Anal. ($C_{29}H_{40}N_2O_7$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl-L-valyl-L-valyl-N^7-benzyl-N^7-(benzyloxycarbonyl)-4(*S*),7-diamino-3(*R*,*S*)-hydroxyheptanoic Acid Ethyl Ester.** Compound **27ab** (100 mg, 0.19 mmol) was deprotected according to general procedure A. The resulting hydrochloride was coupled with Boc-valine anhydride (0.4 mmol) according to general procedure D with methylene chloride as solvent. The crude product obtained was chromatographed over 10 g of silica gel eluting with 40% ethyl acetate in methylene chloride. Product was isolated as an oil in about 80% yield: TLC R_f (B) 0.50, R_f (G) 0.25; NMR ($CDCl_3$) δ 0.79–1.18 (m, 6 H), 1.23 (t, J = 7.5 Hz, 3 H), 1.30–1.71 (m, 13 H), 2.05 (m, 1 H), 2.39 (m, 2 H), 3.11–3.56 (m, 3 H), 3.62–4.30 (m, 5 H, includes quartet δ 4.13, J = 7.5 Hz), 4.45 (s, 2 H), 5.0 (m, 1 H), 5.14 (s, 2 H), 6.39 (br m, 1 H), 7.08–7.45 (m, 10 H).

***N*-(*tert*-Butyloxycarbonyl)-L-valyl-L-valyl-N^7-benzyl-N^7-(benzyloxycarbonyl)-4(*S*),7-diamino-3(*R*,*S*)-hydroxyheptanoic Acid Ethyl Ester.** Boc-Val-[OrnSta]-OEt (66 mg, 0.105 mmol) was deprotected according to general procedure A. The resulting hydrochloride was coupled with Boc-valine anhydride (0.2 mmol) according to general procedure D with methylene chloride as solvent. Silica gel column purification (10 g) eluting with 40% ethyl acetate in methylene chloride afforded pure compound as an oil in 75% yield: TLC R_f (G) 0.13; NMR ($CDCl_3$) δ 0.75–1.11 (m, 12 H), 1.15–1.62 (m, 16 H, includes triplet δ 1.26, J = 7 Hz), 1.83–2.25 (m, 2 H), 2.43 (m, 2 H), 3.22 (m, 2 H), 3.60–4.40 (m, 7 H, includes quartet δ 4.18, J = 7 Hz), 4.45 (s, 2 H), 5.18 (s, 2 H), 5.38 (d, J = 8 Hz, 1 H), 6.65–7.10 (m, 2 H), 7.10–7.52 (m, 10 H).

***N*-Isovaleryl-L-valyl-L-valyl-N^7-benzyl-N^7-(benzyloxycarbonyl)-4(*S*),7-diamino-3(*R*,*S*)-hydroxyheptanoic Acid Ethyl Ester (28ab).** Boc-Val-Val-[OrnSta]-OEt (37 mg, 0.051 mmol) was deprotected according to general procedure A. The resulting hydrochloride was coupled to isovaleric anhydride according to general procedure D with DMF as solvent. Precipitation from ethyl ether gave product as a white powder in about 96% yield: mp 148–152 °C; TLC R_f (A) 0.61; NMR (MeOH-d_4) δ 0.77–1.13 (m, 18 H), 1.16–1.64 (m, 7 H, includes triplet δ 1.28, J = 7 Hz), 1.82–2.26 (m, 5 H), 2.45 (m, 2 H), 3.10 (m, 2 H), 3.61–4.60 (m, 8 H), 5.28 (s, 2 H), 7.30 (m, 10 H). Anal. ($C_{39}H_{58}N_4O_8$) C, H, N.

***N*-Isovaleryl-L-valyl-L-valyl-4(*S*),7-diamino-3(*R*,*S*)-hydroxyheptanoic Acid Ethyl Ester Acetate (27a).** Compound **28ab** (28 mg, 0.04 mmol) was dissolved in methanol (3 mL) and 3–4 drops of acetic acid were added. The solution was then purged of oxygen with nitrogen before 20% palladium hydroxide on carbon (10 mg) was added. The mixture was put on a Parr hydrogenation apparatus at 30 psi for 3 h. After this, the catalyst was removed by filtration over Celite and washed with methanol. The solvent was concentrated in vacuo (~1 mL) and product was precipitated with ethyl ether and collected as a white powder about 88% yield: mp 228–230 °C; TLC R_f (I) 0.52; NMR (MeOH-d_4) δ 0.91–1.10 (m, 18 H), 1.23 (t, J = 7 Hz, 3 H), 1.65 (m, 4 H), 1.80–2.21 (m, 8 H), 2.46 (m, 2 H), 2.90 (m, 2 H), 3.63–4.41 (m, 6 H). Anal. ($C_{26}H_{50}N_4O_8$) C, H, N.

Acknowledgment. This work was supported at the University of Wisconsin by research grants from the National Institutes of Health (AM 20100) and from Merck Sharp and Dohme. We thank Drs. David Davies and Richard Bott for a generous sample of *R. chinensis* pepsin and Dr. Jürgen Maibaum and Karen Rebholz for additional kinetic data on compound **16a**.

Registry No. **9**, 82689-16-5; **10a**, 98045-10-4; **10b**, 98063-00-4; **12a**, 105562-69-4; **12b**, 105562-70-7; **13a**, 105562-71-8; **13b**, 105617-26-3; **14a**, 105562-72-9; **14b**, 105617-27-4; **15a**, 91416-63-6; **15b**, 91464-93-6; **16a**, 105617-28-5; **16a** (free base), 91416-61-4; **16b**, 105660-65-9; **16b** (free base), 105617-32-1; **17**, 105562-73-0; **18**, 105562-74-1; **19**, 105617-29-6; **19** (free base), 91416-62-5; **20**, 2480-95-7; **21**, 105562-75-2; **22**, 105562-76-3; **23**, 105562-77-4; **24**, 105562-78-5; **25**, 105562-79-6; **26**, 105562-80-9; **27a**, 105562-81-0; **27b**, 105562-82-1; **28a**, 105562-83-2; **28b**, 105617-30-9; **29a**, 105562-85-4; **29a** (free base), 105562-84-3; **29b**, 105660-66-0; **29b** (free base), 105617-33-2; **33**, 105562-86-5; $ClCO_2CH_2CCl_3$, 17341-93-4; Boc-Val anhydride, 33294-55-2; H-Phe-OMe, 2577-90-4; Boc-L-Val-L-Val-N^8-Cbz-(3*S*,4*S*)-[LySta]-L-Phe-OMe, 105562-87-6; ZCl, 501-53-1; AcOEt, 141-78-6; Boc-L-Val-N^7-Bzl-N^7-Cbz-(3*S*,4*S*)-[OrnSta]-OEt, 105660-72-8; Boc-L-Val-N^7-Bal-N^7-Cbz-(3*R*,4*S*)-[OrnSta]-OEt, 105562-88-7; Boc-L-Val-L-Val-N^7-Bzl-N^7-Cbz-(3*S*,4*S*)-[OrnSta]-OEt, 105562-89-8; Boc-L-Val-L-Val-N^7-Bzl-N^7-Cbz-(3*R*,4*S*)-[OrnSta]-OEt, 105617-31-0; isovaleric anhydride, 1468-39-9; benzaldehyde, 100-52-7; penicillopepsin, 9074-08-2; aspartic proteinase, 78169-47-8; pepsin, 9001-75-6.

Inhibitors of Cyclic AMP Phosphodiesterase. 1. Analogues of Cilostamide and Anagrelide[1]

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Evaluation of a series of lactam heterocyclic analogues of cilostamide (**2**) as inhibitors of cyclic AMP phosphodiesterase derived from both human platelets and rat heart in comparison with their corresponding methoxy-substituted heterocycles has revealed that the *N*-cyclohexyl-*N*-methyl-4-oxybutyramide side chain of **2** is an important lipophilic and/or steric pharmacophore. Attachment of this side chain to the parent heterocycle of the potent cyclic AMP phosphodiesterase inhibitor anagrelide (**3**) afforded the hybrid structure RS-82856 (**1**), shown to be more potent than either of its progenitors as an inhibitor of cyclic AMP phosphodiesterase or of ADP-induced platelet aggregation. The available in vitro data suggest that **1** possesses potentially useful antithrombotic and cardiotonic properties.

