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.
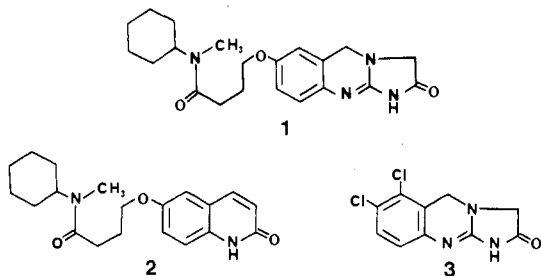
# Inhibitors of Cyclic AMP Phosphodiesterase. 2. Structural Variations of N-Cyclohexyl-N-methyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (RS-82856)[1]

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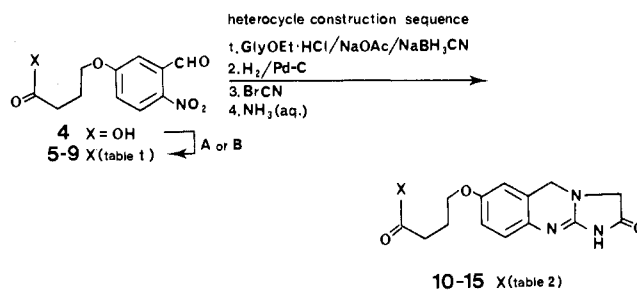*Received June 2, 1986*

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

**Scheme I**

heterocycle construction sequence

1. GlyOEt·HCl/NaOAc/NaBH$_3$CN
2. H$_2$/Pd-C
3. BrCN
4. NH$_3$(aq.)

**4** X = OH
**5-9** X (table 1)

**10-15** X (table 2)

**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 mol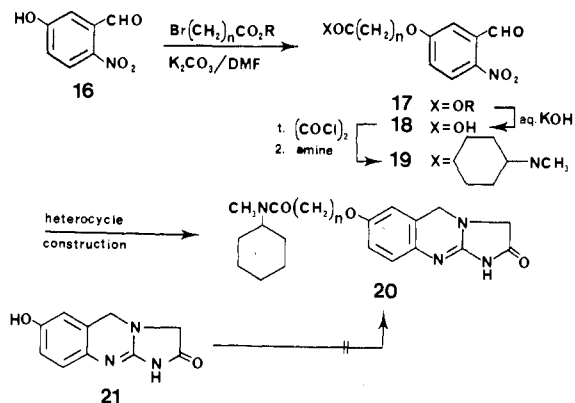ecular features of **1** in order to probe the structural requirements for activity as an inhibitor of cyclic AMP phosphodiesterase. To this end, seven types of changes

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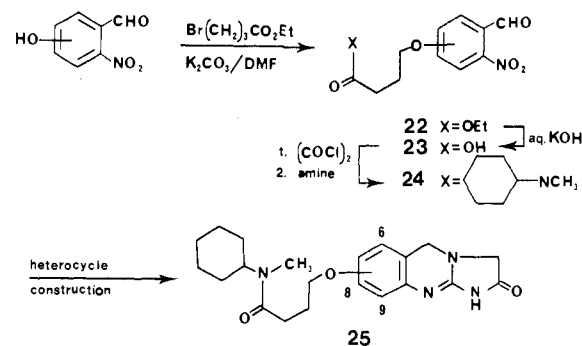
**Scheme II**



**Scheme III**



**Scheme IV**



in the structure of 1 were examined: amide analogues; side-chain homologues; side-chain positional isomers; side-chain linkages and other side-chain functional variations; carbonyl isomers; ring substitutions; and other related analogues.

## Chemistry

Variations of the N-cyclohexyl-N-methyl amide portion of 1 were prepared most efficiently by the sequence used for the synthesis of 1 (Scheme I). The precursor nitro aldehydes (5a-m, 6a-h, 7a-g, 8a-e, and 9a,b, Table I) were prepared from the oxybutyric acid 4,[4] using the acid chloride and the appropriate amine under either Schotten–Baumann (method A) or anhydrous (method B) acylation conditions. Conversion to the corresponding amide analogues (acyclic amides 10a-m, cyclic amides 11a-h, hydroxyalkyl amides 12a-g, other functionalized amides 13a-e, and esters 14a,b) listed in Table II was accomplished by sodium cyanoborohydride reductive amination with glycine ethyl ester, hydrogenation, and ring closure with cyanogen bromide as described previously.[4] Further transformations of some of the amide analogues could be effected directly on these products. Acylation of the hydroxyethyl analogue 12a with the appropriate anhydride and pyridine provided esters 15a-d. Methyl acetate analogue 13a was saponified to give the acetic acid analogue 13f. Finally, ethyl and cyclohexyl ester analogues 14a and 14b were prepared from the corresponding precursor nitro aldehydes (9a,b). Saponification of 14a gave the side chain acid analogue 14c, while treatment with ammonia gave butyramide 10n. Attempts to prepare amide analogues, and indeed 1 itself, from acid 14c using various amide-forming reagents (e.g., isobutyl chloroformate, DCC) were disappointing, due to the extremely low solubility of 14c in suitable solvents at the proper working temperatures.

The length and position of the cilostamide oxyalkyl amide side chain were then varied. Homologues of 1 were prepared by beginning with alkylation of phenol 16[18] with
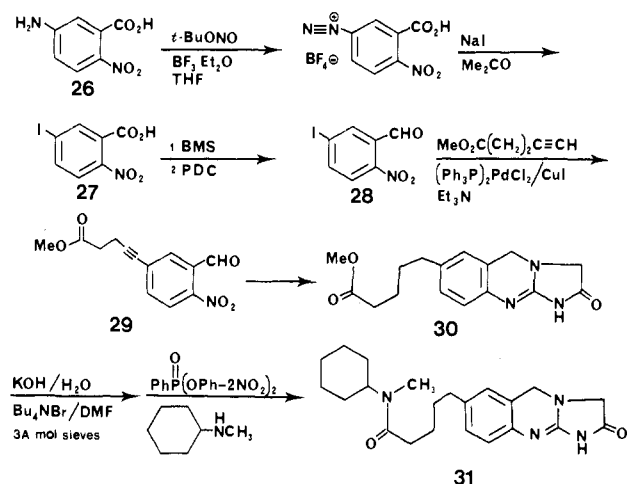
$C_2$ and $C_5$ to $C_7$ ω-bromoalkanoates and using conditions described for the parent[4] (Scheme II, Table III). The propensity for β-elimination in the $C_3$ case precluded preparation of the propionate analogue. Saponification of esters 17a-d to acids 18a-d, followed by Schotten–Baumann acylation, gave nitro aldehydes 19a-d, which were transformed into side-chain homologues 20a-d by the standard ring-construction sequence. Attempts to alkylate 7-hydroxy-1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-2-one (21)[19] under a number of conditions lead to mixtures of N(1), O(2), and O(7) alkylated products. Side-chain positional isomers of 1 were prepared by alkylation of the known isomeric 6-,[20] 4-,[21] and 3-hydroxy-2-nitrobenzaldehydes[22] to give the isomeric esters 22a-c (Scheme III, Table IV), which were converted via the corresponding acids (23a-c) and amides (24a-c) to the desired 6-, 8-, and 9-positional isomers (25a-c), respectively.

The oxygen of the side-chain ether linkage was replaced by –CH$_2$– as outlined in Scheme IV. 5-Iodo-2-nitrobenzoic acid (27)[23] was prepared in 56% yield from the available 5-amino-2-nitrobenzoic acid (26) via anhydrous diazotization[24] followed by treatment with NaI in acetone. Aqueous diazotizations under either standard (NaNO$_2$/H$_2$SO$_4$) or modified Witt conditions (HNO$_3$/Na$_2$S$_2$O$_5$)[25]

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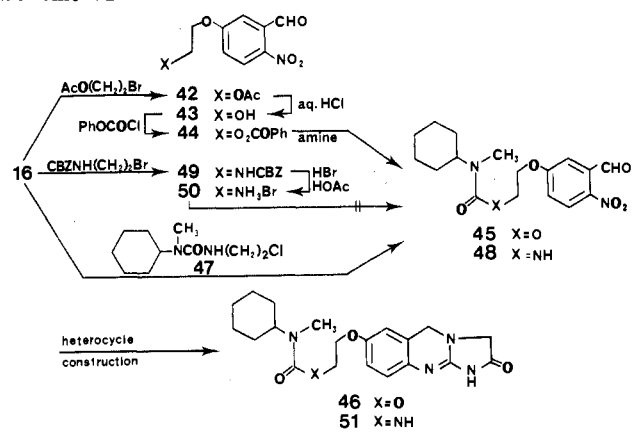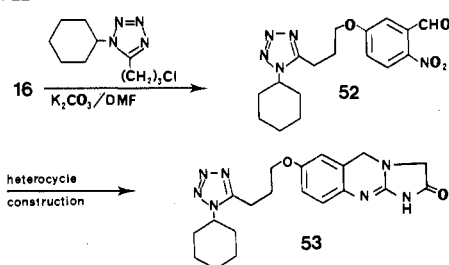
Scheme V

Scheme VI

Scheme VII

Scheme VIII
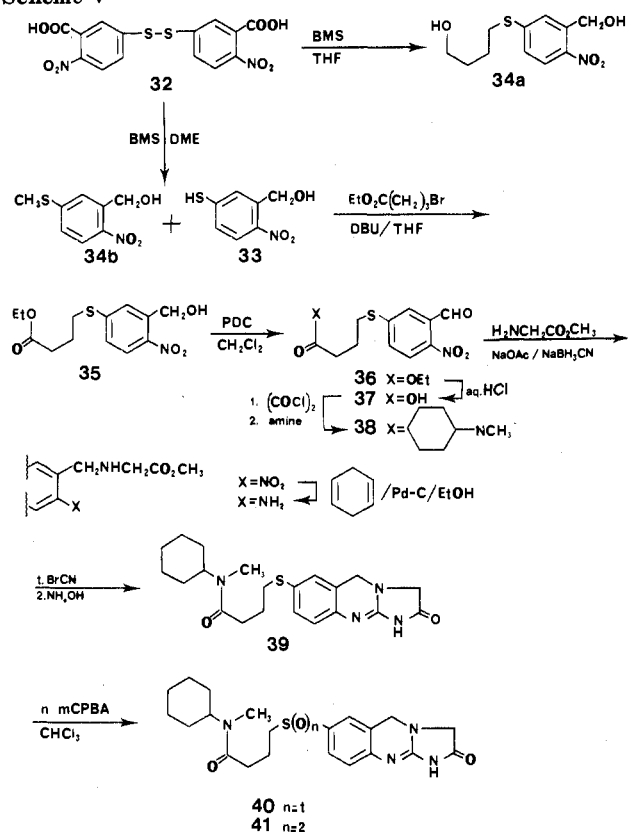
gave unacceptably low yields (6% and 27%, respectively). Borane–methyl sulfide reduction followed by PDC oxidation[26] gave aldehyde 28. Palladium-catalyzed coupling[27] of 28 with methyl 4-pentynoate[28] afforded the alkyne ester 29 in 80% yield. Attempts to hydrolyze 29 under either acidic or basic conditions uniformly failed, however, likely due to intramolecular cyclization of the resulting acid into the $p$-$NO_2$-activated alkyne. Ester 30 was obtained in 31% yield via the standard ring construction sequence from 29, with concomitant alkyne reduction at the hydrogenation step. Conversion of 30 to amide 31 was accomplished in 43% yield by the procedure of Mukaiyama,[29] utilizing bis(o-nitrophenyl)phenylphosphonate as coupling reagent after anhydrous ester cleavage in DMF.

Replacement of the oxygen linkage with sulfur proceeded from Ellman's reagent (32) as outlined in Scheme V. Borane–methyl sulfide reduction[30] of 32 in DME gave 2-nitro-5-mercaptobenzyl alcohol (33). Attempts to use THF as solvent gave the 4-hydroxybutyl sulfide 34a as the major product, presumably due to thiolate cleavage of borane-complexed THF. Only small amounts of the methyl sulfide (34b) were detected from reduction in DME. Alkylation of thiol 33 with ethyl 4-bromobutyrate and DBU in THF afforded 35 in 46% yield after chromatography, oxidation of which with pyridinium dichromate in dichloromethane gave aldehyde 36 in 73% yield. Hydrolysis of 36 to acid 37, followed by Schotten–
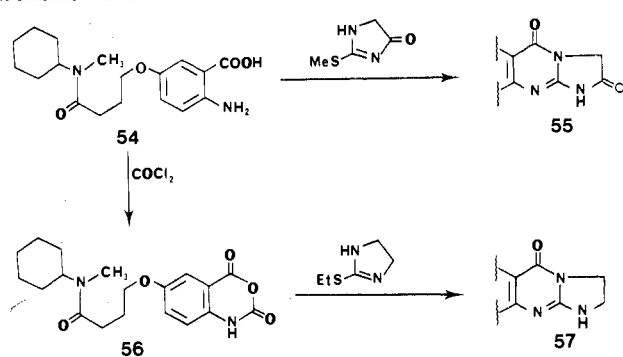
Baumann acylation, gave amide 38, which was subjected to the ring-construction sequence, substituting catalytic transfer hydrogenation (1,4-cyclohexadiene/Pd–C)[31] for the usual hydrogenation, to afford sulfide 39 in 36% yield. Oxidation of 39 with either 1 or 2 equiv of m-CPBA gave sulfoxide 40 or sulfone 41, respectively.

