from whole blood by centrifugation, transferred to polypropylene tubes, and stored at −20 °C prior to analysis for **1**. Concentrations of **1** in plasma samples were determined by HPLC assay[26] with a sensitivity of 5 ng 1/plasma mL. Area under the plasma concentration–time curves (AUC) values were calculated by the trapezoidal rule from time 0 to 24 h.

The concentration of **1** in plasma is determined by the following method. Compound **1** and an added internal standard chosen from the previously reported set of analogues of **1**[12] are extracted from the plasma sample prior to HPLC by application of a portion of the plasma sample (1 mL) to a Bond-Elute SCX column. The column is then washed with phosphate buffer (0.01 M, pH 7), followed by a 1:1 mixture of the same phosphate buffer with methanol to elute **1** and the internal standard. Further purification is carried out on a C-18 Sep-Pak column, from which **1** and the internal standard are eluted with a 4:1 mixture of methanol and water. The solvent is evaporated from the eluate, and the dry residue is reconstituted in a mixture of methanol and phosphate buffer (62:38). An aliquot of this mixture is injected onto a C-8 reverse-phase HPLC column using a mixture of methanol and phosphate buffer (62.5:37.5) as mobile phase. The concentration of **1** is determined at 280 nm. The quantitation limit of this method is 5 ng 1/mL plasma, and the calibrated range of the method extends from 5 ng to 500 ng per plasma mL. The reproducibility of the method over this range has been determined: the coefficient of variation for the recovery of **1** from spiked samples is less than 9%.

Acknowledgment. We thank Drs. John Moffatt, Gordon Jones, Arthur Kluge, Eric Benjamin, Gary Visor, and George Grass, Syntex Research, for helpful suggestions throughout this program, and Suzanne Leeman for her coordination of this effort as Manager of the lixazinone Project Team. We also gratefuly acknowledge Ronnie Chin, Institute of Bio-Organic Chemistry, for the preparation of some of the glycine ester hydrochlorides, Kurt Liittschwager, Linda Osborne, and Louise Drinkwater, Institute of Biological Sciences, for carrying out the in vitro bioassays, Lowell Johnson, Institute of Experimental Pharmacology, for conducting the in vivo inotropic evaluations, and Stephanie Sweetana, Institute of Pharmaceutical Sciences, for the preparation of the formulated capsules used in the bioavailability studies.

Registry No. **1**, 94192-59-3; **2a**, 94193-05-2; **2b**, 108609-34-3; **2c**, 116005-57-3; **2d**, 116005-58-4; **2e**, 108609-36-5; **if**, 116025-42-4; **4a**, 116005-63-1; **4b**, 116005-62-0; **5**, 86647-91-8; **6a**, 116005-59-5; **6b**, 116005-60-8; **7**, 116005-61-9; **8a**, 116005-64-2; **8b**, 116005-65-3; **8c**, 116005-66-4; **8d**, 116025-43-5; **8e**, 116005-67-5; **9b**, 116005-68-6; **9c**, 116005-69-7; **10**, 94193-38-1; **11**, 116005-70-0; **11** (methyl carbamate), 116005-71-1; **11** (phenyl carbamate), 116025-44-6; **12a**, 116005-72-2; **12b**, 116005-73-3; **13**, 116005-74-4; **15**, 116005-82-4; **16a**, 27532-96-3; **16b**, 623-33-6; **16c**, 14019-62-6; **16d**, 116005-75-5; **16e**, 116005-76-6; **16f**, 116005-77-7; **16g**, 114703-79-6; **16h**, 24152-96-3; **16i**, 5437-48-9; **16j**, 4801-39-2; **16k**, 1668-10-6; **17a**, 114703-82-1; **17b**, 114703-76-3; **17c**, 114703-84-3; **17d**, 116005-78-8; **17e**, 114703-86-5; **17f**, 114703-85-4; **17g**, 114703-83-2; **17h**, 114703-87-6; **17i**, 116005-79-9; **17j**, 116005-80-2; **17k**, 116005-81-3; **18**, 115623-42-2; GlyOEt·HCl, 623-33-6; $ClCH_2OCOBu$-t, 18997-19-8; $ClCH_2OCOPh$, 3587-60-8; $HOCH(CH_3)_2$, 67-63-0; $HOCH_2C(CH_3)_3$, 75-84-3; $HOCH(CH_3)C(CH_3)_3$, 464-07-3; $HOCH(CH_3)C_6H_5$, 98-85-1; ethyl malonyl chloride, 36239-09-5; *N*-methylcyclohexylamine, 100-60-7; 1,2-dianilinoethane, 150-61-8; glycinamide hydrochloride, 1668-10-6; methyl chloroformate, 79-22-1; phenyl chloroformate, 1885-14-9; chloroacetyl chloride, 79-04-9; cyclohexanol, 108-93-0; cyclohexyl chloroacetate, 6975-91-3; cyclohexyl azidoacetate, 114703-78-5; BOC-glycine, 4530-20-5; BOC-glycine morpholinylamide, 114703-81-0; BOC-glycine piperidinylamide, 88621-47-0; BOC-glycine anilide, 27904-92-3; cyclic AMP phosphodiesterase, 9036-21-9.