Current therapeutic approaches to the treatment of heart failure rely on the stimulation of cardiac contractility with the administration of cardiac glycosides or sympathomimetic agents. The absence of a safe, orally active, positive inotropic agent has prompted the search for such drugs. Recently, considerable interest has focused on the properties of some inhibitors of cyclic AMP phosphodiesterase (PDE).[2,3] Phosphodiesterase inhibitors have been described with cardiotonic and vasodilatory properties

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(1) Contribution No. 239 from the Institute of Bio-organic Chemistry; presented in part at the 188th National Meeting of the American Chemical Society, Philadelphia, PA, 26–31 August 1984; paper MEDI 12.
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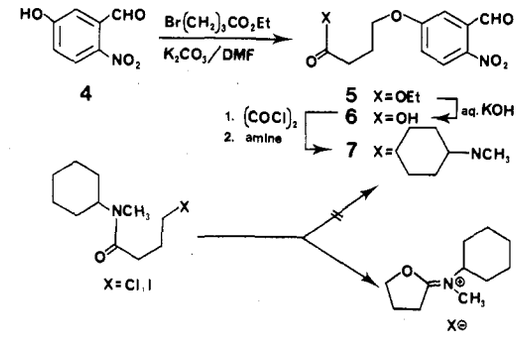
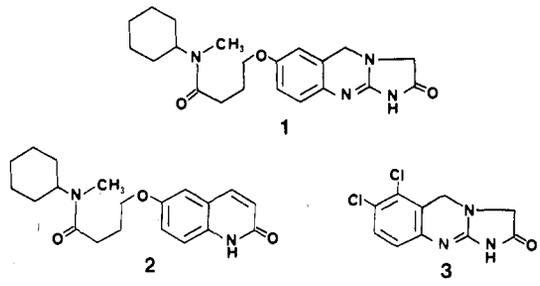
Scheme I



while others have potential antithrombotic effects.[4-7] From a therapeutic perspective, it may be advantageous to administer a cardiotonic agent that also has antithrombotic properties to those patients with a history of myocardial infarction and an increased risk of thrombosis.

Numerous biochemical studies have revealed the presence of several distinct molecular forms of phosphodiesterase in mammalian cells. The relative abundance of these forms varies in different cell types and they can be selectively inhibited by various compounds.[2,3] These findings provide a rationale for the development of tissue-selective drugs with a reduced incidence of adverse side effects.

In this paper we present the biochemical evaluation of a series of lactam heterocyclic analogues of cilostamide. The results indicate that *N*-cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)-oxy]butyramide (RS-82856, 1)[8] is a potent and tissue-selective inhibitor of cyclic AMP phosphodiesterase. This compound (1) contains essential structural elements of cilostamide (**2**)[9] and anagrelide (**3**)[10] and has potential cardiotonic and antithrombotic properties.[11]



Chemistry

Reports by two groups[5,12,13] that indicated that cilostamide (**2**) is a potent inhibitor of platelet aggregation, operating by selective inhibition of the low-K_m cyclic AMP specific PDE III (type IV),[14] prompted an examination of the molecular features necessary for the observed specificity. Cilostamide has as its two major structural components the heteroaromatic lactam, carbostyril, and a bulky lipophilic oxybutyramide side chain. Since the carbostyril moiety itself is not an effective inhibitor of cAMP phosphodiesterase (vide infra), we sought to elucidate the role that the *N*-cyclohexyl-*N*-methyloxybutyramide side chain plays in enhancing the potency of an otherwise inactive heterocycle. To this end, a series of heterocyclic lactam analogues of **2** were prepared.

Most analogues of **2** were efficiently synthesized from a common nitro aldehyde intermediate (**7**), itself readily available in three steps (Scheme I) from the known 5-hydroxy-2-nitrobenzaldehyde (**4**).[15] Alkylation of **4** with ethyl 4-bromobutyrate in DMF using potassium carbonate as base gave ester **5** in 80–95% yield as a distillable syrup. Saponification to acid **6** followed by Schotten–Baumann acylation provided amide **7** in 90% yield from **5**. Attempts to alkylate **4** directly using *N*-cyclohexyl-*N*-methyl-4-halobutyramides uniformly failed, presumably due to intramolecular cyclization and decomposition of the halobutyramide reagents under the conditions of the reaction. Nitro aldehyde **7** was converted to the corresponding nitrocinnamic acid (**8**) by using malonic acid and pyridine in ethanol (Scheme II).[15] Ferrous sulfate–ammonium hydroxide reduction of the nitro group,[16] followed by acid-catalyzed ring closure of the intermediate aminocinnamic acid, provided **2** for reference purposes. Simultaneous catalytic reduction and ring closure of **8** using 10% Pd–C in ethanol afforded dihydrocilostamide (**9**). Oxidation of **7** with tetrabutylammonium permanganate in pyridine[17] gave the nitrobenzoic acid **10**, which was catalytically reduced to the anthranilic acid **11**. Treatment of **11** with potassium cyanate in the presence of aqueous acid, followed by brief heating at reflux, gave the quinazoline-2,4-dione **12**. Condensation of **11** with phosgene in dioxane provided the isatoic anhydride **13**, which afforded the benzodiazepine-2,5-dione (**14**) upon DMAP-catalyzed condensation with glycine ethyl ester hydrochloride in refluxing pyridine.[18,19] Reduction of **7** with sodium borohydride in ethanol afforded the benzyl alcohol **15**, which was converted to the corresponding bromide **16** with (bromomethylene)dimethylammonium bromide in dioxane.[20] Alkylation of potassium phthalimide with **16** in DMF gave the nitrobenzyl phthalimide **17**. Deprotection of **17** using hydrazine in refluxing ethanol, followed by direct treatment with phenyl chloroformate and catalytic reduction with 10% Pd–C in ethanol, gave **18**, which, upon brief heating in DMF, closed to provide the cyclic urea **19**. Reduction of **15** with sodium borohydride–

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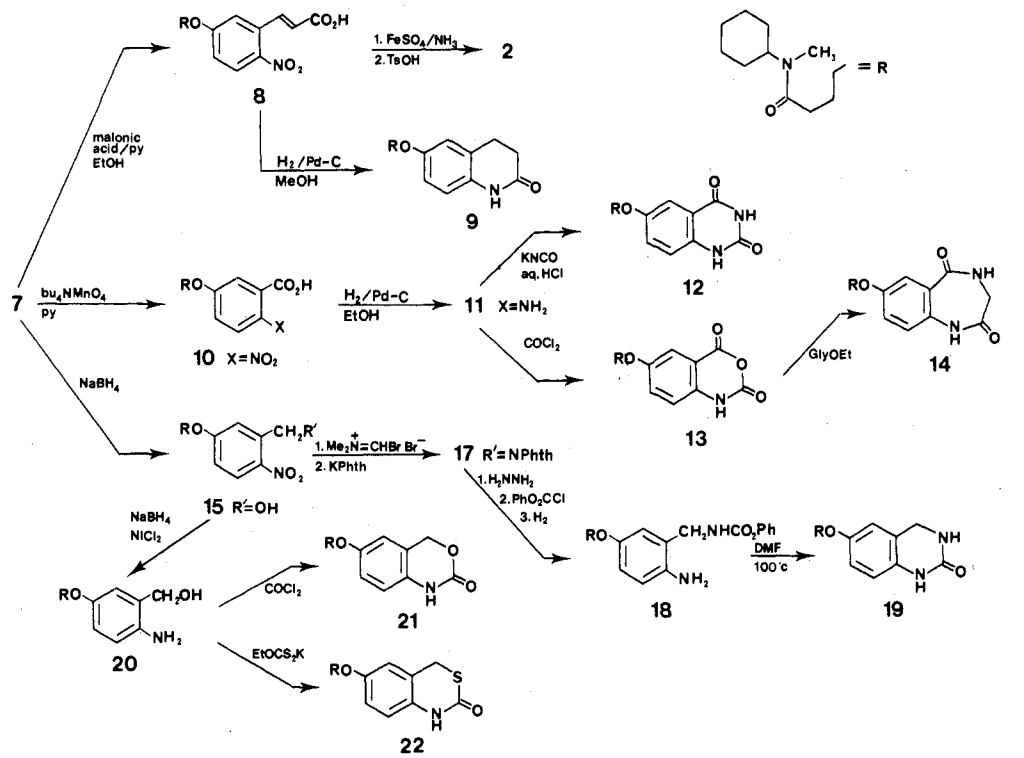
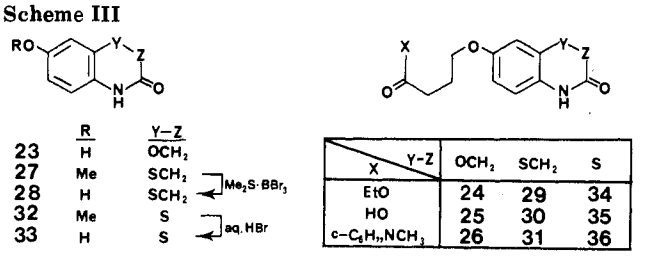
Scheme II



Scheme III



	R	Y–Z
23	H	OCH_2
27	Me	SCH_2
28	H	SCH_2
32	Me	S
33	H	S

X \ Y–Z	OCH_2	SCH_2	S
EtO	24	29	34
HO	25	30	35
c-$C_5H_{10}NCH_2$	26	31	36

nickelous chloride[21] provided **20**, treatment of which with phosgene afforded the cyclic carbamate **21**.[22] Alternatively, reaction of **20** with potassium ethyl xanthate, followed by oxidation with hydrogen peroxide,[23] yielded the cyclic thiocarbamate **22**.