Three variations of the amide portion of the side chain of 1 were also prepared. Phenol 16 was alkylated with 2-bromoethyl acetate (Scheme VI) to afford 42, which was hydrolyzed to alcohol 43 and condensed with phenyl chloroformate to afford carbonate 44. Treatment of 44 with N-methylcyclohexylamine gave 45, carried through the ring-construction sequence to give the urethane analogue 46. Alternatively, alkylation of 16 with 1-(2-chloroethyl)-3-cyclohexyl-3-methylurea (47), prepared from 2-chloroethyl isocyanate,[32] gave 48, albeit in 27% yield after chromatography. Attempts to condense amine 50, itself prepared from the corresponding CBZ-protected amine 49, with N-cyclohexyl-N-methylcarbamoyl chloride gave only trace amounts of 48. Transformation of 48 via the ring-construction sequence afforded the urea analogue

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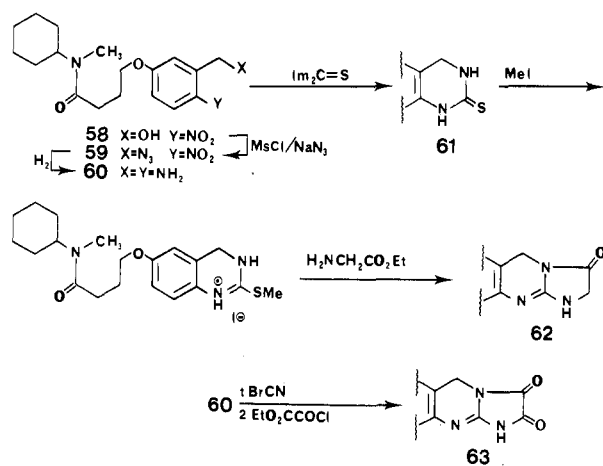(29) Mukaiyama, T.; Morito, N.; Watanabe, Y. Chem. Lett. 1979, 1305. Watanabe, Y.; Mukaiyama, T. Chem. Lett. 1981, 285.
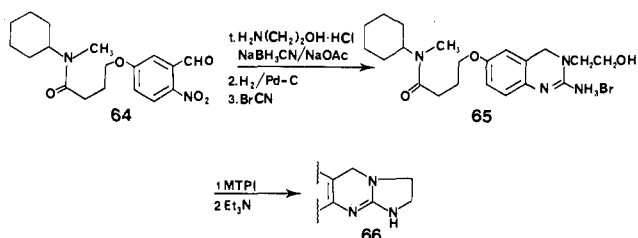(30) Hutchins, R. O.; Cistone, F. Org. Prep. Proc. Int. 1981, 13, 225.

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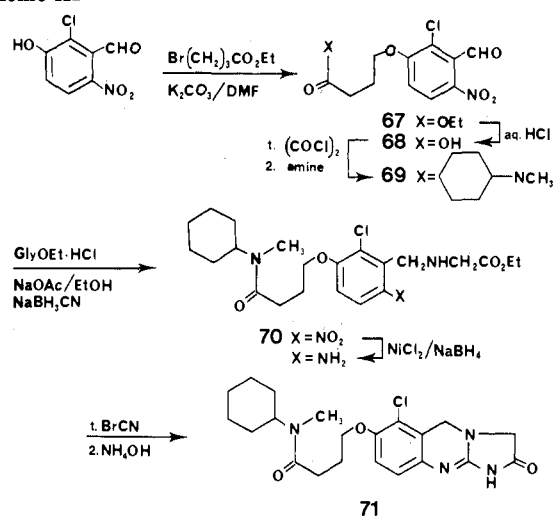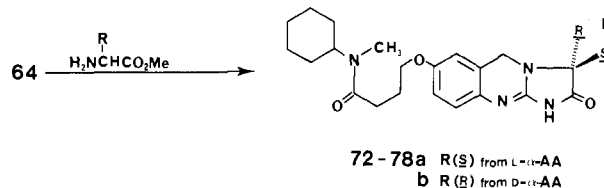
**Scheme IX**



**Scheme X**



**Scheme XI**



**Scheme XII**



72-78a R(S) from L-α-AA
    b R(R) from D-α-AA

**Scheme XIII**



(51). Alkylation of **16** with 5-(3-chloropropyl)-1-cyclo-hexyltetrazole[33] gave **52**, which was converted to the tetrazole analogue **53** by the standard sequence (Scheme VII). This last side chain is of a type found in a series of cilostamide analogues, of which cilostazol is the parent.[33,34]

Carbonyl analogues of the heterocyclic portion of **1** were prepared as outlined in Scheme VIII. Condensation of anthranilic acid **54**[4] with 2-(methylthio)hydantoin[35] afforded the 2,5-dione analogue **55**. A similar condensation[36] utilizing the corresponding isatoic anhydride **56**[4] and 2-(ethylthio)imidazoline[37] gave the 5-oxo isomer (**57**). Treatment of the mesylate of alcohol **58**[4] (Scheme IX) with sodium azide in aqueous THF afforded the nitrobenzyl azide **59**, reduction of which over Palladium gave the diamine **60**. Condensation of **60** with 1,1'-thiocarbonyldiimidazole gave the cyclic thiourea **61**, which was converted by treatment with methyl iodide followed by glycine methyl ester to the 3-oxo isomer (**62**).[38] Alternatively, sequential treatment of **60** with cyanogen bromide followed by ethyl oxalyl chloride afforded the 2,3-dione analogue (**63**). Finally, reductive amination of aldehyde **64**[4] with ethanolamine, followed by hydrogenation and treatment with cyanogen bromide, gave the (hydroxyethyl)-guanidinium bromide **65** (Scheme X). Conversion of the alcohol to the corresponding iodide using methyltriphenoxyphosphonium iodide (MTPI, Rydon reagent),[39] followed by treatment with an excess of triethylamine to
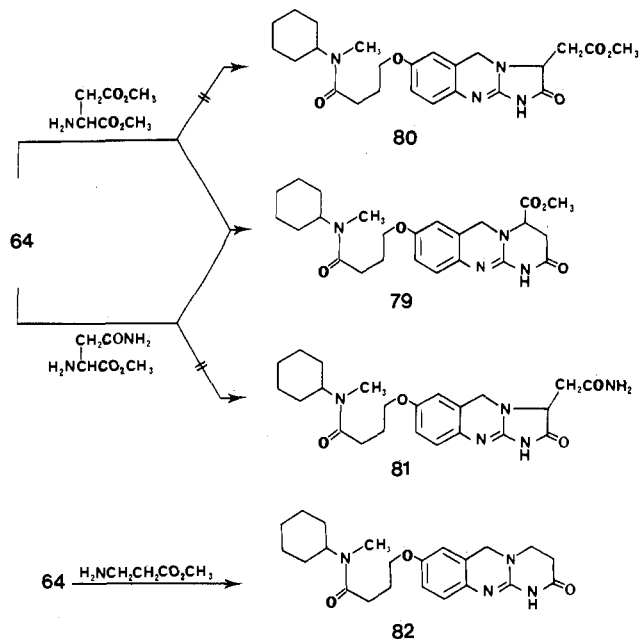
induce cyclization, afforded the desoxy analogue (**66**).

Ring substitution was accomplished at two positions relatively straightforwardly. The 6-chloro substituent present in **3** was introduced by utilization of the known 2-chloro-3-hydroxy-6-nitrobenzaldehyde[40] in place of the usual phenol (**16**) as starting material (Scheme XI). Conversion to **69** via ester **67** and acid **68** and subsequent reductive amination with glycine ethyl ester were accomplished by the precedented route.[4] Reduction of the nitro group of **70** using NaBH$_4$/NiCl$_2$ to avoid hydrogenolysis of the chloro substituent,[41] followed by sequential treatment of the resulting diamine with cyanogen bromide and

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Table I. 4-(3-Formyl-4-nitrophenoxy)butyramides **5a–m, 6a–h, 7a–g,** and **8a–e** and Esters **9a,b** from Scheme I

| no. | X[a] | yield, % | method[b] | mp, °C | anal. (C, H, N) |
|---|---|---|---|---|---|
| 5a | $CH_3NH$ | 86 | B | 92–93 | $C_{12}H_{14}N_2O_5$ |
| 5b | $(CH_3)_2N$ | 87 | B | 66–67 | $C_{13}H_{16}N_2O_5$ |
| 5c | $c\text{-}C_6H_{11}NH$ | 59 | A | 116–117 | $C_{17}H_{22}N_2O_5$ |
| 5d | $c\text{-}C_6H_{11}NCH_2CH_3$ | 56 | A | 82–83 | $C_{19}H_{26}N_2O_5$ |
| 5e | $c\text{-}C_6H_{11}NCH(CH_3)_2$ | 40 | A | 59–61[c] | $C_{20}H_{28}N_2O_5$ |
| 5f | $c\text{-}C_6H_9NCH_3$ | 57 | A | 69–70[c] | $C_{17}H_{22}N_2O_5$ |
| 5g | $c\text{-}C_7H_{13}NCH_3$ | 96 | A | syrup[c] | $C_{19}H_{26}N_2O_5 \cdot H_2O$[d] |
| 5h | $c\text{-}C_8H_{15}NCH_3$ | 66 | A | syrup[c] | $C_{20}H_{28}N_2O_5$ |
| 5i | $PhNCH_3$ | 83 | B | 72–73 | $C_{18}H_{18}N_2O_5$ |
| 4j | $PhCH_2NCH_3$ | 95 | A | syrup[c] | $C_{19}H_{20}N_2O_5$ |
| 5k | $(PhCH_2)_2N$ | 78 | A | 76–77 | $C_{25}H_{24}N_2O_5$ |
| 5l | $(c\text{-}C_6H_{11})_2N$ | 28 | A | 107–108 | $C_{23}H_{32}N_2O_5$ |
| 5m | $Ph_2CHNCH_3$[e] | 62 | A | 117–118 | $C_{25}H_{24}N_2O_5$ |
| 6a | 4-morpholinyl | 91 | A | 106–107 | $C_{15}H_{18}N_2O_6$ |
| 6b | 1-piperidinyl | 76 | A | 98–99 | $C_{16}H_{20}N_2O_5$ |
| 6c | 1-pyrrolidinyl | 97 | A | 82–83 | $C_{15}H_{18}N_2O_5$ |
| 6d | 1-tetrahydroquinolinyl | 48 | B | 95–96 | $C_{20}H_{20}N_2O_5$ |
| 6e | 2-tetrahydroisoquinolinyl | 78 | A | 99–100 | $C_{20}H_{20}N_2O_5$ |
| 6f | 1-indolinyl | 54 | B | 155–156 | $C_{19}H_{18}N_2O_5$ |
| 6g | 1-(±)-*trans*-decahydroquinolinyl[f] | 88 | A | 77–78 | $C_{20}H_{26}N_2O_5$ |
| 6h | 1-perhexylenyl[g] | 76 | A | amorph[c] | $C_{30}H_{44}N_2O_5$ |
| 7a | $c\text{-}C_6H_{11}N(CH_2)_2OH$ | 85 | A | 108–110 | $C_{19}H_{26}N_2O_6 \cdot 0.25H_2O$ |
| 7b | $c\text{-}C_6H_{11}N(CH_2)_3OH$[h] | 95 | A | amorph[c] | $C_{20}H_{28}N_2O_6 \cdot H_2O$ |
| 7c | $c\text{-}C_6H_{11}N(CH_2)_4OH$[b] | 81 | A | syrup[c] | $C_{21}H_{30}N_2O_6$ |
| 7d | $c\text{-}C_6H_{11}N(CH_2)_5OH$[i] | 50 | A | amorph[c] | $C_{22}H_{32}N_2O_6$ |
| 7e | $c\text{-}C_6H_{11}N(CH_2)_6OH$[b] | 58 | A | syrup[c] | $C_{23}H_{34}N_2O_6$ |
| 7f | $c\text{-}C_6H_{11}NCH(CH_2OH)_2$[b] | 30 | B | amorph[c] | $C_{20}H_{28}N_2O_7$ |
| 7g | $4\text{-}HO\text{-}c\text{-}C_6H_{11}NCH_3$[b] | 99 | B | amorph[c] | $C_{18}H_{24}N_2O_6 \cdot 0.5H_2O$ |
| 8a | $c\text{-}C_6H_{11}NCH_2CO_2CH_3$[j] | 74 | B | syrup[c] | $C_{20}H_{26}N_2O_7 \cdot 0.5H_2O$ |
| 8b | $c\text{-}C_6H_{11}NCH_2CONH_2$[k] | 75 | A | 104–105 | $C_{19}H_{25}N_3O_6 \cdot 0.33H_2O$ |
| 8c | $c\text{-}C_6H_{11}N(CH_2)_2\overline{N(CH_2)_2OCH_2CH_2}$[b] | 66 | A | syrup[c] | $C_{23}H_{33}N_3O_6 \cdot 0.25H_2O$ |
| 8d | $c\text{-}C_6H_{11}N(CH_2)_2OCH_3$[l] | 46 | A | syrup[c] | $C_{20}H_{28}N_2O_6 \cdot 0.25H_2O$ |
| 8e | $c\text{-}C_6H_{11}N(CH_2)_2\overline{NCH=NCH=CH}$[b] | 65 | B | syrup[c] | $C_{22}H_{28}N_4O_5$ |
| 9a | $CH_3CH_2O$[m] | | | | |
| 9b | $c\text{-}C_6H_{11}O$[n] | 100 | B | syrup | $C_{17}H_{21}NO_6$ |

[a] If not commercially available, amine source noted. [b] See Experimental Section. [c] After chromatography over silica gel with 10% ethyl acetate in dichloromethane. [d] H: calcd, 7.41; found, 6.94. [e] Reference 10. [f] Reference 11. [g] 1,1-Dicyclohexyl-2-(2-piperidyl)ethane; ref 12. [h] Reference 13; prepared by $NaBH_3CN$ reductive amination of cyclohexanone with 3-amino-1-propanol. [i] Reference 14; prepared by $NaBH_3CN$ reductive amination of cyclohexanone with 5-amino-1-pentanol. [j] Reference 15; prepared by alkylation of cyclohexylamine with methyl bromoacetate and DBU in THF. [k] Reference 16; prepared by treatment of methyl ester (amine component of **8a**) with anhydrous ammonia. [l] Reference 17; prepared by $NaBH_3CN$ reductive amination of cyclohexanone with 2-methoxyethylamine. [m] Synthetic precursor of acid 4; reference 4. [n] Cyclohexanol substituted for amine in method B.

base, afforded the 6-chloro analogue (**71**).

Introduction of substituents at position 3 was carried out by utilizing the appropriate α-amino acid ester in place of glycine ethyl ester in the reductive amination of **64**[4] (Scheme XII). In this manner, analogues **72a,b–78a,b** (Table V) were prepared from the corresponding L- or D-α-amino acids, respectively. However, in the cases of both aspartic acid β-methyl ester and asparagine, the undesired six-membered cyclization occurred to give the same pyrimido[2,1-*b*]quinazolin-2-one (**79**) rather than the corresponding ester (**80**) or amide (**81**, Scheme XIII). The parent analogue of this ring system (**82**) was prepared by using β-alanine methyl ester in place of glycine ethyl ester in the reductive amination step. Both methyl α-amino-isobutyrate and methyl 1-aminocyclopropanecarboxylate[42] were also incorporated into the sequence, providing the 3,3-disubstituted analogues **83** and **84**, respectively (Scheme XIV).