Three additional analogues of **2** not available from **7** were also prepared (Scheme III). Alkylation of 2,3-dihydro-7-hydroxy-1,4-benzoxazin-3-one (**23**)[24] with ethyl 4-bromobutyrate gave **24**, converted via acid **25** to amide **26**. The thio analogue of **26** was prepared by an analogous procedure. Acylation of 2-amino-5-methoxybenzenethiol[25] with chloroacetyl chloride followed by base-induced closure gave **27**, which was demethylated under mild conditions with boron tribromide–dimethyl sulfide[26] to provide phenol **28**. Alkylation, saponification, and acylation via **29** and **30** gave amide **31**. Benzothiazolone **36** was prepared by a route similar to that for **31** from **32** via phenol **33**, ester **34**, and acid **35**.

Attachment of the *N*-cyclohexyl-*N*-methyl-4-oxybutyramide side chain of **2** to 1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one, the parent heterocycle of anagrelide (**3**), provided RS-82856 (**1**). Preparation of **1** (Scheme IV) was straightforward, once again utilizing nitro aldehyde **7** as starting material. Reductive amination of **7** using glycine ethyl ester hydrochloride, sodium acetate, and sodium cyanoborohydride gave nitro ester **37**. Catalytic reduction of the nitro group, followed by sequential treatment of the intermediate diamine with BrCN and base,[27] afforded **1** in 50–60% yield.

Scheme IV

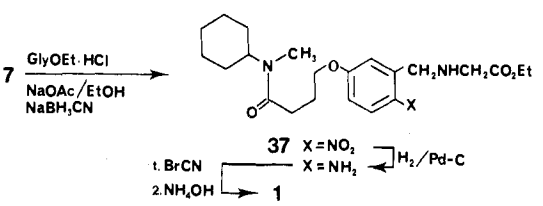


Table I. Inhibition of Cyclic AMP Phosphodiesterase by Cilostamide Analogues[a]

compd	human platelet IC_{50}, μM	rat heart IC_{25}, μM	methoxy heterocycle[b]	human platelet IC_{50}, μM
1	0.01	0.6	**48**	1.5
2	0.17	0.7	**38**	I[c]
3	0.08	>100[e]		
9	1.35	1.50	**39**	I[c]
12	1.15	1.30	**40**	53
13	4.40	>30	**41**	I[c]
14	3.60	50	**42**	I[c]
19	1.30	0.88	**43**	35
21	1.15	1.0	**45**	100
22	0.19	0.38	**46**	13
26	3.4	1.30	**47**	I[c]
31	1.80	1.40	**27**	I[c]
36	10	>10	**32**	21
49[d]	0.29	0.32		

[a] Refer to Experimental Section for assay methods and statistical interpretation of data. [b] *N*-Cyclohexyl-*N*-methylbutyramide side chain (R) of analogue replaced by methyl ether (R = CH_3). [c] Inactive at 10^{-4} M. [d] For lipophilicity comparison with **1** and **48**, R = *n*-hexyl. [e] Weak, partial inhibition obtained with **3**; maximum 22% inhibition at 10 μM at saturation.

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Table II. Inhibition of ADP-Induced Platelet Aggregation[a]

compd	IC_{50}, μM	compd	IC_{50}, μM
1	0.11	48	4
2	1.9	49	62
3	2.6		

[a] Refer to Experimental Section for assay method and statistical interpretation of data.

Methoxy-substituted heterocycles corresponding to this series of cilostamide analogues were prepared by literature procedures or by routes analogous to those used above. Details of the preparation of these heterocycles (**38–49**) are included in the Experimental Section.

Biological Evaluation

Biological evaluation of the cilostamide analogues as inhibitors of cyclic AMP phosphodiesterase was carried out in two assays using enzyme derived from human platelets and from rat heart. Results are expressed in Table I as IC_{50} (μM) values for human platelet PDE and IC_{25} (μM) values for rat heart PDE. In addition, the corresponding methoxy-substituted heterocycle for each cilostamide analogue was assayed as an inhibitor of the human platelet enzyme.

Examination of the PDE inhibition data reveals the structural requirements for activity within this series. Of the bicyclic lactam analogues of **2** (**9**, **12–14**, **19**, **21**, **22**, **26**, **31**, and **36**), none are as potent as **2** itself, but all do possess reasonable PDE inhibitory activity, with cyclic thiocarbamate **22** being most potent in both enzymes examined. More importantly, however, is the fact that, in every case examined, addition of the oxybutyramide side chain of cilostamide to a heterocycle dramatically increased the PDE inhibition observed over that seen for the methoxy-substituted heterocycle. None of the methoxy heterocycles corresponding to **2** itself or the bicyclic analogues enumerated above (**38–43**, **45–47**, **27**, and **32**, respectively) possess any notable PDE inhibitory activity (Table I). In fact, in most cases, no inhibition was observed at 100 μM. This sharp contrast in activity can only be due to the addition of the *N*-cyclohexyl-*N*-methyl-4-oxybutyramide side chain. Since the side chain itself, represented by *N*-cyclohexyl-*N*-methyl-4-hydroxybutyramide (**50**),[28] possesses no intrinsic PDE inhibitory activity in these assays (up to 100 μM), the obviously lipophilic and sterically demanding nature of this side chain likely confers an additional mode of binding to the otherwise inactive bicyclic lactam moieties of each of the analogues of **2** and, indeed, to **2** itself.

The value of the oxybutyramide side chain as a pharmacophore was confirmed when it was appended to the parent heterocycle of the potent PDE inhibitor anagrelide (**3**). Compound **1**, the combination of the major structural features of both **2** and **3**, possesses greater PDE inhibitory activity than either of its progenitors, being 17 and 8 times more potent than **2** and **3**, respectively, in inhibiting the FIII (type IV) soluble human platelet enzyme. Compound **1** also exhibited increased potency over both **2** and **3**, by factors of 17 and 24, respectively, in ADP-induced platelet aggregation (Figure 1, Table II). The expected synergy with PGE_1 was also observed with **1**.

The nature of the observed enhancement in activity of **1** over **3** was further investigated by comparison of **1** with the 7-methoxy- and 7-*n*-hexyloxy-substituted analogues **48** and **49**. As had been the case in the cilostamide analogue series, attachment of the oxybutyramide side chain

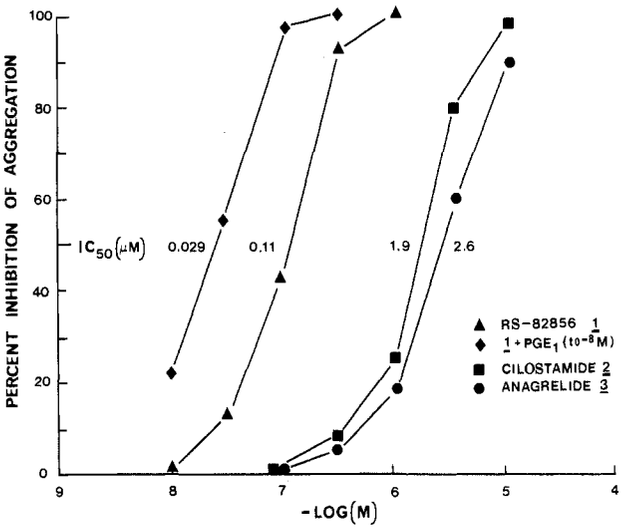

Figure 1. Inhibition of ADP-induced platelet aggregation in vitro by phosphodiesterase inhibitors **1–3** and synergistic action of **1** with exogenous PGE_1.

provided a compound possessing an unambiguous advantage over the parent heterocycle. In the platelet PDE assay (Table I), **1** was 150 and 29 times more potent that **48** and **49**, respectively, indicating that, while an increase in lipophilicity alone leads to an enhancement of activity (**48** vs. **49**), a compound of approximately the same lipophilicity but possessing the oxybutyramide side chain (**1** vs. **49**) is still more potent likely due to an additional mode of binding to the enzyme imparted by the oxybutyramide side chain.

While **1** maintained an overall potency advantage over both **48** and **49** as an inhibitor of platelet aggregation (Table II), a significant reversal in order was observed: **1** was 36 and 563 times more potent than **48** and **49**, respectively. This trend points out that, although an increase in net lipophilicity was favorable in the in vitro PDE assay, the steric demands of the oxybutyramide moiety contribute more to the observed pattern of augmented potency for **1** than does the simple increase in lipophilicity. The relevance of these observations to the mapping of the PDE FIII binding site using analogues of **1** designed to prove these characteristics are discussed in the accompanying paper.[29]

Further investigations[11] into the PDE inhibitory profile of **1** indicate that it possesses high selectivity for the low-K_m, high-affinity form of cAMP PDE, with only weak effects on both the nonspecific and cyclic GMP sensitive phosphodiesterases. The compound also exhibits a pattern of tissue selectivity, having significant inhibitory activity on human platelet and dog heart membrane-bound PDE, while possessing little or no activity against PDE derived from spleen, intestine, kidney, stomach, lung, and skeletal muscle at concentrations as high as 100 μM. In addition, administration of **1** to instrumented, anesthetized dogs by either intravenous or intraduodenal routes increases cardiac contractile force and reduces afterload.[11] Taken together, these data suggest that **1** may be of potential use as an agent to increase cardiac output in the treatment of congestive heart failure.

Conclusion

Dissection of **2** in a systematic manner revealed that the *N*-cyclohexyl-*N*-methyl-4-oxybutyramide side chain was

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(29) Venuti, M. C.; Jones, G. H.; Alvarez, R.; Bruno, J. J. *J. Med. Chem.*, following paper in this issue.

essential to the observed PDE inhibitory potency of **2**. The attachment of this apparent steric and/or lipophilic pharmacophore to a heterocycle possessing intrinsic PDE inhibitory activity afforded **1**, which has proved to be a potent inhibitor of cAMP PDE and of platelet aggregation. Further modifications of this parent structure (**1**) have been carried out, including optimization of the length, position, and substitution of the amide side chain, heterocycle substituents, ring isomers, and others. These results, reported in the accompanying paper,[29] encompass a wide range of phosphodiesterase inhibitory activities and provide a clearer picture of the structural features required for potency, tissue selectivity, and enhanced in vivo activity.

Experimental Section

Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H 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. Mass spectra were determined on an Atlas CH-4 or CH-7 instrument. All compounds exhibited NMR, IR, and mass spectral data consistent with the proposed structures. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA, on samples dried 24 h at ambient temperature and high vacuum and were within 0.4% of theoretical values. All organic extracts were dried over sodium sulfate prior to evaporation.

Ethyl 4-(3-Formyl-4-nitrophenoxy)butyrate (5). Potassium carbonate (76.0 g, 550 mmol) was added to a solution of 5-hydroxy-2-nitrobenzaldehyde (**4**)[15] (84.0 g, 500 mmol) and ethyl 4-bromobutyrate (Aldrich; 86 mL, 600 mmol) in dry DMF (500 mL) blanketed under dry nitrogen. The reaction mixture was heated to 100 °C for 1 h with mechanical stirring, at which time TLC (10% methanol in dichloromethane) of an acid-washed aliquot showed that all starting **4** had been consumed. The reaction mixture was cooled, and the DMF was removed by evaporation to give a dark brown syrup. The residue was partitioned between ethyl acetate and saturated sodium carbonate (500 mL each). The organic layer was washed with additional saturated sodium carbonate (3 × 500 mL) and brine (2 × 500 mL), dried, filtered, and thoroughly evaporated to give a dark brown syrup free of **3** by TLC (CH_2Cl_2).[30] Kugelrohr distillation (180 °C, 0.05 mm) afforded **5** (133.4 g, 474 mmol, 95%) as a bright yellow syrup, which slowly darkens upon standing. Anal. ($C_{13}H_{15}NO_6$) C, H, N.

4-(3-Formyl-4-nitrophenoxy)butyric Acid (6). A solution of potassium hydroxide (80 g) in water (200 mL) was added dropwise to a solution of **5** (262 g, 933 mmol) in ethanol (500 mL). After TLC (dichloromethane) indicated clean conversion to product, the reaction mixture was acidified with concentrated HCl, and the ethanol was evaporated. The precipitate was collected by filtration, washed with water, and dried in vacuo over P_2O_5 for 72 h to afford **6** (226 g, 893 mmol, 96%), mp 109–110 °C. Anal. ($C_{11}H_{11}NO_6$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-(3-formyl-4-nitrophenoxy)butyramide (7).** Oxalyl chloride (28.4 mL, 325 mmol) was added dropwise to a stirred suspension of **6** (55 g, 217 mmol) in benzene (300 mL) and DMF (0.5 mL). The reaction mixture was stirred at room temperature for 1 h, at which time **6** had completely dissolved. The solution was evaporated to a thick syrup, redissolved in dry THF (100 mL), and reevaporated twice. The final residue was dissolved in THF (200 mL) and was added dropwise to a well-stirred mixture of *N*-methylcyclohexylamine (29.5 mL, 260 mmol) and sodium carbonate (28.8 g, 270 mmol) in THF (250 mL) and water (500 mL) cooled to 0 °C. When the addition was complete, the ice bath was removed, and the reaction mixture was allowed to stir at room temperature for 1 h. Most of the THF was removed by evaporation, and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate (500 mL each). The organic layer was washed with additional saturated sodium bicarbonate (2 × 200 mL), water (100 mL), 1 M HCl (2 × 200 mL), and brine (2 × 200 mL) and then dried, filtered, and evaporated to give **7** (75 g, 215 mmol, 99%) as a yellow solid, mp 98–100 °C. Anal. ($C_{18}H_{24}N_2O_5 \cdot 0.5H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[3-(2-carboxyvinyl)-4-nitrophenoxy]butyramide (8).** A mixture of **7** (10.5 g, 30 mmol), malonic acid (4.7 g, 45 mmol), and pyridine (0.75 mL) in absolute ethanol (20 mL) was heated to reflux, at which time a homogeneous solution was obtained. After heating overnight, the reaction mixture was cooled and partitioned between ethyl acetate and water (100 mL each). The organic layer was washed with saturated sodium bicarbonate (3 × 100 mL), and the combined aqueous layers were washed with ether (2 × 100 mL). The aqueous layer was carefully acidified with concentrated HCl and was then extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with brine (2 × 100 mL), dried, filtered, and evaporated at high vacuum to give a syrup, which, upon reevaporation from dichloromethane, afforded **8** (9.70 g, 24.8 mmol, 83%) as an amorphous foam.

***N*-Cyclohexyl-*N*-methyl-4-[(1,2-dihydro-2-oxoquinolin-2-6-yl)oxy]butyramide (Cilostamide, 2).** Iron(II) sulfate heptahydrate (56 g, 200 mmol) was added in one amount to a solution of **8** (9.7 g, 25 mmol) in concentrated ammonium hydroxide (60 mL) at reflux. The dark mixture was maintained at reflux for 15 min and was then filtered through a pad of Celite. The precipitated mass was washed with hot 1 M sodium hydroxide (600 mL), and the green filtrates were combined. Glacial acetic acid (36 mL) was added, and the aqueous layer was covered with ethyl acetate (300 mL) and acidified to pH 4–5 with concentrated HCl. The aqueous layer was extracted with additional ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (2 × 200 mL), dried, filtered, and evaporated to give a red-brown syrup (9.6 g). The crude amino acid was dissolved in toluene–THF (250 mL, 4:1), and to the solution was added *p*-toluenesulfonic acid (9.5 g, 50 mmol). The solution was maintained at reflux for 2 days and then cooled and thoroughly extracted with saturated sodium bicarbonate (4 × 200 mL) and brine (2 × 200 mL). The organic layer was dried, filtered, evaporated, and recrystallized from ethyl acetate to yield **2** (1.80 g, 5.25 mmol, 21%), mp 180–181 °C (lit.[9] mp 186–188 °C). Anal. ($C_{20}H_{26}N_2O_3$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(1,2,3,4-tetrahydro-2-oxoquinolin-6-yl)oxy]butyramide (9).** A solution of **8** (3.9 g, 10 mmol) in methanol (75 mL) was hydrogenated at 60 psi over 10% Pd–C (0.5 g) until uptake ceased, approximately 3 h. The reaction mixture was filtered to remove the catalyst, and the filtrate was evaporated. The residue was dissolved in ethyl acetate (100 mL), and the organic layer was washed with saturated sodium bicarbonate (5 × 50 mL) and with brine (2 × 50 mL), dried, filtered, and evaporated to give a crude foam. Chromatography over silica gel (1:1 ethyl acetate–dichloromethane) afforded **9** (0.57 g, 1.66 mmol, 17%) as a crisp foam that crystallized from ether, mp 141–142 °C (lit.[31] mp 144–146 °C). Anal. ($C_{20}H_{28}N_2O_3$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-(3-carboxy-4-nitrophenoxy)butyramide (10).** Solid tetrabutylammonium permanganate[17] (2.53 g, 7 mmol) was added portionwise over 1 h to a solution of **7** (3.5 g, 10 mmol) in dry pyridine (20 mL) under a blanket of nitrogen. The reaction was stirred at room temperature for 1 h and was then poured into ethyl acetate–6 M HCl (100 mL each). Solid sodium bisulfite was added to decolorize the solution, and the layers were separated. The aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with 1 M HCl (3 × 50 mL) and brine (2 × 50 mL), dried, filtered, and evaporated to give a syrup, which foamed at high vacuum from dichloromethane to yield **10** (3.45 g, 9.5 mmol, 95%) as an amorphous solid. Anal. ($C_{18}H_{24}N_2O_6 \cdot 0.5H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-(4-amino-3-carboxyphenoxy)butyramide (11).** A solution of **10** (78.7 g, 216 mmol) in absolute ethanol (750 mL) was hydrogenated at 60 psi over 10% Pd–C (6 g) overnight. The catalyst was removed by filtration through a pad of Celite and was thoroughly washed with additional ethanol

(30) Incomplete removal of **4** from the crude product will result in extensive and potentially explosive decomposition during distillation.