Manipulation of the 3-hydroxymethyl analogue (**75a**) was also investigated (Scheme XV). Dehydration of **75a** could be carried out by elimination of the corresponding mesylate with diisopropylethylamine in pyridine, on the basis of similar conversions in serine-containing peptides,[43]
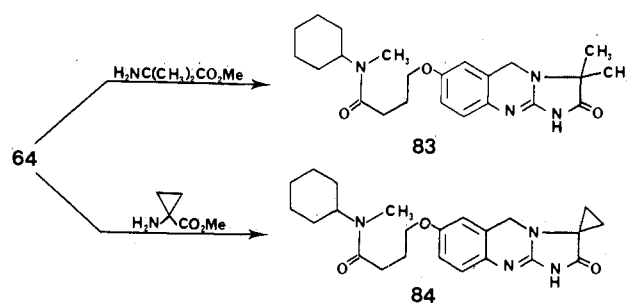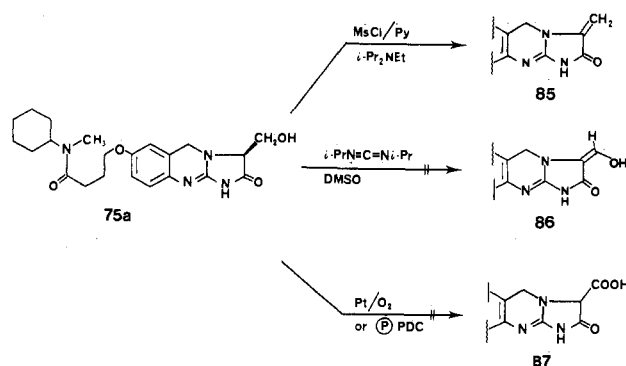
(42) From the BOC derivative: Rich, D. H.; Tam, J. P. *Synthesis* 1978, 46.
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**Scheme XIV**



**Scheme XV**

**Table II.** 4-[(1,2,3,5-Tetrahydro-2-oxoimidazo[2,1-b]quinazolin-7-yl)oxy]butyramides 10a–n, 11a–h, 12a–g, 13a–f, and 15a–d and Acid Derivatives 14a–c from Scheme I and Other Analogues (46, 51, 53) from Schemes VI and VII

| no. | X | yield,[a] % | mp, °C | anal. (C, H, N) |
|---|---|---|---|---|
| 10a | $CH_3NH$ | 36 | 269–270 | $C_{15}H_{18}N_4O_3 \cdot H_2O$ |
| 10b | $(CH_3)_2N$ | 21 | 265–266 | $C_{16}H_{20}N_4O_3$ |
| 10c | $c\text{-}C_6H_{11}NH$ | 19 | 255–256 | $C_{20}H_{26}N_4O_3 \cdot H_2O$ |
| 10d | $c\text{-}C_6H_{11}NCH_2CH_3$ | 26 | 220–221 | $C_{22}H_{30}N_4O_3$ |
| 10e | $c\text{-}C_6H_{11}NCH(CH_3)_2$ | 23 | 244–246 | $C_{23}H_{32}N_4O_3 \cdot 0.5H_2O$ |
| 10f | $c\text{-}C_5H_9NCH_3$ | 59 | 262–263 | $C_{20}H_{28}N_4O_3$ |
| 10g | $c\text{-}C_7H_{13}NCH_3$ | 27 | 226–228 | $C_{22}H_{30}N_4O_3 \cdot 0.33H_2O$ |
| 10h | $c\text{-}C_8H_{15}NCH_3$ | 28 | 234–235 | $C_{23}H_{32}N_4O_3$ |
| 10i | $PhNCH_3$ | 10 | 223–224 | $C_{21}H_{22}N_4O_3 \cdot H_2O$ |
| 10j | $PhCH_2NCH_3$ | 11.5 | 232–234 | $C_{22}H_{24}N_4O_3 \cdot 0.75H_2O$ |
| 10k | $(PhCH_2)_2N$ | 26 | 194–196 | $C_{28}H_{28}N_4O_3$ |
| 10l | $(c\text{-}C_6H_{11})_2N$ | 11 | 242–244 | $C_{26}H_{36}N_4O_3$ |
| 10m | $Ph_2CHNCH_3$ | 34 | 232–234 | $C_{28}H_{28}N_4O_3 \cdot H_2O$ |
| 10n | $NH_2$ | 91[b] | 280–282 | $C_{14}H_{16}N_4O_3$ |
| 11a | 4-morpholinyl | 15.4 | 288–290 | $C_{18}H_{22}N_4O_4$ |
| 11b | 1-piperidinyl | 13 | 276–278 | $C_{19}H_{24}N_4O_3 \cdot H_2O$ |
| 11c | 1-pyrollidinyl | 16 | 278–280 | $C_{18}H_{22}N_4O_3 \cdot 0.5H_2O$ |
| 11d | 1-tetrahydroquinolinyl | 2[c] | 203–204 | $C_{23}H_{24}N_4O_3 \cdot H_2O$ |
| 11e | 2-tetrahydroisoquinolinyl | 13 | 216–218 | $C_{23}H_{24}N_4O_3 \cdot 0.75H_2O$ |
| 11f | 1-indolinyl | 3[c] | 264–266 | $C_{22}H_{22}N_4O_3 \cdot 0.75H_2O$ |
| 11g | 1-(±)-*trans*-decahydroquinolinyl | 4 | 218–220 | $C_{23}H_{30}N_4O_3 \cdot 1.25H_2O$ |
| 11h | 1-perhexylenyl | 12 | 217–218 | $C_{33}H_{48}N_4O_3$ |
| 12a | $c\text{-}C_6H_{11}N(CH_2)_2OH$ | 37 | 185–186 | $C_{22}H_{30}N_4O_4 \cdot H_2O$ |
| 12b | $c\text{-}C_6H_{11}N(CH_2)_3OH$ | 17 | 170–172 | $C_{23}H_{32}N_4O_4 \cdot 0.75H_2O$ |
| 12c | $c\text{-}C_6H_{11}N(CH_2)_4OH$ | 40 | 199–200 | $C_{24}H_{34}N_4O_4 \cdot 0.25H_2O$ |
| 12d | $c\text{-}C_6H_{11}N(CH_2)_5OH$ | 18 | 194–195 | $C_{25}H_{36}N_4O_4 \cdot 0.25H_2O$ |
| 12e | $c\text{-}C_6H_{11}N(CH_2)_6OH$ | 32 | 179–180 | $C_{26}H_{38}N_4O_4$ |
| 12f | $c\text{-}C_6H_{11}NCH(CH_2OH)_2$ | 13 | 189–190 | $C_{23}H_{32}N_4O_5 \cdot H_2O$ |
| 12g | $4\text{-}HO\text{-}c\text{-}C_6H_{11}NCH_3$ | 19 | 253–255 | $C_{21}H_{28}N_4O_4 \cdot 0.5H_2O$ |
| 13a | $c\text{-}C_6H_{11}NCH_2CO_2CH_3$ | 21[d] | 185–186 | $C_{23}H_{30}N_4O_5 \cdot 0.5H_2O$ |
| 13b | $c\text{-}C_6H_{11}NCH_2CONH_2$ | 28 | 207–208 | $C_{22}H_{29}N_5O_4 \cdot 1.5H_2O$ |
| 13c | $c\text{-}C_6H_{11}N(CH_2)_2\overline{N(CH_2)_2OCH_2CH_2}$ | 13[c] | 115–117 | $C_{26}H_{37}N_5O_4 \cdot 0.75H_2O$ |
| 13d | $c\text{-}C_6H_{11}N(CH_2)_2OCH_3$ | 41 | 176–177 | $C_{23}H_{32}N_4O_4 \cdot 0.5H_2O$ |
| 13e | $c\text{-}C_6H_{11}N(CH_2)_2\overline{NCH=NCH=CH}$ | 15[c,e] | 176–177 | $C_{25}H_{32}N_6O_3 \cdot 1.75H_2O$ |
| 13f | $c\text{-}C_6H_{11}NCH_2COOH$ | 92[f] | 194–195 | $C_{22}H_{28}N_4O_5 \cdot 2H_2O$ |
| 14a | $CH_3CH_2O$ | 31 | 243–244 | $C_{16}H_{19}N_3O_4$ |
| 14b | $c\text{-}C_6H_{11}O$ | 28 | 224–226 | $C_{20}H_{25}N_3O_4 \cdot 0.2H_2O$ |
| 14c | $HO$ | 74[g] | >300 | $C_{14}H_{14}N_3O_4Na \cdot 2.5H_2O$ |
| 15a | $c\text{-}C_6H_{11}N(CH_2)_2O_2CCH_3$ | 66[h] | 164–166 | $C_{24}H_{32}N_4O_5$ |
| 15b | $c\text{-}C_6H_{11}N(CH_2)_2O_2CCH(CH_3)_2$ | 86[h] | 154–155 | $C_{26}H_{36}N_4O_5$ |
| 15c | $c\text{-}C_6H_{11}N(CH_2)_2O_2CC(CH_3)_3$ | 70[h] | 152–153 | $C_{27}H_{38}N_4O_5 \cdot 1.5H_2O$ |
| 15d | $c\text{-}C_6H_{11}N(CH_2)_2O_2CPh$ | 81[h] | 94–95 | $C_{29}H_{34}N_4O_5 \cdot H_2O$ |
| 46 | | 31[i] | 242–243 | $C_{20}H_{26}N_4O_4$ |
| 51 | | 27[j] | 230–231 | $C_{20}H_{27}N_5O_3 \cdot H_2O$ |
| 53 | | 28[k] | 258–259 | $C_{20}H_{25}N_7O_2 \cdot 0.25H_2O$ |

[a] Overall yield from nitro aldehyde precursor (Table I) except as noted. [b] From 14a by treatment with anhydrous ammonia. [c] Isolated by silica gel chromatography (10% methanol in dichloromethane). [d] Methanol and glycine methyl ester hydrochloride substituted into ring-construction sequence to prevent transesterification. [e] Crystallized from acetone. [f] From 13a by saponification. [g] From 14a by saponification, isolated as the sodium salt. [h] From 12a by treatment with the corresponding anhydride and DMAP in pyridine. [i] From nitro aldehyde 45. [j] From nitro aldehyde 48. [k] From nitro aldehyde 52.

**Table III.** Chain-Length Homologues 20a–d and Synthetic Precursors 17a–d, 18a–d, and 19a–d from Scheme II

| no. | X | n | yield, % | mp, °C | anal. (C, H, N) |
|---|---|---|---|---|---|
| 17a | $CH_3CH_2O$ | 1 | 89 | 57–58 | $C_{11}H_{11}NO_6$ |
| 17b | $CH_3O$ | 4 | 98 | oil[a] | $C_{13}H_{15}NO_6$ |
| 17c | $CH_3CH_2O$ | 5 | 94 | oil[a] | $C_{15}H_{19}NO_6$ |
| 17d | $CH_3CH_2O$ | 6 | 94 | oil[a] | $C_{16}H_{21}NO_6$ |
| 18a | $HO$ | 1 | 78 | 147–148 | $C_9H_7NO_6$ |
| 18b | $HO$ | 4 | 75 | 96–98 | $C_{12}H_{13}NO_6$ |
| 18c | $HO$ | 5 | 77 | 95–97 | $C_{13}H_{15}NO_6$ |
| 18d | $HO$ | 6 | 61 | 88–89 | $C_{14}H_{17}NO_6$ |
| 19a | $c\text{-}C_6H_{11}NCH_3$ | 1 | 74 | 128–129 | $C_{16}H_{20}N_2O_5$ |
| 19b | $c\text{-}C_6H_{11}NCH_3$ | 4 | 98 | oil[b] | $C_{19}H_{26}N_2O_5$ |
| 19c | $c\text{-}C_6H_{11}NCH_3$ | 5 | 63 | 96–97 | $C_{20}H_{28}N_2O_5$ |
| 19d | $c\text{-}C_6H_{11}NCH_3$ | 6 | 95 | amorph[b] | $C_{21}H_{30}N_2O_5$ |
| 20a | | 1 | 21 | 237–239 | $C_{19}H_{24}N_4O_3 \cdot 1.5H_2O$ |
| 20b | | 4 | 22 | 204–206 | $C_{22}H_{30}N_4O_3 \cdot 1.5H_2O$ |
| 20c | | 5 | 18 | 208–209 | $C_{23}H_{32}N_4O_3$ |
| 20d | | 6 | 11 | 148–150 | $C_{24}H_{34}N_4O_3 \cdot 0.5H_2O$ |

[a] Isolated by silica gel chromatography with dichloromethane as eluant. [b] Isolated by silica gel chromatography with 10% ethyl acetate in dichloromethane as eluant.

**Table IV.** Side-Chain Positional Isomers 25a–c and Synthetic Precursors 22a–c, 23a–c, and 24a–c from Scheme III

| no. | X | isomer | yield, % | mp, °C | anal. (C, H, N) |
|---|---|---|---|---|---|
| 22a | $CH_3CH_2O$ | 6 | 94 | 50–51 | $C_{13}H_{15}NO_6$ |
| 22b | $CH_3CH_2O$ | 4 | 90 | oil[a] | $C_{13}H_{15}NO_6$ |
| 22c | $CH_3CH_2O$ | 3 | 38 | 54–55[a] | $C_{13}H_{15}NO_6$ |
| 23a | $HO$ | 6 | 91 | 109–110 | $C_{11}H_{11}NO_6$ |
| 23b | $HO$ | 4 | 69 | 96–97 | $C_{11}H_{11}NO_6$ |
| 23c | $HO$ | 3 | 51 | 135–136 | $C_{11}H_{11}NO_6 \cdot 0.2H_2O$ |
| 24a | $c\text{-}C_6H_{11}NCH_3$ | 6 | 85 | 69–70 | $C_{18}H_{24}N_2O_5$ |
| 24b | $c\text{-}C_6H_{11}NCH_3$ | 4 | 75 | 75–76 | $C_{18}H_{24}N_2O_5$ |
| 24c | $c\text{-}C_6H_{11}NCH_3$ | 3 | 69 | amorph | $C_{18}H_{24}N_2O_5 \cdot 0.66H_2O$ |
| 25a | | 6 | 14 | 256–258 | $C_{21}H_{28}N_4O_3 \cdot 0.5H_2O$ |
| 25b | | 8 | 14 | 113–114 | $C_{21}H_{28}N_4O_3 \cdot 1.75H_2O$ |
| 25c | | 9 | 7 | 110–111 | $C_{21}H_{28}N_4O_3 \cdot 0.5H_2O$ |

[a] Isolated by silica gel chromatography with dichloromethane as eluant.

**Table V.** 3-Substituted 1,2,3,5-Tetrahydroimidazo[2,1-*b*]quin-azolin-2-ones **72–78** from Scheme XII and 3,3-Disubstituted Analogues **83** and **84** from Scheme XIV

| no. | $R^a$ | yield,$^b$ % | mp, °C | anal. (C, H, N) |
|-----|-------|-------------|--------|-----------------|
| 72a | (S)-CH$_3$ | 35 | 119–120 | C$_{22}$H$_{30}$N$_4$O$_3$·0.5H$_2$O |
| 72b | (R)-CH$_3$ | 29 | 120–121 | C$_{22}$H$_{30}$N$_4$O$_3$·0.5H$_2$O |
| 73a | (S)-CH$_2$CH$_3$ | 14$^c$ | 184–185 | C$_{23}$H$_{32}$N$_4$O$_3$·0.25H$_2$O |
| 73b | (R)-CH$_2$CH$_3$ | 10$^c$ | 185–186 | C$_{23}$H$_{32}$N$_4$O$_3$ |
| 74a | (S)-CH(CH$_3$)$_2$ | 6$^c$ | 176–177 | C$_{24}$H$_{34}$N$_4$O$_3$·0.25H$_2$O |
| 74b | (R)-CH(CH$_3$)$_2$ | 7$^c$ | 178–179 | C$_{24}$H$_{34}$N$_4$O$_3$·0.5H$_2$O |
| 75a | (S)-CH$_2$OH | 26 | 219–220 | C$_{22}$H$_{30}$N$_4$O$_4$ |
| 75b | (R)-CH$_2$OH | 31 | 218–219 | C$_{22}$H$_{30}$N$_4$O$_4$ |
| 76a | (S)-CH(OH)CH$_3$ | 29 | 210–211 | C$_{23}$H$_{32}$N$_4$O$_4$·0.5H$_2$O |
| 76b | (R)-CH(OH)CH$_3$ | 29 | 211–212 | C$_{23}$H$_{32}$N$_4$O$_4$ |
| 77a | (S)-Ph | 21 | 201–202 | C$_{27}$H$_{32}$N$_4$O$_3$·0.25H$_2$O |
| 77b | (R)-Ph | 15 | 201–202 | C$_{27}$H$_{32}$N$_4$O$_3$ |
| 78a | (S)-PhCH$_2$ | 55 | 228–229 | C$_{28}$H$_{34}$N$_4$O$_3$ |
| 78b | (R)-PhCH$_2$ | 31 | 228–229 | C$_{28}$H$_{34}$N$_4$O$_3$ |
| 83 | d | 60 | 180–181 | C$_{23}$H$_{32}$N$_4$O$_3$ |
| 84 | e | 48 | 234–235 | C$_{23}$H$_{30}$N$_4$O$_3$ |

$^a$ Derived from appropriate L-(S) or D-(R) amino acid methyl ester hydrochloride. $^b$ Overall yield from **64**. $^c$ Isolated by crystallization after aqueous extraction. $^d$ From methyl α-aminoisobutyrate. $^e$ From methyl 1-aminocyclopropane-1-carboxylate.

**Scheme XVI**
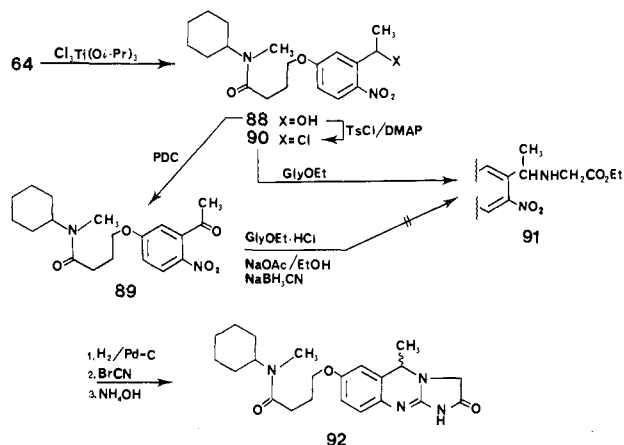


to give the 3-methylene analogue (**85**). However, attempts to oxidize **75a** to the corresponding aldehyde (**86**) or acid (**87**) met with no success with use of a variety of reagents and conditions, likely due to the reported propensity of structurally related peptides to undergo cleavage to glycolic acid derivatives upon oxidation.[44] Indeed, the 2,3-dione (**63**) was detected as the major product among many upon Moffatt oxidation of **75a**.

Introduction of a methyl group in the 5-position was accomplished by the synthetic sequence shown in Scheme XVI. Treatment of nitro aldehyde **64** with CH$_3$Ti(O-*i*-Pr)$_3$[45] in THF gave carbinol **88**, which chould be oxidized to acetophenone **89** with pyridinium dichromate.[46] Unfortunately, **89** did not undergo Pd–C-catalyzed or NaBH$_3$CN-induced reductive amination with glycine ethyl ester. Conversion of **88** into the corresponding chloride (**90**) using *p*-toluenesulfonyl chloride/DMAP in acetonitrile (rather than dichloromethane),[47] followed by alkylation of glycine ethyl ester,[48] gave **91**, which was reduced

**Scheme XVII**
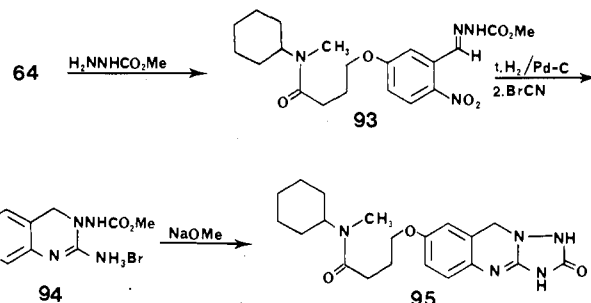


**Scheme XVIII**
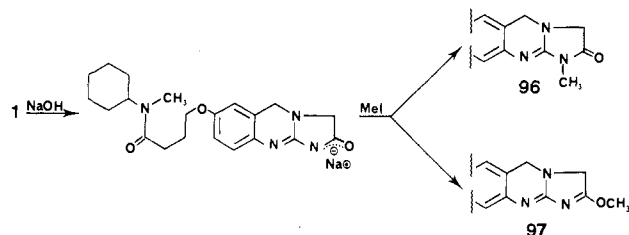


and treated with cyanogen bromide to afford **92**.

Two other major changes in the heterocyclic portion of **1** were also completed. Introduction of a fourth nitrogen, at position 3 of the heterocyclic system, was carried out as described in Scheme XVII. Aldehyde **64** was converted to hydrazone **93**, which was catalytically reduced and treated with cyanogen bromide to afford the stable ester **94**. Treatment of **94** with an excess of sodium methoxide in methanol at reflux induced cyclization to yield the 3-aza analogue (**95**). Finally, alkylation of the sodium salt of **1** with methyl iodide in DMF afforded a mixture of N- and O-methylated analogues (**96** and **97**) as major products, which were separated by chromatography (Scheme XVIII).

### Biological Evaluation

In vitro evaluation of the various analogues of **1** as inhibitors of cyclic AMP phosphodiesterase was carried out in two assays, with enzyme derived from human platelets (IC$_{50}$, nM) and from rat or dog heart (IC$_{25}$, nM) by the method described in the preceding paper.[4] A ratio of these two values, IC$_{25}$ (heart)/IC$_{50}$ (platelet), can be taken to indicate relative cardioselectivity if significantly less than 1.0 and relative platelet selectivity if significantly greater than 1.0. Inhibition of ADP-induced platelet aggregation (IC$_{50}$, μM)[4] and a ratio of this value with the IC$_{50}$ (platelet PDE) were used as indicators of the potential in vivo efficacy of each agent vs. its intrinsic PDE inhibitory activity (Table VI).

### Structure–Activity Correlations

Several major trends emerge upon examination of the platelet PDE data, both within individual groups and across the range of analogues of **1**. Where only the amide substituent(s) is varied, an increase in net lipophilicity is, in general, accompanied by an increase in platelet PDE inhibition. For example, in the progression R$_1$R$_2$N = NH$_2$ (**10n**), CH$_3$NH (**10a**), (CH$_3$)$_2$N (**10b**), C$_6$H$_{11}$NH (**10c**), C$_6$H$_{11}$NCH$_3$ (**1**), C$_6$H$_{11}$NCH$_2$CH$_3$ (**10d**), C$_6$H$_{11}$NCH(CH$_3$)$_2$ (**10e**), and (C$_6$H$_{11}$)$_2$N (**101**), inhibition increases 1000-fold to the nanomolar range. Similar trends can be observed within the series of cycloalkyl (**10f**, **1**, **10g,h**) and hydroxyalkyl (**12a–e**) homologues. Derivatives of the hydroxyethyl amide (**12b**) also exhibit increasing activity within both the ester series (**15a–d**) and the functionalized

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**Table VI.** Biological Evaluation of 4-[(1,2,3,5-Tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramides and Analogues[a]

| no. | cAMP phosphodiesterase inhibition | | | inhibition of ADP-induced platelet aggregation: IC$_{50}$, $\mu$M | efficacy index[d] |
|---|---|---|---|---|---|
| | human platelet: IC$_{50}$, nM | rat or dog heart:[b] IC$_{25}$, nM | selectivity index[c] | | |
| 1 | 10.2 | 4.1 (B) | 0.4 | 0.11 | 10.8 |
| 10a | 1600 | 1300 (B) | 0.8 | I[e] | |
| 10b | 1200 | 360 (B) | 0.3 | 90 | 75 |
| 10c | 72 | 180 (B) | 2.5 | 10 | 139 |
| 10d | 1.5 | 5.2 (B) | 3.5 | 0.18 | 120 |
| 10e | 3.2 | 15 (B) | 4.7 | 0.26 | 81 |
| 10f | 48 | 25 (B) | 0.5 | 1.45 | 30 |
| 10g | 1.0 | 4.2 (B) | 4.2 | 0.25 | 250 |
| 10h | 2.2 | 1.6 (B) | 0.7 | 0.19 | 86 |
| 10i | 26 | 350 (A) | 13.5 | 0.39 | 15 |
| 10j | 26 | 240 (A) | 9.2 | 0.98 | 38 |
| 10k | 9.2 | 60 (A) | 6.5 | 1.05 | 11.4 |
| 10l | 1.45 | 16 (A) | 11 | 0.42 | 300 |
| 10m | 15 | 60 (A) | 4.0 | 9.2 | 613 |
| 10n | 690 | 380 (B) | 0.55 | 47 | 68 |
| 11a | 1600 | 7000 (A) | 4.4 | 53 | 33 |
| 11b | 260 | 1500 (A) | 5.8 | 14.5 | 56 |
| 11c | 340 | 1700 (A) | 5 | 82 | 241 |
| 11d | 10 | 160 (A) | 16 | 1.05 | 105 |
| 11e | 180 | 520 (A) | 2.9 | 2.6 | 14 |
| 11f | 76 | 260 (A) | 3.4 | 90 | 1184 |
| 11g | 13.5 | 200 (A) | 15 | 0.88 | 65 |
| 11h | 1.85 | 36 (A) | 19.5 | 2.3 | 1282 |
| 12a | 12.5 | 70 (A) | 5.6 | 2.85 | 228 |
| 12b | 6.0 | 17 (B) | 2.8 | 1.7 | 283 |
| 12c | 6.4 | 7.2 (B) | 1.1 | 4.0 | 625 |
| 12d | 4.9 | 2.3 (B) | 0.47 | 2.7 | 551 |
| 12e | 3.6 | 2.0 (B) | 0.56 | 4.0 | 1111 |
| 12f | 12.0 | 26.0 (B) | 2.2 | 82 | 6833 |
| 12g | 120 | 290 (B) | 2.4 | 25 | 208 |
| 13a | 2.0 | 10 (B) | 5 | 0.7 | 350 |
| 13b | 11 | 100 (B) | 9.1 | 20 | 1818 |
| 13c | 4.1 | 32 (B) | 7.8 | 1.0 | 243 |
| 13d | 1.3 | <10 (B) | 7.7 | 0.105 | 81 |
| 13e | 5.6 | 7.0 (B) | 1.25 | 3.6 | 642 |
| 13f | 5 | 100 (A) | 20 | 5.2 | 1040 |
| 14a | 240 | 440 (B) | 1.8 | 1.6 | 6.7 |
| 14b | 18 | 43 (B) | 2.4 | 3.4 | 188 |
| 14c | 280 | 130 (B) | 0.46 | I[e] | |
| 15a | 1.45 | 100 (A) | 69 | 0.16 | 114 |
| 15b | 1.1 | 100 (A) | 91 | 0.29 | 264 |
| 15c | 1.0 | 100 (A) | 100 | 0.50 | 500 |
| 15d | 0.94 | 80 (A) | 85 | 1.35 | 1436 |
| 20a | 820 | 4400 (A) | 5.4 | 7.0 | 8.5 |
| 20b | 4.6 | 22 (A) | 4.8 | 0.86 | 187 |
| 20c | 12 | 21 (A) | 1.75 | 1.9 | 158 |
| 20d | 16 | 45 (A) | 2.8 | 4.6 | 287 |
| 25a | 2000 | 1500 (B) | 0.75 | 60 | 30 |
| 25b | 150 | 64 (B) | 0.43 | 26 | 173 |
| 25c | >10$^5$[f] | 1600 (B) | <0.016 | I[e] | |
| 31 | 200 | 220 (B) | 1.1 | 6.6 | 33 |
| 39 | 140 | 260 (B) | 1.86 | 25 | 178 |
| 40 | 1700 | 6200 (B) | 3.6 | I[e] | |
| 41 | 3100 | 6600 (B) | 2.1 | 58 | 19 |
| 46 | 13 | 58 (B) | 4.5 | 1.5 | 115 |
| 51 | 17 | 780 (B) | 46 | 8.6 | 506 |
| 53 | 4.5 | 18 (B) | 4 | 1.0 | 222 |
| 55 | 5400 | 2900 (A) | 0.53 | I[e] | |
| 57 | 25000 | 3700 (A) | 0.15 | I[e] | |
| 62 | 15000 | 1600 (B) | 0.11 | 120 | 8 |
| 63 | 4400 | 1700 (B) | 0.4 | 150 | 34 |
| 66 | 27000 | 21000 (B) | 0.8 | I[e] | |
| 71 | 14 | 100 (B) | 7.1 | 1.1 | 79 |
| 72a | 26 | 78 (B) | 3 | 0.47 | 18 |
| 72b | 5.4 | 27 (B) | 5 | 0.36 | 66 |
| 73a | 580 | 450 (B) | 0.78 | 8.2 | 14 |
| 73b | 370 | 620 (B) | 1.68 | 5.6 | 15 |
| 74a | >10$^4$ | 6400 (B) | <0.64 | I[e] | |
| 74b | 10$^4$ | 6000 (B) | 0.6 | I[e] | |
| 75a | 27.5 | 90 (B) | 3.27 | 15.5 | 563 |
| 75b | 18.5 | 72 (B) | 3.9 | 6.9 | 373 |
| 76a | 1000 | 1700 (B) | 1.7 | 22 | 22 |
| 76b | 1600 | 3700 (B) | 2.3 | 54 | 34 |
| 77a | 3800 | 4200 (B) | 1.1 | I[e] | |