(31) Nishi, T.; Ueda, H.; Nakagawa, K. Jpn. Kokai Tokkyo Koho 79 168,825, 26 Dec 1979; *Chem. Abstr.* **1980**, *93*, 26291r.

(250 mL). The combined filtrates were thoroughly evaporated to give a thick syrup, which precipitated from hexane–dichloromethane to afford 11 (42.0 g, 126 mmol, 58%) as a yellow powder, mp 175–176 °C. Anal. ($C_{18}H_{26}N_2O_4$) C, H, N. Hydrogenation in the presence of HCl gave 11·HCl salt as a hygroscopic solid in 95% yield.

***N*-Cyclohexyl-*N*-methyl-4-[(1,3-dihydro-2,4-dioxoquinazolin-6-yl)oxy]butyramide (12).** A suspension of 11 (2.17 g, 6.5 mmol) in water (10 mL) and THF (5 mL) was treated with 1 M HCl (6.5 mL) and potassium cyanate (0.65 g, 7.8 mmol). After the mixture was stirred at room temperature for 30 min, all material had dissolved, and the reaction mixture was extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with brine (2 × 20 mL) and then evaporated. The residue was dissolved in concentrated HCl–ethanol (20 mL, 1:1) and heated at 90 °C for 1 h. The mixture was cooled, poured into water (50 mL), and extracted with ethyl acetate (3 × 50 mL). The organic extract was washed with brine (2 × 20 mL), dried, filtered, and evaporated to give 12 (0.85 g, 2.37 mmol, 36%) as a brown solid, mp 202–204 °C. Anal. ($C_{19}H_{25}N_3O_4·H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(1,4-dihydro-2,4-dioxo-2*H*-3,1-benzoxazin-6-yl)oxy]butyramide (13).** Condensed phosgene (10 mL) was added to a suspension of 10 (3.30 g, 10 mmol) in dioxane (50 mL), and the resulting mixture was heated to 60 °C with mechanical stirring. After 1 h, the thick suspension was poured into THF–ethyl acetate (200 mL, 1:1) and was thoroughly shaken with water (100 mL) to hydrolyze any iminium chloride formed. The organic layer was washed with brine (2 × 100 mL) and evaporated to give a solid, which recrystallized from THF to yield 13 (3.50 g, 9.7 mmol, 97%), mp 223–224 °C dec. Anal. ($C_{19}H_{24}N_2O_5$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(2,5-dioxo-1,4-benzodiazepin-7-yl)oxy]butyramide (14).** A mixture of 13 (1.8 g, 5 mmol), glycine ethyl ester hydrochloride (0.75 g, 6 mmol), and 4-(dimethylamino)pyridine (61 mg, 0.5 mmol) in dry pyridine was heated at 140 °C (oil bath temperature) for 4 days and then cooled and poured into ethyl acetate (100 mL). The organic phase was washed with 6 M HCl (2 × 25 mL), 1 M HCl (2 × 25 mL), and brine (2 × 25 mL) and then was dried, filtered, and evaporated. Recrystallization of the residue from ethyl acetate–ether provided 14 (420 mg, 1.12 mmol, 22%), mp 95–96 °C. Anal. ($C_{20}H_{27}N_3O_4·0.5H_2O$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[3-(hydroxymethyl)-4-nitrophenoxy]butyramide (15).** A suspension of 7 (35 g, 100 mmol) and sodium borohydride (1.20 g, 32 mmol) in absolute ethanol (250 mL) was stirred at room temperature until TLC (CH_2Cl_2) showed complete conversion, approximately 1 h. The ethanol was removed by evaporation, and the residue was partitioned between ethyl acetate and water (200 mL each). The mixture was carefully quenched with 6 M HCl, the layers were separated, and the aqueous layer was washed with ethyl acetate (2 × 100 mL). The combined organic layers were washed with 1 M HCl (2 × 100 mL) and brine (2 × 100 mL), dried, filtered, and evaporated to give an amber oil, which crystallized upon trituration with ethyl acetate–ether to afford 15 (35 g, 100 mmol, 100%), mp 95–96 °C. Anal. ($C_{18}H_{26}N_2O_5$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(3,4-dihydro-2-oxo-1*H*-quinazolin-6-yl)oxy]butyramide (19).** A solution of 15 (24.5 g, 70 mmol) in dioxane (100 mL) was added dropwise to a suspension of (bromomethylene)dimethylammonium bromide[20] (19 g, 87.5 mmol) in dioxane (100 mL) cooled to 0 °C under a blanket of dry nitrogen. The mixture was then heated at reflux for 30 min, at which time TLC indicated complete conversion. The reaction mixture was cooled and most of the dioxane was removed by evaporation. The residue was partitioned between ether and water (100 mL each), and the organic layer was washed with additional water (2 × 100 mL) and brine (2 × 100 mL), dried, filtered, and evaporated to give crude, and somewhat unstable, bromide 16 (29 g, 70 mmol, 100%) as an amber syrup. A small sample was purified by silica gel chromatography (dichloromethane) to afford 16 as a yellow syrup. Anal. ($C_{18}H_{25}BrN_2O_4·0.25H_2O$) C, H, N.

A suspension of potassium phthalimide (5.5 g, 30 mmol) and 16 (10.4 g, 25 mmol) in dry DMF (100 mL) was heated at 100 °C overnight. The resulting chocolate-brown mixture was poured into ethyl acetate (500 mL) and was then washed with water (4 × 100 mL) and brine (2 × 100 mL), dried, filtered, and evaporated. The residue was crystallized from ethyl acetate–ether to yield 17 (6.50 g, 13.6 mmol, 54%), mp 156–158 °C. Anal. ($C_{26}H_{29}N_3O_6$) C, H, N.

A mixture of 17 (10 g, 20.8 mmol) and anhydrous hydrazine (20 mL) in absolute ethanol (500 mL) was brought to reflux. Within minutes, a thick precipitate of phthalazinedione had been formed. After 30 min, the reaction was cooled to 0 °C and filtered to remove the precipitated byproduct. Most of the ethanol was removed by evaporation, and the residue was dissolved in ethyl acetate (200 mL). The organic layer was thoroughly washed with water (5 × 100 mL) and brine (2 × 100 mL), dried, filtered, and evaporated to give the crude benzylamine, which was used immediately. A solution of the benzylamine (6.60 g, 19 mmol) and sodium carbonate (2.76 g, 26 mmol) in THF (50 mL) and water (100 mL) cooled to 0 °C was treated with a solution of phenyl chloroformate (4.07 g, 26 mmol) in THF (25 mL) added dropwise. When the addition was complete, the ice bath was removed, and the reaction was allowed to stir at room temperature for 30 min. The THF was removed by evaporation, and the aqueous residue was extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with saturated sodium bicarbonate (2 × 50 mL), 1 M HCl (2 × 50 mL), and brine (2 × 50 mL), dried, filtered, and evaporated to give crude nitrocarbamate. Hydrogenation of the crude intermediate in ethanol (100 mL) over 10% Pd–C (1.0 g) at 50 psi was complete overnight. The ethanol was evaporated, and the residue was dissolved in ethyl acetate (200 mL). The organic layer was washed with brine (2 × 50 mL), dried, filtered, and evaporated to give a solid. Recrystallization from ethyl acetate–ether afforded 18 (5.60 g, 16.2 mmol, 85%), mp 128–129 °C. Anal. ($C_{25}H_{31}N_3O_4·H_2O$) C, H, N.

A solution of 18 (1.10 g, 2.5 mmol) in DMF (10 mL) was heated to 110 °C for 30 min. The reaction mixture was poured into ethyl acetate (50 mL), and the organic layer was washed with water (3 × 10 mL) and brine (2 × 10 mL), dried, filtered, and evaporated to give a syrup, which crystallized on trituration with ether to yield 19 (850 mg, 2.46 mmol, 90%), mp 160–161 °C. Anal. ($C_{19}H_{27}N_3O_3$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[4-amino-3-(hydroxymethyl)phenoxy]butyramide (20).** Nickel(II) chloride hexahydrate (10.31 g, 43.4 mmol) was added to a suspension of 15 (7.60 g, 21.7 mmol) in methanol (150 mL). The mixture was cooled to 0 °C, and sodium borohydride (3.28 g, 86.8 mmol) was added in small portions over 30 min. The ice bath was removed, and the reaction was allowed to stir at room temperature for 30 min. The methanol was removed by evaporation, and the black residue was dissolved in 1 M HCl (200 mL). Additional concentrated HCl was added to complete the dissolution of the inorganic residues. The solution was made basic with concentrated ammonium hydroxide and was then extracted with ethyl acetate (3 × 20 mL). The organic layer was washed with brine (2 × 100 mL), dried, filtered, and evaporated to give an amber syrup. Chromatography over silica gel (8% methanol in dichloromethane) afforded 20 (6.15 g, 19.2 mmol, 88.5%) as a thick syrup, which crystallized on standing, mp 60–61 °C. Anal. ($C_{18}H_{28}N_2O_3$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(2-oxo-4*H*-3,1-benzoxazin-6-yl)oxy]butyramide (21).** Condensed phosgene (5 mL) was added to a solution of 20 (3.2 g, 10 mmol) in THF (25 mL), and the mixture was stirred at 50 °C for 1 h. The THF was removed by evaporation, and the residue was partitioned between ether and water (50 mL each). The organic layer was washed with brine (2 × 50 mL), dried, filtered, and evaporated to give a brown foam. Chromatography over silica gel (ethyl acetate–dichloromethane gradient from 1:3 to 1:1) gave 21 (0.7 g, 2 mmol, 20%), as a white foam, which crystallized from ethyl acetate–ether, mp 139–140 °C. Anal. ($C_{19}H_{26}N_2O_4$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(2-oxo-4*H*-3,1-benzothiazin-6-yl)oxy]butyramide (22).** A solution of 20 (0.30 g, 0.94 mmol) and potassium ethyl xanthate (1.80 g, 11.2 mmol) in dry DMF (10 mL) was heated at 100 °C for 5 days under a blanket of nitrogen. The DMF was removed by evaporation, the residue was dissolved in 1 M NaOH (15 mL), and 3% H_2O_2 (15 mL) was added. After 15 min at room temperature, the mixture was extracted with ethyl acetate (25 mL). The organic extract was washed with brine (2 × 10 mL), dried, filtered, and evaporated to give a residue, which was chromatographed over silica gel (ethyl

acetate–dichloromethane, 1:1) to yield **22** (30 mg, 0.083 mmol, 9%) as an amorphous solid. Anal. ($C_{19}H_{26}N_2O_3S\cdot0.5H_2O$) C, H, N, S.

***N*-Cyclohexyl-*N*-methyl-4-[(2,3-dihydro-3-oxo-1,4-benzoxazin-7-yl)oxy]butyramide (26).** Alkylation of 2,3-dihydro-7-hydroxy-1,4-benzoxazin-3-one (**23**)[24] was carried out as for **5** on a 50-mmol scale. Chromatography of the crude ester over silica gel (ethyl acetate–dichloromethane gradient 1:2 to 1:1) afforded **24** (5.02 g, 18 mmol, 36%), mp 112–113 °C. Anal. ($C_{14}H_{17}NO_5$) C, H, N. Saponification of **24** was carried out as for **6** on an 18-mmol scale to yield acid **25** (1.77 g, 7.1 mmol, 41%), mp 198–200 °C. Anal. ($C_{12}H_{13}NO_5$) C, H, N. Schotten–Baumann acylation was carried out as for **7** on a 2-mmol scale to give **26** (0.31 g, 0.9 mmol, 45%), mp 172–173 °C. Anal. ($C_{19}H_{26}N_2O_4\cdot0.25H_2O$) C, H, N.

7-Methoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (27). A solution of chloroacetyl chloride (3.75 mL, 47 mmol) in THF (20 mL) was added to an ice-cooled solution of 2-amino-5-methoxybenzenethiol[25] (7.50 g, 39 mmol) and potassium carbonate (11.35 g, 82 mmol) in THF (80 mL) and water (60 mL). The cooling bath was removed, and the reaction was allowed to stir overnight. Sodium hydroxide (2 N, 50 mL) was added to the mixture in three portions to bring the pH to 12. After stirring an additional hour at room temperature, the mixture was acidified to pH 2 with concentrated HCl and evaporated to remove THF, upon which the product crystallized. Filtration and drying afforded **27** (5.89 g, 30 mmol, 77%), mp 181–183 °C. Anal. ($C_9H_9NO_2S$) C, H, N, S.

***N*-Cyclohexyl-*N*-methyl-4-[(3,4-dihydro-3-oxo-2*H*-1,4-benzothiazin-7-yl)oxy]butyramide (31).** To a suspension of boron tribromide–methyl sulfide complex[26] (32 g, 102 mmol) in toluene (200 mL) was added **27** (5.50 g, 28.2 mmol). The mixture was heated to reflux under nitrogen and was maintained there overnight. The reaction was cooled, diluted with ethyl acetate (300 mL), and then quenched with water (100 mL). The organic layer was washed with saturated sodium bicarbonate (2 × 500 mL), followed by thorough extraction with 4% sodium hydroxide (4 × 100 mL). The combined basic layers were acidified to pH 2 and extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine (2 × 50 mL), dried, filtered, and evaporated to give **28** (3.9 g, 21.5 mmol, 76%), as an amorphous solid. Alkylation of **28** with ethyl 4-bromobutyrate was carried out as for **24** on a 21.5-mmol scale. Column chromatography (15% ethyl acetate in dichloromethane) afforded **29** (2.38 g, 8.06 mmol, 37.5%), mp 117–118 °C. Anal. ($C_{14}H_{17}NO_4S$) C, H, N, S. Saponification of **29** was carried out on a 9-mmol scale as for **25** to yield **30** (2.21 g, 8.3 mmol, 92%), mp 198–199.5 °C. Anal. ($C_{12}H_{13}NO_4S$) C, H, N, S. Schotten–Baumann acylation of **30** on a 2-mmol scale as for **26** gave **31** (0.10 g, 0.28 mmol, 14%) as an amorphous solid. Anal. ($C_{19}H_{26}N_2O_3S\cdot0.25H_2O$) C, H, N, S.

6-Methoxy-1,3-benzothiazol-2(3*H*)-one (32). 2-Amino-6-methoxybenzothiazole (Aldrich; 18.0 g, 100 mmol) was diazotized at −10 °C in a mixture of formic acid (50 mL), acetic acid (20 mL), and concentrated HCl (40 mL) with sodium nitrite (7.0 g, 100 mmol) in water (10 mL) as described.[32] The mixture was allowed to warm to room temperature, with some bubbling evident, and was then heated to reflux for 24 h. The reaction mixture was cooled, diluted with water (500 mL), and extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with brine (2 × 50 mL), dried, filtered, and evaporated to give a crude solid. Chromatography over silica gel (10% ethyl acetate in dichloromethane) afforded **32** (7.1 g, 39 mmol, 39%), mp 163–165 °C. Anal. ($C_8H_7NO_2S$) C, H, N, S.