Table VI (Continued)

| no. | cAMP phosphodiesterase inhibition | | | inhibition of ADP-induced platelet aggregation: IC$_{50}$, $\mu$M | efficacy index[d] |
|---|---|---|---|---|---|
| | human platelet: IC$_{50}$, nM | rat or dog heart:[b] IC$_{25}$, nM | selectivity index[c] | | |
| 77b | 3300 | 3300 (B) | 1.0 | I[e] | |
| 78a | >10$^4$ | >10$^4$ (B) | | I[e] | |
| 78b | >10$^4$ | >10$^4$ (B) | | I[e] | |
| 82 | 130 | 100 (B) | 0.77 | 1.9 | 14.6 |
| 83 | 470 | 2400 (B) | 5.1 | 4.3 | 9 |
| 84 | 760 | 3700 (B) | 4.9 | 50 | 66 |
| 85 | 2.7 | 45 (B) | 16.7 | 0.15 | 55.5 |
| 92 | 5.9 | 14 (B) | 2.4 | 0.13 | 22 |
| 95 | 500 | 180 (B) | 0.36 | I[e] | |
| 96 | 76000 | 39000 (B) | 0.5 | I[e] | |
| 97 | 9600 | 2700 (B) | 0.3 | I[e] | |

[a] Both cAMP phosphodiesterase and platelet aggregation inhibition studies and statistical interpretation thereof were carried out by methods described in ref 4. IC$_{50}$ or IC$_{25}$ values were determined by measuring the inhibiting effects of each agent at five different inhibitor concentrations in triplicate and determined graphically from the resulting dose–response curve. [b] Rat heart (A) or dog heart (B) as tissue source. [c] IC$_{25}$ (heart)/IC$_{50}$ (platelet). [d] IC$_{50}$ (platelet aggregation inhibition)/IC$_{50}$ (platelet PDE inhibition). [e] Inactive up to 100 $\mu$M. [f] 20% inhibition observed at 10$^{-4}$ M.
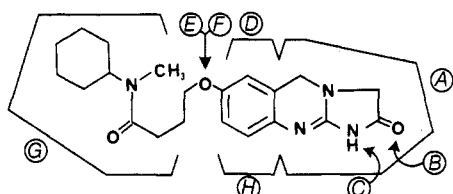


**Figure 1.** Characteristics of the human platelet type IV cyclic AMP phosphodiesterase binding site in relation to the structure of 1: A, planar, sterically demanding binding site; B, polarizable functionality; C, acidic proton; D, area of bulk tolerance; E, hydrogen-bonding functionality; F, regiospecific connection; G, lipophilic, sterically tolerant secondary binding site; and H, alternate site of bulk tolerance in cardiac enzyme.

series (13a–f) that are correlated with lipophilicity. In contrast, the less lipophilic 4-hydroxycyclohexyl analogue (12g), a likely metabolite based on the recently observed hydroxylation pattern for the side chain of cilostazole,[49] exhibited a significant decrease in activity vs. 1. In general, however, aromatic substituents (10i–k,m) or incorporation of the amide nitrogen into a ring (11a–h) tend to decrease activity, except in cases where net lipophilicity is extremely high (e.g., 11h). Replacement of the amide substituent altogether by the corresponding acid (14c), ethyl (14a) or cyclohexyl (14b) ester, urethane (46), urea (51), or tetrazole (53) afforded a series of nearly isosteric compounds exhibiting increasing activity in the order given, although only 53 was more active than the parent.

The overall topography of the binding site was deduced by examining the activity of compounds with a number of additional structural variations. Within the series of chain-length homologues of 1, the optimal length is found in the valeramide (20b). Modest decreases in activity are observed for the two next higher homologues (20c,d), but a precipitous decrease in activity is seen for the acetamide (20a), indicating an obvious requirement for spacing between the heterocycle and the amide functionality. The positional isomer series (25a–c) provided the strongest evidence for a steric requirement in the arrangement of the side chain relative to the heterocycle. While the position-8 isomer (25b) lost a significant degree of activity in comparison with 1, attachment of the side chain to either positions 6 (25a) or 9 (25c) resulted in near abolition of the PDE inhibition, indicating that little tolerance exists for substantial aryl heterocycle substitution in any place but position 7. The 6-chloro analogue (71) was nearly as

(49) Nishi, T.; Tabusa, F.; Tanaka, T.; Shimizu, T.; Nakagawa, K. *Chem. Pharm. Bull.* **1985**, *33*, 1140.
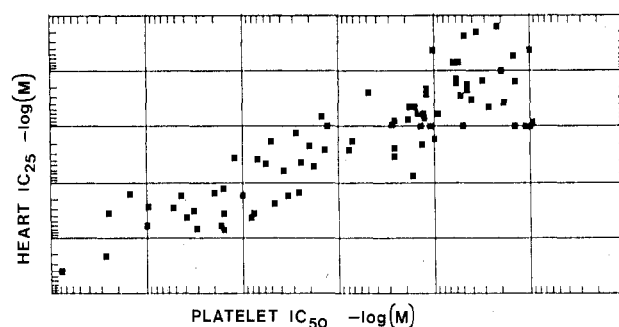


**Figure 2.** Human platelet IC$_{50}$ values vs. cardiac IC$_{25}$ values for compounds in Table VI (except for 25c). Correlation coefficient = 0.912.

**Table VII.** Inhibition of Human Platelet PDE by Cardiotonic Agents

| compound | IC$_{50}$,[a] nM | IC$_{50}$/IC$_{50}$ (1)[b] |
|---|---|---|
| 98 | 150 | 14.7 |
| 99 | 3800 | 373 |
| 100 | 6200 | 608 |
| 101 | 860 | 84 |

[a] Experimental method as in Table VI. [b] From Table VI.

active as the parent, however, indicating that, at least at C(6), smaller substituents are better accommodated, a result consistent with previous findings.[38] Replacement of the oxygen connecting the side chain to the heterocycle by sulfur as sulfide (39), sulfoxide (40), or sulfone (31) reduced activity in that order. With carbon as a connecting atom, analogue 31 was slightly less potent than the sulfide (39), indicating a preference for an electron-donating substituent at C(7).

Substitution at other positions on the heterocycle were examined to define the steric environment immediately surrounding the acylguanidine moiety. Amongst a series of analogues substituted at position 3 (72–78), only those possessing methyl (72a,b) or hydroxymethyl (75a,b) substituents were tolerated with any facility. In both these cases, as had been noted by previous workers in the anagrelide heterocycle series,[38] a preference for the R configuration (derived from the D-$\alpha$-amino acid) was observed. Ring expansion (82) or 3,3-geminal disubstitution with methyl (83) or spirocyclopropane (84) decreased activity 1 order of magnitude over the monomethyl analogue (72). Notably, the 3-methylene analogue (85), the dehydro derivative of 75, exhibited activity 4 times that of the parent structure. Addition of a (racemic) methyl substituent at position 5 (92) also showed enhanced activity. These re-

sults define a very restricted and near-planar binding site with little tolerance for substitution.

Modification of the acylguanidine itself consistently led to significant, if not total, abolition of activity. Both N(1)- and O(2)-methylated analogues (96 and 97, respectively) were essentially inactive in comparison with 1. Acylguanidine isomers (57, 62), diones (55, 63), and the guanidine itself (66) were uniformly 2–4 orders of magnitude less active than the parent structure. The 3-aza analogue (95) was also significantly less active. These observations indicate that the acylguanidine must be left completely intact. This functional requirement produces a molecule (1) with $pK_a$ values of 3.25 (as a base) and 11.50 (as an acid)[50] capable of hydrogen bonding as either a proton acceptor or source at the active site.

The structure–activity overview derived from this systematically varied series of analogues of 1 correlates highly with the "hypothetical" topography of the high-affinity cAMP binding site previously described by Wells et al.[51] for the enzyme from pig coronary arteries and later refined by Bristol et al.[52] The cyclic AMP specific PDE active site proposed therein consists of a fairly planar, sterically demanding binding locus (A) requiring both a polarizable functionality (B) and an acidic proton (C) directly adjacent to an area of bulk tolerance (D) with hydrogen-bonding potential (E) connected in a regiospecific manner (F) to a remote area of more tolerant lipophilic and steric secondary binding (G), features easily related to the structure of 1 (Figure 1) and the limitations imposed upon that structure as defined by the analogue series. In addition to anagrelide and its analogues, other PDE type IV inhibitors, such as CI-930 (98), enoximone (99), OPC-8212 (100), and, to a lesser degree, milrinone (101), all of which



98

99

100

101
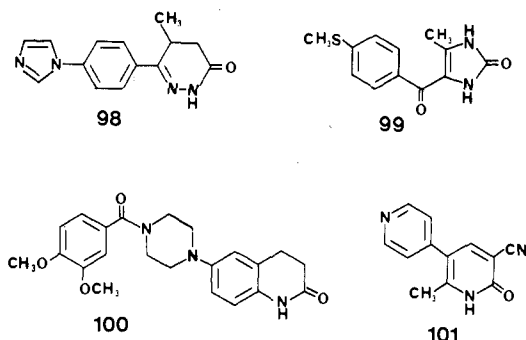
are cardiotonic agents, also exhibit these same characteristics, but all lack the secondary binding capability imparted by the oxybutyramide side chain. This enhanced degree of binding is reflected in the $IC_{50}$ values obtained for inhibition of human platelet PDE (Table VII), which indicate that 1 is from 15 to 600 times more potent than these other compounds as an inhibitor of this enzyme.

The steric and functional demands of the binding site of the platelet and cardiac enzymes were found to be essentially operationally equivalent, since compounds exhibiting significant activity as inhibitors of human platelet PDE were also without exception inhibitors of the corresponding cardiac enzyme. The variation in activity observed was in almost all cases less than 1 order of magnitude, as indicated by selectivity index values between 0.1 and 10 (Table VI) and graphically depicted in Figure 2. Significant exceptions to this generalization, however, were found to be the bulky, lipophilic 2-hydroxyethyl esters (15a–d) and the 9-oxy side chain isomer (25c). This last compound (25c), almost inactive against human platelet PDE (20% inhibition at $10^{-4}$ M), was found to be reasonably potent as an inhibitor of the cardiac enzyme ($IC_{25}$ = 1.6 $\mu$M). This single example of steric bulk in this position, certainly unoptimized with regard to chain length and substitution, strongly suggests that a major difference between the high-affinity cardiac and platelet enzymes involves steric tolerance (or the lack thereof, respectively) at the site corresponding to position 9. The capacity to bind compounds substituted in this manner (site H, Figure 1) correlates highly with the observed pattern of binding of lipophilically substituted xanthines that defined the original active-site topographical map proposed by Wells for the enzyme from pig coronary arteries.[51] Any significant distinctions at this last site may allow for the preparation of either platelet- or cardiac-specific PDE inhibitors, leading to selective antithrombotic or cardiotonic agents.

In the platelet aggregation assay, most analogues of 1 reasonably active as inhibitors of human platelet PDE were also effective as inhibitors of ADP-induced aggregation. Lipophilicity was found to play a role in distinguishing amongst analogues in this assay, however. Within the series of hydroxyethyl esters (15a–d) cited above, a reversal in the order potency is observed; i.e., the most lipophilic is now the least active. This reversal is reminiscent of the similar one observed in the comparison of two much simpler anagrelide analogues.[4] In addition, in the series of amide analogues of increasing lipophilicity (10n, 10a, 10b, 10c, 1, 10d, 10e, and 10l), an actual maximum of activity is reached with the parent structure 1, with compounds both more and less polar being less potent. This correlation is significant to any potential in vivo application, since it defines an optimal lipophilic window for access and/or binding to the enzyme. Indeed, most of the very polar or lipophilic compounds that exhibit little activity in platelet aggregation inhibition showed virtually no activity as inotropic agents in the anesthetized dog model, under experimental conditions where 1 has been shown to be quite potent.[6]

## Conclusion

Two recent detailed studies of the patterns of PDE inhibition displayed by many of the newer cardiotonic agents,[53,54] along with our own results regarding 1 cited previously,[6] indicate that selective inhibition of the cardiac PDE III (type IV) enzyme is the likely shared mechanism of action of these drugs. Examination of a wide variety of analogues of 1 as inhibitors of PDE type IV from two tissue sources, and as inhibitors of ADP-induced platelet aggregation, has revealed further information regarding the active site of this important enzyme. The correlation of inhibition of the cardiac PDE enzyme of this type with positive inotropic action demonstrated previously[52] indeed holds true for 1, shown to be a potent cardiotonic agent in both anesthetized and conscious dogs.[6] The information gleaned from this analogue series has further defined some

(50) We thank Dr. D. Johnson, Institute of Pharmaceutical Sciences, for the spectrophotometric determination of the $pK_a$ values for 1; also see: Gu, L.; Huynh, O.; Becker, A.; Peters, S.; Nguyen, H.; Chu, N. *Drug Dev. Ind. Pharm.*, in press.

(51) Wells, J. N.; Garst, J. E.; Kramer, G. L. *J. Med. Chem.* 1981, 24, 954.