***N*-Cyclohexyl-*N*-methyl-4-[(2,3-dihydro-2-oxo-1,3-benzothiazol-6-yl)oxy]butyramide (36).** A suspension of **32** (4.05 g, 22.3 mmol) in 48% HBr (120 mL) was brought to reflux for 1 h. The reaction mixture was cooled, diluted with saturated brine (100 mL), and extracted with ethyl acetate (3 × 100 mL). The organic extracts were washed with brine (2 × 50 mL), dried, filtered, and evaporated to yield **33** (3.54 g, 21.2 mmol, 95%), mp 233–235 °C. Anal. ($C_7H_5NO_2S\cdot0.2H_2O$) C, H, N, S. Alkylation of **33** (0.80 g, 4.8 mmol) was carried out with ethyl 4-bromobutyrate (0.82 mL, 5.7 mmol) and potassium carbonate (0.73 g, 5.3 mmol) in DMF (10 mL) as for **5** to yield **34** (0.66 g, 2.35 mmol, 49%) after chromatography over silica gel (10% ethyl acetate in dichloromethane), mp 108–110 °C. Anal. ($C_{13}H_{15}NO_4S$) C, H, N, S. Saponification of ester **34** on a 3.7-mmol scale as for **25** gave **35** (0.87 g, 3.4 mmol, 93%), mp 168–171 °C. Anal. ($C_{11}H_{11}NO_4S$) C, H, N, S. Dicyclohexylcarbodiimide (0.27 g, 1.3 mmol) was added to a solution of **35** (0.30 g, 1.2 mmol) and 1-hydroxybenzotriazole (0.36 g, 2.6 mmol) in dry THF (10 mL) cooled to 0 °C. After the mixture was stirred for 1 h, *N*-methylcyclohexylamine (0.17 mL, 1.3 mmol) and *N*-methylmorpholine (0.26 mL, 2.4 mmol) were added. The mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. Ethyl acetate (20 mL) was added, and the mixture was stirred for 1 h and then filtered to remove DCU. The organic filtrate was washed with 1 M HCl (2 × 10 mL), saturated sodium bicarbonate (2 × 10 mL), and brine (2 × 10 mL), dried, filtered, and evaporated. The residue was crystallized from ethyl acetate–ether to yield **36** (0.16 g, 0.46 mmol, 38%), mp 107–110 °C. Anal. ($C_{18}H_{24}N_2O_3S\cdot0.25H_2O$) C, H, N, S.

***N*-Cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (1).** Anhydrous sodium acetate (4.1 g, 50 mmol) was added to a warm solution of glycine ethyl ester hydrochloride (8.4 g, 60 mmol) in absolute ethanol (200 mL). The resulting mixture was stirred overnight at room temperature and was then filtered. Nitro aldehyde **7** (8.7 g, 25 mmol) was added, followed by sodium cyanoborohydride (0.95 g, 15 mmol) in 30 min. After 3 h the solution was evaporated, and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate (300 mL each). The organic extract was washed with additional aqueous sodium bicarbonate (4 × 250 mL) and brine (2 × 250 mL) and then dried, filtered, and evaporated to give **37** as a thick syrup. A small amount of the syrup was dissolved in ether and was treated with dry HCl to afford **37**·HCl as an amorphous foam. Anal. ($C_{22}H_{33}N_3O_6\cdot HCl\cdot2H_2O$) C, H, N. Crude **37** was dissolved in absolute ethanol (100 mL) and was hydrogenated over 10% Pd–C (1.0 g) until uptake ceased, approximately 4 h. The catalyst was removed by filtration through a pad of Celite, and the pad was washed clean with additional absolute ethanol (50 mL). The combined filtrates were treated with cyanogen bromide (3.20 g, 30 mmol), and the resulting solution was stirred at room temperature for 16 h. Concentrated ammonium hydroxide (5 mL) was added, and the solution was stirred at room temperature for 30 min, during which time the product crystallized. Filtration, followed by ethanol and ether washes and drying, afforded **1** (5.09 g, 13.25 mmol, 53%), mp 243–244 °C. Anal. ($C_{21}H_{28}N_4O_3$) C, H, N.

1,2-Dihydro-6-methoxyquinolin-2-one (38). 5-Methoxy-2-nitrobenzaldehyde was converted via 5-methoxy-2-nitrocinnamic acid[15] and 5-methoxy-2-aminocinnamic acid[16] to give **38**, mp 208–210 °C (lit.[33] mp 210 °C).

1,2,3,4-Tetrahydro-6-methoxyquinolin-2-one (39). 5-Methoxy-2-nitrocinnamic acid[15] (11.25 g, 50 mmol) was subjected to reductive cyclization using triethylamine–formic acid and 5% palladium on charcoal according to literature procedure[34] to give **39** (4.20 g, 23.7 mmol, 47%) after chromatography, mp 144–145 °C (lit.[35] mp 143–144 °C).

6-Methoxyquinazolin-2,4(1*H*,3*H*)-dione (40). 5-Methoxyanthranilic acid hydrochloride[36] (10.2 g, 50 mmol) was treated with potassium cyanate (4.90 g, 60 mmol) in water (50 mL) according to literature procedure[37] to give **40** (6.25 g, 32.5 mmol, 65%), mp >270 °C (lit.[37] mp 316–318 °C).

5-Methoxy-2*H*-3,1-benzoxazin-2,4(1*H*)-dione (41). A suspension of 5-methoxyanthranilic acid (17.6 g, 105 mmol) in dioxane (150 mL) was treated with condensed phosgene (22 mL, 315 mmol). The resulting solution was stirred at 60 °C for 3 h, cooled,

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and filtered to yield 41 (18.0 g, 93 mmol, 89%), mp 246–247 °C (lit.[38] mp 244–246 °C).

7-Methoxy-1,4-benzodiazepine-2,5-dione (42). Treatment of 41 with glycine ethyl ester hydrochloride and DMAP in pyridine as for 14, on a 5-mmol scale, gave 42 (0.67 g, 3.25 mmol, 65%), mp 263–265 °C. Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

3,4-Dihydro-6-methoxy-1*H*-quinazolin-2-one (43). 5-Methoxy-2-nitrobenzylamine[39] was treated with phenyl chloroformate, reduced, and cyclized in hot DMF as for 19 to yield 43 (0.46 g, 52%), mp 224–225 °C. Anal. ($C_9H_{10}N_2O_2$) C, H, N.

2-Amino-5-methoxybenzyl Alcohol (44). 5-Methoxyanthranilic acid (10.2 g, 50 mmol) was reduced with borane–methyl sulfide according to the procedure of Lane[40] to give 44 (4.25 g, 27.8 mmol, 55%), mp 88–90 °C. Anal. ($C_8H_{11}NO_2$) C, H, N.

6-Methoxy-4*H*-3,1-benzoxazin-2-one (45). Alcohol 44 (3.06 g, 20 mmol) was treated with condensed phosgene (1.8 mL, 25 mmol) in THF (25 mL) to give 45 (2.40 g, 13.4 mmol, 67%), mp 161–162 °C. Anal. ($C_9H_9NO_3$) C, H, N.

6-Methoxy-4*H*-3,1-benzothiazin-2-one (46). Amino alcohol 44 (0.57 g, 3.7 mmol) was treated with potassium ethyl xanthate (2.7 g, 16.8 mmol) in DMF (30 mL), followed by hydrogen peroxide oxidation, according to literature procedure,[23] to yield 46 (0.20 g, 1.25 mmol, 34%), mp 163–164 °C. Anal. ($C_9H_9NO_2S$) C, H, N, S.

2,3-Dihydro-7-methoxy-1,4-benzoxazin-3-one (47). Alkylation of 2,3-dihydro-7-hydroxy-1,4-benzoxazin-3-one (23)[24] with dimethyl sulfate–potassium hydroxide in ethanol provided 47, mp 158–159 °C. Anal. ($C_9H_9NO_3$) C, H, N.

7-Methoxy-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one (48). The title compound 48 was prepared by the general method of Yamaguchi and Ishikawa for the construction of the parent heterocycle.[41] 2,4-Dichloro-6-methoxyquinazoline (10 g, 50 mmol), prepared from 40 by literature methods,[37] was reduced to 2-chloro-3,4-dihydro-6-methoxyquinazoline with sodium borohydride in chloroform–ethanol. Alkylation with ethyl bromoacetate/potassium carbonate in 2-butanone, followed by ring closure with anhydrous ammonia in ethylene glycol, afforded 48 (0.95 g, 4.4 mmol, 8.8% overall yield), mp >300 °C (lit.[27] mp 267–270 °C). Anal. ($C_{11}H_{11}N_3O_2$) C, H, N. Additional spectral evidence (NMR, IR, MS) confirmed the title structure despite the difference in the observed and reported melting points.

7-(Hexyloxy)-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one (49). Phenol 4 (33.4 g, 200 mmol) was alkylated with 1-bromohexane (33.7 mL, 240 mmol) by the procedure used for 5. Aqueous workup and crystallization of the residue from petroleum ether afforded 5-(hexyloxy)-2-nitrobenzaldehyde (41.5 g, 165 mmol, 83%), mp 40–41 °C. Anal. ($C_{13}H_{17}NO_4$) C, H, N. Following the sequence used for the preparation of 1, the title compound 49 was obtained in 30% yield, mp 266–268 °C. Anal. ($C_{16}H_{21}N_3O_2$) C, H, N.

Biological Evaluation. [G-^{3}H]- or [^{32}P]adenosine 3′:5′-monophosphate (10–50 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). Other reagent chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Phosphodiesterase Preparations. Human Platelet. Blood was obtained from donors who had not taken aspirin or similar medications for at least 2 weeks and was collected by venipuncture into evacuated glass tubes (Vacutainer, Becton, Dickinson, Rutherford, NJ) containing EDTA (7.7 mM, final concentration). Platelet-rich plasma (PRP) was obtained by centrifuging the blood in polycarbonate tubes at 200*g* for 15 min at 4 °C. All subsequent steps were performed at 4 °C. A platelet pellet was obtained by further centrifugation of the PRP at 1000*g* for 15 min. The pellet was resuspended in a volume of buffer A (0.137 M NaCl, 12.3 mM Tris-HCl buffer, pH 7.4 at 37 °C, 1.54 mM EDTA, and 20 mM glucose) equal to the original PRP volume. The suspension was centrifuged at 1100*g* for 15 min and the pellet was resuspended in buffer A. The pellet was centrifuged at 1100*g*, and the pellets were resuspended in 0.5 mL of 50 mL Tris-HCl buffer, pH 7.7, containing 1 mM $MgCl_2$. The hypotonically lyzed platelet suspension was centrifuged at 48000*g* for 15 min and the supernatant was saved. The pellets were frozen on dry ice and briefly thawed at 22 °C. The supernatant was combined with the pellet fraction and the resulting suspension was centrifuged at 48000*g* for 30 min. The pellet and supernatant fractions were used as the crude membrane-bound and soluble enzyme preparations, stored frozen in aliquots at −20 °C. Freshly prepared enzyme was found to be more sensitive to inhibition by 1 than the frozen preparations. Thus, the IC_{50} value for freshly prepared enzyme is 1.2 nM for 1 as compared to 10 nM for the frozen preparation. The frozen preparation, however, had the advantage of a uniform enzyme source to assay the potency of a large number of inhibitors. Although three distinct molecular forms of cyclic nucleotide phosphodiesterase are present in human platelets,[42] when enzyme activity is assayed at low substrate concentrations (1 μM), the predominant form detected is the FIII, high-affinity, cyclic AMP specific enzyme.[11] This observation made it unnecessary to isolate the FIII enzyme by column chromatography.

Dog and Rat Heart. The left ventricle was dissected, minced, washed free of blood, and homogenized for 1 min in a Waring blender in 10 volumes of cold 0.01 M Tris-HCl buffer, pH 7.7. The homogenate was passed through two layers of cheesecloth and centrifuged at 12000*g* for 20 min. All steps were performed at 4 °C. The supernatant was used as a source of enzyme and was stored frozen in aliquots at −20 °C. Dog heart homogenates contain two high-affinity forms of cyclic AMP phosphodiesterase. Previous studies have shown that only the FIII enzyme is significantly inhibited by 1.[11] Inhibition of this enzyme was only partial, and consequentially it was necessary to express the data in terms of IC_{25} rather than IC_{50} values. Crude enzyme preparations were used in this study to detect structural modifications that may exhibit activity against both high-affinity enzyme forms. Although potent inhibition of cardiac phosphodiesterase activity was obtained with compounds in this series, efficacy was invariably low. This observation suggests that only one of the high-affinity enzyme forms (probably FIII) in these cardiac preparations was significantly inhibited by the compounds described in this paper.

Cyclic AMP Phosphodiesterase Assay. The phosphodiesterase incubation medium containing 12 mM Tris-HCl buffer, pH 7.7, 0.5 mM $MgCl_2$, 0.137 M NaCl, 20 mM glucose, and appropriate concentrations of [^{3}H]cyclic AMP (0.2 μCi) in a total volume of 1.0 mL was added to a Me_2SO solution (10 μL) of the test compound. Following addition of the enzyme, the contents were mixed and incubated for 10 min at 30 °C. The reaction was terminated by adding 10 μL of 0.1 M EDTA, pH 7.0, mixing, and immediately immersing the tubes in a boiling water bath for 90 s. Labeled adenosine was isolated from alumina columns according to the method of Filburn and Karn.[43] 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} (platelet) or IC_{25} (heart) values reported in Table I were determined graphically. Standard deviations from mean values in each experiment were generally less than ±5%. IC_{50} values presented are from representative experiments. IC_{50} values were highly reproducible and varied by less than a factor of 0.5 to 2 times of the initial determination.

Human Platelet Aggregation Studies. Blood from donors who had not taken aspirin for 2 weeks was collected by venipuncture into evacuated tubes containing sodium citrate (30 mM, final concentration). Platelet rich plasma (PRP) was collected after centrifugation for 15 min at 200*g* at room temperature. Platelet concentration was determined with a Royco cell counter (Cell-Crit 921). Siliconized glassware or plastic test tubes were used in all procedures. Aggregation was followed by the turbidimetric procedure of Born[44] with a Payton aggregation module. Test compounds dissolved in either DMF or Me_2SO were added to stirred PRP (450 rpm) at 37 °C and incubated for 5 min prior to induction of aggregation by ADP (5 μM). The total volume was 1 mL. The degree of inhibition was determined by measuring

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the rate of change in percentage transmission of the primary phase of ADP-induced aggregation after 5 min of incubation. Experiments were repeated at least twice with platelets obtained from different donors. IC_{50} values and statistical interpretation were determined graphically as above and are reported in Table II.

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Inhibitors of Cyclic AMP Phosphodiesterase. 2. Structural Variations of *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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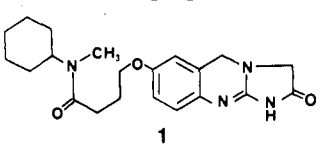
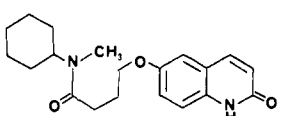
A series of analogues of the cyclic AMP phosphodiesterase (PDE) inhibitor *N*-cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (RS-82856, 1) was prepared by systematic variation of the side-chain substituent, length, position, connecting atom, and the parent heterocycle itself. The compounds were evaluated as inhibitors of cyclic AMP phosphodiesterase from both human platelets and rat or dog heart tissue and as inhibitors of ADP-induced platelet aggregation. Structure–activity correlations for the analogue series revealed significant limitations on the steric bulk of substituents on the 1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one heterocycle and the position and length of the side chain. As inhibitors of cyclic AMP phosphodiesterase (PDE), potency steadily increased with increasingly lipophilic side chains. In platelet aggregation inhibition studies, however, a maximum in activity was reached with 1, while more lipophilic compounds were significantly less active. Major changes in the heterocycle itself, represented by isomeric and other carbonyl variations, also decreased activity. The molecular features defined by this series of analogues of 1 correlate to a high degree with current understanding of the chemical and topographical requirements of the active site of the FIII (type IV) form of cyclic AMP PDE. Selective inhibition of this enzyme has been proposed as the principal component of the positive inotropic action of a number of cardiotonic agents.

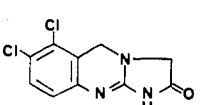
Selective inhibitors of cyclic AMP phosphodiesterase (PDE) have potential utility as therapeutic agents. The inotropic and cardiotonic properties of several newer PDE inhibitors appear to result from selective inhibition of the high-affinity, cyclic AMP specific (FIII or type IV) enzyme.[2,3] In the preceding paper[4] and elsewhere[5,6] we described the synthesis and biological evaluation of RS-82856 (1), a potent and selective inhibitor of a high-affinity form of cyclic AMP PDE, which exhibits potential cardiotonic and antithrombotic properties. This compound, a combination of the major structural elements of cilostamide (2)[7,8] and anagrelide (3),[9] was prepared after the realization



1



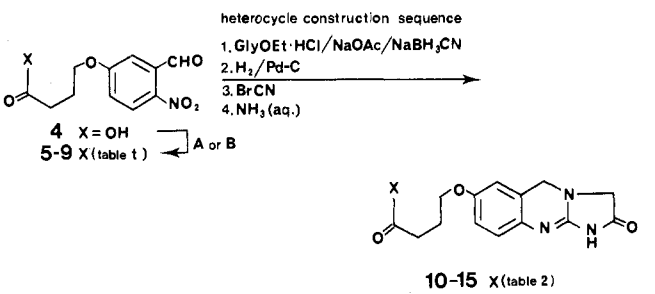
2



3

that the *N*-cyclohexyl-*N*-methyl-4-oxybutyramide side chain of 2 was of significant value as a steric and/or lipophilic pharmacophore within a series of lactam analogues of 2. Attachment of this side chain to 1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one, the parent heterocycle of the potent PDE inhibitor anagrelide (3), conferred activity upon 1 well in excess of either of its progenitors. In this paper we present the preparation and biological evaluation of a wide range of variations of the molecular features of 1 in order to probe the structural

Scheme I



requirements for activity as an inhibitor of cyclic AMP phosphodiesterase. To this end, seven types of changes

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