(52) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* 1984, 27, 1099.

(53) Weishaar, R. E.; Burrows, S. D.; Kobylarz, D. C.; Quade, M. M.; Evans, D. B. *Biochem. Pharmacol.* 1986, 35, 787.

(54) Ahn, H. S.; Eardley, D.; Watkins, R.; Prioli, N. *Biochem. Pharmacol.* 1986, 35, 1113.

parameters governing the secondary binding site present in the enzyme, exploitation of which greatly enhances the observed in vitro potency and potential in vivo activity of such inhibitors. More importantly, however, differences noted between platelet and cardiac PDEs, and their respective influences on aggregation and inotropism, will no doubt permit the more accurate topographical mapping of the active site(s) involved and eventually lead to the design of highly specific antithrombotic or cardiotonic agents.

## 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 unless otherwise stated. All organic extracts were dried over sodium sulfate prior to evaporation.

**4-(3-Formyl-4-nitrophenoxy)butyramides 5a–m, 6a–h, 7a–g, and 8a–e.** Oxalyl chloride (4.40 mL, 75 mmol) was added to a suspension of acid $4^4$ (12.65 g, 50 mmol) in benzene (50 mL) and DMF (0.5 mL) in small portions. When all 4 had dissolved, the mixture was stirred for an additional 30 min. The solution was evaporated to a thick syrup, redissolved in dry THF (50 mL), and reevaporated twice. The final residue of crude acid chloride was dissolved in THF (50 mL) and used in either of the two methods described below. See Table I for method used, yield, and physical data for each compound.

**Method A.** The THF solution of the acid chloride was added dropwise to a well-stirred solution of amine (60 mmol) and sodium carbonate (6.90 g, 65 mmol) in THF (100 mL) and water (100 mL) cooled to 0 °C in an ice bath. When the addition was complete, the cooling bath was removed, and the mixture was allowed to stir for 1 h at room temperature. Most of 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 a residue, crystallized from ethyl acetate or chromatographed over silica gel (10% ethyl acetate in dichloromethane) followed by crystallization from ether.

**Method B.** The THF solution of the acid chloride was added dropwise to a solution of amine (60 mmol), triethylamine (9.0 mL, 65 mmol), and 4-(dimethylamino)pyridine (0.60 g, 5 mmol) in dry THF (100 mL) blanketed under nitrogen and cooled to 0 °C. When the addition was complete, the reaction was stirred at room temperature for 2 h. After removal of the THF, the residue was partitioned between ethyl acetate and 1 M HCl (300 mL each). The organic layer was washed with 1 M HCl (2 × 100 mL), saturated sodium bicarbonate (3 × 100 mL), and brine (2 × 50 mL), dried, filtered, and evaporated. Purification of the residue was carried out as in method A.

**4-(Cyclohexylamino)butanol (Amine Component for 7c).** A mixture of *N*-cyclohexyl-4-hydroxybutyramide[55] (60 g, 350 mmol) and LAH (13.2 g, 350 mmol) in THF (1 L) was heated at reflux for 18 h and was then cooled and poured into THF (1 L) containing sodium sulfate decahydrate (225 g, 700 mmol). The resulting mixture was heated with stirring until the precipitated solid was white and granular and then was filtered and evaporated. The residue was dissolved in ether, then filtered, and evaporated to give a solid, which was recrystallized from ether–petroleum ether to yield 49 g (287 mmol, 82%), mp 35–36 °C. Anal. ($C_{10}H_{21}NO$) C, H, N.

**6-(Cyclohexylamino)hexanol (Amine Component for 7e).** *N*-Cyclohexyl-6-hydroxyhexanamide, prepared from ε-capro-

lactone and cyclohexylamine,[55] was reduced with LAH by the method described above to yield the title amine as a thick amber syrup. Anal. ($C_{12}H_{25}NO$) C, H, N.

**2-(Cyclohexylamino)-1,3-propanediol (Amine Component for 7f).** Di-*tert*-butyl bromomalonate[56] (57 g, 193 mmol) was added over 2.5 h to cyclohexylamine (88 mL, 772 mmol) at 70 °C. After an additional 1 h, the mixture was cooled, filtered, and evaporated to remove excess amine. The crude amino diester (60 g) was dissolved in THF (100 mL) and added dropwise to a suspension of LAH (14 g) in THF (500 mL), and the resulting mixture was stirred overnight at reflux. After cooling, the mixture was subjected to the workup described above. The residue obtained was crystallized from dichloromethane to yield 16.3 g (94 mmol. 49%), mp 98–99 °C. Anal. ($C_9H_{19}NO_2$·$0.25CH_2Cl_2$) C, H, N.

***trans*-4-(Methylamino)cyclohexanol (Amine Component for 7g).** A mixture of *trans*-4-aminocyclohexanol hydrochloride (37.9 g, 250 mmol) and sodium carbonate (58.3 g, 550 mmol) in THF (500 mL) and water (1 L) was cooled to 5 °C and was treated dropwise with a solution of benzyl chloroformate (39 mL) in THF (50 mL). When the addition was complete, the mixture was stirred at room temperature for 30 min. Diethyl ether (500 mL) was added, and the layers were separated. The organic layer was washed with saturated brine and then dried, filtered, and evaporated. Crystallization from ether afforded 58 g (249 mmol, 100%) of *trans*-4-[(benzyloxycarbonyl)amino]cyclohexanol, mp 164–165 °C. Anal. ($C_{14}H_{19}NO_3$) C, H, N.

A solution of this alcohol (57 g, 230 mmol) in THF (500 mL) was added to a suspension of lithium aluminum hydride (17.4 g, 460 mmol) in THF (600 mL). The resulting mixture was brought to reflux overnight. After cooling, the reaction mixture was added in small portions to a suspension of sodium sulfate decahydrate (180 g) in THF (1 L). The mixture was heated to boiling for 1 h, then cooled, filtered, and evaporated to afford a thick oil, which crystallized from ether to yield 24 g of *trans*-4-(methylamino)-cyclohexanol (185 mmol, 80%), mp 120–121 °C (lit.[57] mp 113–114 °C).

**4-[2-(Cyclohexylamino)ethyl]morpholine (Amine Component for 8c).** Methanol saturated with dry hydrogen chloride (15 mL) was added to a solution of 4-(2-aminoethyl)morpholine (78 mL, 600 mmol) in methanol (300 mL) cooled to 5 °C. Cyclohexanone (10.4 mL, 100 mmol) and 3A molecular sieves (20 g) were added, and the mixture was stirred at room temperature for 1 h. Sodium cyanoborohydride (3.8 g, 60 mmol) was added, and the mixture was stirred overnight, then filtered, and evaporated. The residue was partitioned between ether (500 mL) and saturated sodium bicarbonate. The organic extract was washed with saturated sodium bicarbonate (5 × 200 mL) and brine (2 × 200 mL) and was then dried, filtered, and evaporated. Fractional distillation (105–108 °C, 0.5 mm) provided the title amine as a colorless oil (17.5 g, 82 mmol, 82%). Anal. ($C_{12}H_{24}N_2O$·$0.25H_2O$) C, H, N.

***N*-[2-(Cyclohexylamino)ethyl]imidazole (Amine Component for 8e).** Triethylamine (175 mL, 1.25 mol) was added dropwise to a solution of *N*-(2-chloroethyl)cyclohexylamine hydrochloride[58] (84 g, 500 mmol) and benzyl chloroformate (106.5 mL, 625 mmol) in chloroform, and the resulting mixture was stirred overnight. The solution was extracted with 1 M HCl (3 × 500 mL), saturated sodium carbonate (3 × 500 mL), and brine (2 × 500 mL) and then was dried and evaporated. The residue was crystallized from cold pentane to yield *N*-carbobenzoxy-*N*-(2-chloroethyl)cyclohexylamine (89 g, 300 mmol, 60%), mp 52–53 °C. Anal. ($C_{16}H_{22}ClNO_2$) C, H, N, Cl. A solution prepared from imidazole (23.3 g, 344 mmol) and sodium hydride (7.30 g, 303 mmol, washed free of oil) in DMF (250 mL) was added dropwise to a solution of the above (chloroethyl)amine (81.3 g, 275 mmol) in dry DMF (250 mL) at room temperature. The

(55) Tsuchiki, K., Japanese Patent 6802343, 27 Jan 1968; *Chem. Abstr.* **1968**, *69*, 66979g.

(56) Hollowood, J.; Jansen, A. B. A.; Southgate, P. J. *J. Med. Chem.* **1967**, *19*, 863.

(57) Russo, G.; Danieli, B. *Gazz. Chim. Ital.* **1965**, *95*, 438. These authors prepared a mixture of cis and trans isomers, left unidentified after separation by fractional crystallization.

(58) Cope, A. C.; Nace, H. R.; Hatchard, W. R.; Jones, W. H.; Stahmann, M. A.; Turner, R. B. *J. Am. Chem. Soc.* **1949**, *71*, 554.

mixture was heated at 60 °C overnight and was then evaporated. The residue was partitioned between ether and water (500 mL each), and the organic extract was washed with water (3 × 500 mL). The organic layer was evaporated to one-third volume, resulting in crystallization of *N*-carbobenzoxy-*N*-(2-*N*-imidazolylethyl)cyclohexylamine (69 g, 211 mmol, 77%), mp 101–102 °C. Anal. (C$_{19}$H$_{25}$N$_3$O$_2$) C, H, N. Hydrogenation of the CBZ-protected amine (69 g, 211 mmol) in THF over 10% Pd–C (5 g) at 60 psi afforded an oil (40 g, 207 mmol, 98%). A sample was treated with dry HCl in ethyl acetate/ether to yield the dihydrochloride, mp 225–228 °C. Anal. (C$_{11}$H$_{21}$Cl$_2$N$_3$) C, H, N, Cl.

**4-[(1,2,3,5-Tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramides 10a–m, 11a–h, 12a–g, 13a–e, Esters 14a,b, and Analogues 46, 51, and 53.** Nitro aldehydes 5–9, 45, 48, and 52 were subjected to the reaction sequence described for the preparation of 1,[4] generalized as follows.

Anhydrous sodium acetate (2.0 equiv) was added to a warm solution of glycine ethyl ester hydrochloride (2.4 equiv) in absolute ethanol (3 mL/mmol). The resulting mixture was stirred overnight at room temperature and was then filtered. The appropriate nitroaldehyde (1.0 equiv) was added, and the mixture was stirred for 30 min. Additional absolute ethanol was added to insure complete dissolution at this point. Sodium cyanoborohydride (0.6 equiv) was then added in one portion. After 3 h the solution was evaporated, and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic extract was washed with additional aqueous sodium bicarbonate and with brine, then dried, filtered, and evaporated to give the intermediate amine as a thick syrup. The crude amine was dissolved in absolute ethanol (2 mL/mmol) and was hydrogenated over 10% Pd–C (1.0 g/50 mmol) usually overnight. The catalyst was removed by filtration through a pad of Celite, and the pad was washed clean with additional absolute ethanol. The combined filtrates were treated with cyanogen bromide (1.1 equiv), and the resulting solution was stirred at room temperature overnight. Concentrated ammonium hydroxide (1 mL/mmol) was added, and the solution was stirred at room temperature for 30 min, during which time the product crystallized directly from the reaction mixture. However, in those cases where no product was obtained directly, the ethanol solution was concentrated and the residue was dissolved in ethyl acetate. The organic extract was washed with water and brine and was dried and evaporated. The residue was crystallized from aqueous ethanol or, where noted, subjected to silica gel chromatography to obtain the desired products. Yields and physical data appear in Table II.

The following compounds (10n, 13f, 14c, 15a–d) were prepared from products of the above sequence. Yields and physical data are detailed in Table II.

**4-[(1,2,3,5-Tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (10n).** A suspension of ester 14a (3.17 g, 10 mmol) in ethylene glycol saturated with ammonia (50 mL) was heated in a steel pressure apparatus at 140 °C for 24 h. The mixture was cooled and the resulting precipitate was washed with absolute ethanol to yield 10n (2.62 g, 9.1 mmol, 91%).

*N*-Carboxymethyl-*N*-cyclohexyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (13f). A suspension of 13a (8.1 g, 18 mmol) in methanol (50 mL) was treated with 2 N NaOH (45 mL). After 30 min at room temperature, the mixture was acidified to pH 5 to yield a precipitate of 13f, collected by filtration and dried at high vacuum (7.10 g, 16.5 mmol, 92%).

**4-[(1,2,3,5-Tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyric Acid (14c).** A suspension of 14a (3.2 g, 10 mmol) in ethanol (10 mL) was treated with 2 N NaOH (10 mL), followed by additional water (30 mL). After 30 min, 1 M HCl was added to give a precipitate, which was collected by filtration and dried at high vacuum to afford 14c (2.65 g, 7.4 mmol, 74%) as the sodium salt.

*N*-(2-Acetoxyethyl)-*N*-cyclohexyl-4-[(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (15a). A solution of 12a (207 mg) in acetic anhydride (1 mL) and pyridine (1 mL) was treated with DMAP (6 mg), and the resulting solution was stirred at room temperature for 1 h. The mixture was concentrated, and the residue was dissolved in ethyl acetate (25 mL). The solution was washed with saturated sodium bicarbonate (2

× 10 mL) and brine (2 × 10 mL) and was then dried, filtered, and evaporated. The residue was crystallized from ethyl acetate to afford 15a (150 mg, 0.33 mmol, 66%). Substitution of the appropriate anhydrides in the above procedure gave esters 15b–d.

**Chain-length homologues 20a–d** were prepared by alkylation of 16[18] with the desired ω-bromoalkanoates, followed by conversion according to the sequence described for the preparation of 1[4] (Scheme II). Yields and physical data for 17–20 appear in Table III.

**Positional isomers 25a–c** were prepared by alkylation of the required isomeric hydroxy-2-nitrobenzaldehydes[20–22] with ethyl 4-bromobutyrate, followed by conversion according to the sequence described for the preparation of 1[4] (Scheme III). Yields and physical data for 22–25 appear in Table IV.

**5-Iodo-2-nitrobenzoic Acid (27).** A solution of 5-amino-2-nitrobenzoic acid (Fluka; 36 g, 100 mmol, dried over P$_2$O$_5$) in THF (200 mL) was added over 30 min to neat boron trifluoride etherate (50 mL) cooled in an ice/acetone bath. A solution of *tert*-butyl nitrite (32 mL) in THF (50 mL) was then added dropwise at −10 °C. After 30 min, a fine precipitate appeared. The mixture was allowed to warm to 5 °C, and ether (250 mL) was added. The precipitate was collected by filtration, washed with ether, and air-dried to yield the diazonium tetrafluoroborate salt (56 g). The diazonium salt was added portionwise to a solution of sodium iodide (40 g) in acetone (1 L). The solution was concentrated and the residue was triturated with water to yield 27 (37 g, 126 mmol, 63%), mp 173–174 °C (lit.[23] mp 174 °C).

**5-Iodo-2-nitrobenzaldehyde (28).** Borane–methyl sulfide (Aldrich; 10 M, 20 mL) was added via syringe to a solution of 27 (51.3 g, 175 mmol) in THF (250 mL). When the addition was complete, the mixture was brought to reflux for 2 h with concomitant removal of methyl sulfide to give a precipitate. The mixture was cooled and diluted with ether (500 mL), and the powdery precipitate was collected by filtration. A mixture of the crude intermediate and pyridinium dichromate (99 g, 263 mmol) in dichloromethane (500 mL) was heated at reflux overnight. The mixture was cooled and poured into ether (1.5 L), stirred 24 h, and then filtered through Celite. Evaporation and trituration with hexane afforded 28 (27 g, 97.5 mmol, 56%), mp 64–65 °C. Anal. (C$_7$H$_4$INO$_3$) C, H, N, I.

**Methyl 5-(3-Formyl-4-nitrophenyl)-4-pentynoate (29).** Bis(triphenylphosphine)palladium(II) chloride (Alfa; 1.4 g, 2 mmol) and copper(I) iodide (190 mg, 1 mmol) were added to a solution of 28 (27.6 g, 100 mmol) and methyl 4-pentynoate (13.4 g, 120 mmol) in dry degassed triethylamine (200 mL). After heating at 60 °C for 30 min, the mixture was cooled, and the supernatant was decanted away from precipitated solids and evaporated. The residue was partitioned between ethyl acetate (500 mL) and 1 M HCl (250 mL), and the organic extract was washed with 1 M HCl (3 × 200 mL) and brine (2 × 250 mL), then dried, filtered, and evaporated. The residual amber oil was crystallized from ether–hexane to afford 29 (20.0 g, 76.6 mmol, 77%), mp 92–93 °C. Anal. (C$_{13}$H$_{11}$NO$_5$) C, H, N.

**Methyl 5-(1,2,3,5-Tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)pentanoate (30).** Nitro aldehyde 29 (11.8 g, 45 mmol) was subjected to the reaction sequence utilized for the preparation of 1 to yield 30 (4.25 g, 14.1 mmol, 31%), mp 214–215 °C. Anal. (C$_{16}$H$_{19}$N$_3$O$_3$) C, H, N.

*N*-Cyclohexyl-*N*-methyl-5-(1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)pentanamide (31). A suspension of 30 (3.01 g, 10 mmol) and tetrabutylammonium bromide (9.66 g, 30 mmol) in DMF (100 mL) was treated with a solution of KOH (1.6 g, 25 mmol) in water (5 mL), and the resulting mixture was stirred overnight. Molecular sieves (25 g) were added, followed by *N*-methylcyclohexylamine (2.6 mL, 20 mmol) and bis(*p*-nitrophenyl) phenylphosphonate[29] (12 g, 30 mmol) in 24 h. The mixture was agitated by shaking for 3 days and was then filtered through Celite and evaporated. The residue was dissolved in ethyl acetate (250 mL) and was washed with saturated sodium carbonate (2 × 100 mL) and brine (2 × 100 mL), then dried, filtered, and evaporated. Recrystallization from ethyl acetate–ether gave 31 (1.65 g, 4.31 mmol, 43%), mp 173–174 °C. Anal. (C$_{22}$H$_{30}$N$_4$O$_2$·0.25H$_2$O) C, H, N.

**2-Nitro-5-mercaptobenzyl Alcohol (33).** A solution of 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, 32, 39.6 g, 100 mmol) in dry dimethoxyethane (2 L)[59] was treated with

borane–methyl sulfide (10 M, 40 mL) dropwise with stirring. When hydrogen evolution ceased, the mixture was brought to reflux for 3 h, then cooled, quenched with concentrated HCl, and evaporated. The residue was dissolved in ethyl acetate (500 mL) and was washed with 1 M HCl (3 × 200 mL) and brine (2 × 200 mL), dried, filtered, and evaporated. Crystallization of the resulting oil from ethyl acetate–ether afforded **33** (29.6 g, 160 mmol, 80%), mp 119–120 °C. Anal. ($C_7H_7NO_3S$) C, H, N, S.

With tetrahydrofuran as solvent, the sole product isolated upon BMS reduction of **32** was 5-[(4-hydroxybutyl)thio]-2-nitrobenzyl alcohol (**34a**), mp 70–72 °C. Anal. ($C_{11}H_{15}NO_4S\cdot0.2H_2O$) C, H, N, S. Chromatography (5% ethyl acetate in dichloromethane) of the mother liquors of the BMS reduction carried out in DME afforded 5-(methylthio)-2-nitrobenzyl alcohol (**34b**), mp 105–106 °C. Anal. ($C_8H_9NO_3S$) C, H, N, S.

**Ethyl 4-[[3-(Hydroxymethyl)-4-nitrophenyl]thio]butyrate (35).** A solution of DBU (29.9 mL, 200 mmol) in dry tetrahydrofuran (50 mL) was added via syringe pump over 4 h to a solution of **33** (37 g, 200 mmol) and ethyl 4-bromobutyrate (28.6 mL, 240 mmol). The reaction mixture was evaporated, and the residue was dissolved in ethyl acetate (300 mL). The organic layer was washed with 1 M HCl (4 × 100 mL) and brine (2 × 100 mL), then dried, filtered, and evaporated to give a thick oil. Chromatography over silica gel (5% ethyl acetate in dichloromethane) afforded **35** (27.5 g, 91.9 mmol, 46%) as a thick syrup. Anal. ($C_{13}N_{17}NO_5S$) C, H, N, S.

**Ethyl 4-[(3-Formyl-4-nitrophenyl)thio]butyrate (36).** Solid pyridinium dichromate (50.8 g, 135 mmol) was added to a solution of **35** (26.9 g, 90 mmol) in dichloromethane (150 mL), and the mixture was stirred overnight. Ether (300 mL) was added to the mixture, which was filtered through Celite after being stirred for 1 h. The filtrate was evaporated, and the residue was filtered through silica gel (500 g) with dichloromethane as eluant. Crystallization from petroleum ether afforded **36** (19.5 g, 66 mmol, 73%), mp 35–36 °C. Anal. ($C_{13}H_{15}NO_5S$) C, H, N, S.

**4-[(3-Formyl-4-nitrophenyl)thio]butyric Acid (37).** A solution of **36** (17.8 g, 60 mmol) in dioxane and 6 M HCl (100 mL each) was heated at reflux for 2 h, with removal of ethanol. The mixture was cooled and extracted with ethyl acetate (5 × 100 mL). The organic extract was washed with water (3 × 100 mL) and brine (2 × 100 mL), then dried, filtered, and evaporated. The residue was crystallized from ether to afford **37** (16 g, 59 mmol, 99%), mp 122–123 °C. Anal. ($C_{11}H_{11}NO_5S$) C, H, N, S.

***N*-Cyclohexyl-*N*-methyl-4-[(3-formyl-4-nitrophenyl)thio]butyramide (38).** Conversion of **37** (14.8 g, 55 mmol) to amide **38** was carried out by using the procedure described above (method A). The title compound was isolated as thick oil, which crystallized on standing (19.6 g, 53.8 mmol, 98%), mp 53–54 °C. Anal. ($C_{18}H_{24}N_2O_4S$) C, H, N, S.

***N*-Cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxo-imidazo[2,1-*b*]quinazolin-7-yl)thio]butyramide (39).** Reductive amination of **38** (18.2 g, 50 mmol) with glycine methyl ester and sodium cyanoborohydride by using the procedure for **1**[4] afforded a thick syrup, which was dissolved in ethanol (250 mL) and carefully poured over 10% Pd–C (20 g). The mixture was blanketed under $N_2$ and to it was added 1,4-cyclohexadiene (47.3 mL, 500 mmol). After heating at reflux for 48 h, the mixture was cooled, filtered, and treated with cyanogen bromide (5.8 g, 55 mmol). After 24 h, ammonium hydroxide was added to afford a fine precipitate of **39** (7.2 g, 18 mmol, 36%), mp 217–218 °C. Anal. ($C_{21}H_{28}N_4O_2S$) C, H, N, S.

**Sulfoxide 40 and Sulfone 41.** A solution of **39** (1.0 g, 2.5 mmol) in chloroform (125 mL) cooled to −10 °C was treated with a solution of *m*-CPBA (0.65 g, 2.5 mmol) in chloroform (20 mL) dropwise over 2 h. The mixture was warmed to ambient temperature and was extracted with saturated sodium bicarbonate (3 × 100 mL) and brine (2 × 100 mL), then dried, filtered, and evaporated. The residue was crystallized from ether to yield **40** (0.87 g, 1.98 mmol, 79%), mp 228–230 °C. Anal. ($C_{21}H_{28}N_4O_3S\cdot1.25H_2O$) C, H, N, S.

With use of similar conditions, oxidation of **39** with 2 equiv of *m*-CPBA afforded **41** (0.98 g, 2.27 mmol, 91%), mp 259–260

°C. Anal. ($C_{21}H_{28}N_4O_4S$) C, H, N, S.

**5-(2-Acetoxyethoxy)-2-nitrobenzaldehyde (42).** A mixture of **16** (25 g, 150 mmol), 2-bromoethyl acetate (18.2 mL, 165 mmol), and anhydrous potassium carbonate (25.9 g, 187.5 mmol) in DMF (100 mL) was heated at 60–70 °C overnight. The reaction mixture was cooled, filtered, and evaporated, and the residue was dissolved in ethyl acetate (300 mL). The organic layer was washed with saturated sodium bicarbonate (2 × 100 mL) and brine (2 × 100 mL), then dried, filtered, and evaporated. The residue was crystallized from ether to afford **42** (31.6 g, 125 mmol, 83%), mp 65–66 °C. Anal. ($C_{11}H_{11}NO_6$) C, H, N.

**5-(2-Hydroxyethoxy)-2-nitrobenzaldehyde (43).** A mixture of **42** (30.4 g, 120 mmol) in 6 N HCl (250 mL) was heated at reflux for 1 h and was then cooled and extracted with ethyl acetate (4 × 200 mL). The organic layer was washed with saturated sodium bicarbonate (2 × 200 mL) and brine (2 × 200 mL), then dried, filtered, and evaporated. Crystallization from ether gave **43** (24.5 g, 116 mmol, 97%), mp 67–68 °C. Anal. ($C_9H_9NO_5$) C, H, N.

**5-[2-[(*N*-Cyclohexyl-*N*-methylcarbamoyl)oxy]ethoxy]-2-nitrobenzaldehyde (45).** Solid *p*-nitrophenyl chloroformate (5.53 g, 27.5 mmol) was added portionwise to a chilled solution of **43** (5.3 g, 25 mmol) and triethylamine (4.2 mL, 31.2 mmol) in THF (100 mL). After 1 h, the solution was warmed to room temperature and then was filtered, evaporated, and dissolved in ethyl acetate (200 mL). The organic layer was washed with 1 M HCl (3 × 100 mL) and brine (2 × 100 mL) and then was dried, filtered, and evaporated. Crystallization of the residue from ether afforded carbonate **44** (8.90 g, 23.6 mmol, 95%), mp 108–109 °C. Anal. ($C_{16}H_{13}NO_7$) C, H, N.

Carbonate **44** could be converted into urethane **45** by treatment with 1 equiv of *N*-methylcyclohexylamine in DMF. However, a more convenient procedure avoiding the isolation of **44** was also used. The chilled THF solution of **44** generated from **43** (14 g, 66 mmol) and *p*-nitrophenyl chloroformate (14.5 g, 72 mmol) was treated directly with *N*-methylcyclohexylamine (11 mL, 72 mmol). After 1 h, the mixture was evaporated, and the residue was dissolved in ethyl acetate (500 mL). The organic extract was washed with water (3 × 200 mL), saturated sodium carbonate (5 × 200 mL), 1 M HCl (3 × 200 mL), and brine (2 × 200 mL) and then was dried, filtered, and evaporated. Chromatography over silica gel (0–10% gradient of ethyl acetate in dichloromethane) afforded **45** (11.0 g, 31.3 mmol, 47% overall from **43**) as a thick oil, which crystallized on standing, mp 66–67 °C. Anal. ($C_{17}H_{22}N_2O_6$) C, H, N.

**1-(2-Chloroethyl)-3-cyclohexyl-3-methylurea (47).** A solution of *N*-methylcyclohexylamine (29 mL, 225 mmol) in THF (50 mL) was added dropwise over 1 h to a chilled solution of 2-chloroethyl isocyanate (Aldrich; 25 g, 238 mmol) in THF (200 mL). After stirring at room temeprature overnight, the solution was evaporated at <40 °C to give a semisolid, which was crystallized from petroleum ether to yield **47** (38.5 g, 176 mmol, 78%), mp 82–83 °C. Anal. ($C_{10}H_{19}N_2OCl$) C, H, N, Cl.

**5-[2-[(*N*-Cyclohexyl-*N*-methylcarbamoyl)amino]ethoxy]-2-nitrobenzaldehyde (48).** A mixture of **16** (16.7 g, 100 mmol), **47** (27.3 g, 125 mmol), potassium carbonate (18.0 g, 130 mmol), and sodium iodide (1.5 g, 10 mmol) in dry DMF (100 mL) was heated at 100 °C overnight. The mixture was cooled, poured into ethyl acetate (500 mL), and washed with water (3 × 200 mL), saturated sodium carbonate (4 × 200 mL), 1 M HCl (2 × 200 mL), and brine (2 × 200 mL) and then was dried, filtered, and evaporated. Chromatography of the residue over silica gel (0–25% gradient of ethyl acetate in dichloromethane) afforded **48** (9.5 g, 27 mmol, 27%) after crystallization from ether–petroleum ether, mp 81–82 °C. Anal. ($C_{17}H_{23}N_3O_5$) C, H, N.

**5-[3-(1-Cyclohexyl-5-tetrazolyl)propoxy]-2-nitrobenzaldehyde (52).** Alkylation of **16** (8.35 g, 50 mmol) with 5-(3-chloropropyl)-1-cyclohexyltetrazole[33] (14.3 g, 62.5 mmol) under conditions used for the preparation of **48** afforded **52** (11.8 g, 32.8 mmol, 66%) after silica gel chromatography (0–10% ethyl acetate in dichloromethane) and crystallization from ether–ethyl acetate, mp 84–85 °C. Anal. ($C_{17}H_{21}N_5O_4$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-3,5-dioxo-imidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (55).** A suspension of anthranilic acid **54**[4] (0.50 g, 1.5 mmol) in ethanol (10 mL) was treated with an ethanolic solution of freshly prepared 2-(methylthio)hydantoin[35] (3.4 mmol). The dark mixture was

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(59) The large volume prevents the precipitation of the insoluble intermediate formed upon monoreduction of the diacid.

heated and maintained at reflux for 3 h, then cooled, diluted with water, and triturated to give 55 (620 mg, 1.5 mmol, 100%), mp 222–224 °C after recrystallization from methanol–dichloromethane. Anal. ($C_{21}H_{26}N_4O_4·0.5H_2O$) C, H, N.

**N-Cyclohexyl-N-methyl-4-[(1,2,3,5-tetrahydro-5-oxo-imidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (57).** A mixture of isatoic anhydride 56[4] (1.08 g, 3 mmol) and 2-(ethylthio)imidazoline[37] (0.43 g, 3.3 mmol) in DMF (10 mL) was immersed in an oil bath preheated to 100 °C. Dry nitrogen was bubbled through the mixture from a side arm, and the DMF was returned via a condenser. After 3 h, the mixture was cooled and partitioned between ethyl acetate and water (50 mL each). The organic phase was washed with saturated sodium bicarbonate (2 × 50 mL) and water (50 mL). The combined aqueous layers were backwashed with ethyl acetate (50 mL). The combined organic layers were washed with brine (2 × 50 mL), dried, filtered, and evaporated to give a yellow-tan foam. The residue was crystallized from ethyl acetate–ether to afford 57 (380 mg, 1 mmol, 33%), mp 145–146 °C. Anal. ($C_{21}H_{28}N_4O_3$) C, H, N.

**N-Cyclohexyl-N-methyl-4-[3-(azidomethyl)-4-nitrophenoxy]butyramide (59).** A solution of methanesulfonyl chloride (8.2 mL, 105 mmol) in THF (100 mL) was added dropwise to a chilled solution of 58[4] (35 g, 100 mmol) and triethylamine (15.3 mL, 110 mmol) in THF (700 mL) with mechanical stirring to give a thick precipitate of triethylamine hydrochloride. Sodium azide (7.2 g, 110 mmol) in water (100 mL) was added, and the resulting clear solution was heated at 50 °C for 1 h. The reaction mixture was evaporated to remove THF, and the residue was extracted with ethyl acetate (2 × 200 mL). The organic extract was washed with water (2 × 100 mL), 1 M HCl (2 × 100 mL), and brine (2 × 100 mL) and then was dried, filtered, and evaporated to give an amber oil. Crystallization from ether–petroleum ether afforded 59 (36 g, 96 mmol, 96%), mp 63–64 °C. Anal. ($C_{18}H_{25}N_5O_4$) C, H, N.

**N-Cyclohexyl-N-methyl-4-[(1,2,3,4-tetrahydro-2-thioxoquinazolin-6-yl)oxy]butyramide (61).** A solution of 59 (11.3 g, 30 mmol) in THF (100 mL) was hydrogenated over 10% Pd–C (1.0 g) overnight. The solution of diamine 60 was filtered and treated with solid 1,1'-thiocarbonyldiimidazole (5.35 g, 30 mmol) in small portions over 6 h. The reaction mixture was concentrated to half-volume, and ether was added to crystallize the product 61 (10.5 g, 29 mmol, 97%), mp 212–213 °C. Anal. ($C_{19}H_{27}N_3O_2S$) C, H, N, S.

**N-Cyclohexyl-N-methyl-4-[(1,2,3,5-tetrahydro-3-oxo-imidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (62).** A suspension of 61 (3.61 g, 10 mmol) in THF (150 mL) was treated with methyl iodide (10 mL). After 3 h at room temperature, the resulting solution was evaporated and dried at high vacuum to give a foam, which was directly treated with glycine methyl ester hydrochloride (3.75 g, 30 mmol) and N,N-diisopropylethylamine (15 mL, 90 mmol) in acetonitrile (100 mL). After 24 h at reflux, the reaction mixture was cooled and filtered, and the pH was adjusted to 7 with 6 M HCl to give a precipitate of 62 (1.25 g, 3.25 mmol, 33%) as an amorphous solid. Anal. ($C_{21}H_{28}N_4O_3·1.75H_2O$) C, H; N: Calcd, 13.47; found, 13.00.

**N-Cyclohexyl-N-methyl-4-[(1,2,3,5-tetrahydro-2,3-dioxo-imidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (63).** A solution of 59 (4.7 g, 12.5 mmol) in absolute ethanol (100 mL) was hydrogenated over 10% Pd–C (500 mg) at 60 psi overnight. The solution of diamine 60 was filtered and directly treated with cyanogen bromide (1.46 g, 13.75 mmol). After stirring overnight, the solution was evaporated, and the crude guanidinium hydrobromide was dissolved in chloroform (150 mL) and was treated sequentially with N,N-diisopropylethylamine (6.66 mL, 37.5 mmol) and ethyl oxalyl chloride (1.4 mL, 12.5 mmol). The mixture was stirred overnight and was then diluted with ether to give a pale yellow precipitate, collected by filtration and dried to afford 63 (3.13 g, 7.8 mmol, 63%), mp 233–234 °C. Anal. ($C_{21}H_{26}N_4O_4·1.47H_2O$) C, H, N.

**N-Cyclohexyl-N-methyl-4-[(1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (66).** Reductive amination of 64 (17.4 g, 50 mmol) with ethanolamine hydrochloride (11.7 g, 120 mmol), sodium acetate (8.4 g, 100 mmol), and sodium cyanoborohydride (1.89 g, 30 mmol), followed by reduction ($H_2$/Pd–C) and treatment with cyanogen bromide (5.83 g, 55 mmol) by procedures analogous to those used previously,[4] afforded

crude 65 as a foam. The residue was dissolved in DMF (100 mL) and was treated with methyltriphenoxyphosphonium iodide (Fluka; 22.6 g, 50 mmol). After the mixture was stirred at room temperature overnight, triethylamine (20 mL, 275 mmol) was added. The reaction mixture was stirred at room temperature for 3 days and then was evaporated to a thick syrup. The residue was diluted with dichloromethane (100 mL) and was poured into ethyl acetate (1 L). The solution was washed with water (5 × 500 mL), 1 M NaOH (3 × 500 mL), and brine (2 × 500 mL) and was then dried, filtered, and evaporated. Crystallization of the residue from ethyl acetate afforded 66 (1.65 g, 4.45 mmol, 9%), mp 146–147 °C. Anal. ($C_{21}H_{30}N_4O_2$) C, H, N.

**Ethyl 4-(2-Chloro-3-formyl-4-nitrophenoxy)butyrate (67).** Alkylation of 2-chloro-3-hydroxy-6-nitrobenzaldehyde[40,60] (30.2 g, 150 mmol) with ethyl 4-bromobutyrate (23.6 mL, 165 mmol) and potassium carbonate (24.8 g, 180 mmol) in DMF (200 mL) according to the usual procedure[4] gave 67 (38 g, 120 mmol) after crystallization from ether, mp 69–70 °C. Anal. ($C_{13}H_{14}ClNO_6$) C, H, N, Cl.

**4-(2-Chloro-3-formyl-4-nitrophenoxy)butyric Acid (68).** A solution of 67 (35 g, 110 mmol) in 6 N HCl/dioxane (150 mL each) was heated at reflux for 2 h and was then cooled and extracted with ethyl acetate (3 × 200 mL). The organic layer was washed with brine (2 × 200 mL) and then was dried, filtered, and evaporated. Crystallization of the residue from ether gave 68 (26.0 g, 90.4 mmol, 82%), mp 128–129 °C. Anal. ($C_{11}H_{10}ClNO_6$) C, H, N, Cl.

**N-Cyclohexyl-N-methyl-4-(2-chloro-3-formyl-4-nitrophenoxy)butyramide (69).** Acid 68 (23.0 g, 80 mmol) was converted by method A (vide supra) to 69 (27.0 g, 70.5 mmol, 88%), mp 135–136 °C. Anal. ($C_{18}H_{23}ClN_2O_5$) C, H, N, Cl.

**N-Cyclohexyl-N-methyl-4-[(6-chloro-1,2,3,5-tetrahydro-2-oxoimidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (71).** Reductive amination of 69 (24.9 g, 65 mmol) with glycine methyl ester and sodium cyanoborohydride using the procedure for 1[4] afforded crude 70. A solution of 70 (28 g) and nickel(II) chloride hexahydrate (30.9 g, 130 mmol) in methanol (500 mL) cooled to −10 °C was treated portionwise with solid sodium borohydride (5.0 g). The cold reaction was acidified with 6 M HCl and then was made basic by the addition of concentrated ammonium hydroxide. The mixture was extracted with ethyl acetate (4 × 200 mL), and the resulting organic layer was washed with saturated sodium bicarbonate (4 × 100 mL) and brine (2 × 100 mL) and then dried, filtered, and evaporated. The residue containing the diamine was dissolved in absolute ethanol (350 mL) and was treated with cyanogen bromide (7.58 g, 71.5 mmol). After stirring at room temperature overnight, the solution was treated with ammonium hydroxide to afford 71 (4.80 g, 11.5 mmol, 18%), mp 244–245 °C. Anal. ($C_{21}H_{27}N_4O_3Cl·0.25H_2O$) C, H, N, Cl.

**Analogues Substituted at Position 3 (72–78, 83, 84).** Substitution of the apropriate L- or D-α-amino acid methyl ester hydrochloride salts for glycine ethyl ester hydrochloride in the reductive amination of 64, followed by hydrogenation and treatment with cyanogen bromide, afforded 3-substituted analogues 72–78, 83, and 84 (Table V). Attempts to use either methyl asparaginate or dimethyl aspartate in this sequence did not give the desired 80 or 81, respectively; both resulted in formation of 79, mp 130–131 °C. Anal. ($C_{24}H_{32}N_4O_5·0.75H_2O$) C, H, N.

**N-Cyclohexyl-N-methyl-4-[(1,3,4,6-tetrahydro-2-oxo-2H-pyrimido[2,1-b]quinazolin-8-yl)oxy]butyramide (82).** Reductive amination of 64 (12.2 g, 35 mmol) with β-alanine methyl ester and sodium cyanoborohydride, followed by reduction over 10% Pd on C and treatment with cyanogen bromide and base as above, gave 82 (0.54 g, 1.36 mmol, 4%), mp 171–173 °C. Anal. ($C_{22}H_{30}N_4O_3·0.5H_2O$) C, H, N.

**N-Cyclohexyl-N-methyl-4-[(3-methylene-1,2,3,5-tetrahydro-2-oxoimidazo[2,1-b]quinazolin-7-yl)oxy]butyramide (85).** A clear solution of 75a (4.14 g, 10 mmol) in freshly distilled pyridine (500 mL) cooled to 0–5 °C was treated with methanesulfonyl chloride (0.9 mL, 11 mmol) followed in 30 min by N,N-diisopropylethylamine (7.12 mL, 40 mmol). The resulting solution

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(60) One of us (M.C.V.) thanks Dr. J. J. Plattner, Abbott Laboratories, for helpful suggestions regarding the nitration and subsequent isomer purification described in ref 40.

was stirred at ambient temperature for 3 h and then was evaporated. The residue was triturated with water, and the resulting precipitate was collected by filtration to yield **85** (3.25 g, 8.16 mmol, 82%), mp 176–178 °C. Anal. ($C_{22}H_{28}N_4O_3$) C, H, N.

*N*-Cyclohexyl-*N*-methyl-4-[3-(1-hydroxyethyl)-4-nitrophenoxy]butyramide (88). A solution of chlorotitanium(IV) triisopropoxide (Aldrich; 14.3 mL, 60 mmol) in ether (100 mL) cooled to −45 °C was treated with methyllithium (35 mL, 1.7 M, 60 mmol) dropwise over 20 min. The mixture was allowed to warm to ambient temperature for 30 min and then was added to a solution of **64** (17.4 g, 50 mmol) in THF (300 mL). After 3 h at room temperature, the reaction mixture was concentrated, and the residue was partitioned between ethyl acetate (300 mL) and 1 M HCl (200 mL). The organic extract was washed with 1 M HCl (3 × 200 mL) and brine (2 × 200 mL) and then was dried, filtered, and evaporated at high vacuum to afford **88** (18.5 g, 50 mmol, 100%) as an amorphous foam. Anal. ($C_{19}H_{28}N_2O_5$) C, H, N.

*N*-Cyclohexyl-*N*-methyl-4-(3-acetyl-4-nitrophenoxy)-butyramide (89). Oxidation of **88** (16.4 g, 45 mmol) using pyridinium dichromate (18.6 g, 49.5 mmol) in dichloromethane according to the procedure used for **36** afforded **89** (13.5 g, 37.3 mmol, 83%) as a thick syrup. Anal. ($C_{19}H_{26}N_2O_5 \cdot 0.5H_2O$) C, H, N.

*N*-Cyclohexyl-*N*-methyl-4-[3-(1-chloroethyl)-4-nitrophenoxy]butyramide (90). A solution of **88** (27.3 g, 75 mmol), *p*-toluenesulfonyl chloride (17.2 g, 90 mmol), and 4-(dimethylamino)pyridine (10.0 g, 82.5 mmol) in dry acetonitrile (100 mL) was stirred at ambient temperature overnight. The resulting mixture was poured into ether (500 mL), and the solution was washed with water (4 × 100 mL), saturated sodium bicarbonate (3 × 100 mL), 1 M HCl (3 × 100 mL), and brine (2 × 100 mL). The organic layer was dried, filtered, and evaporated to a thick syrup. Chromatography over silica gel (dichloromethane as eluant) afforded **90** (20.1 g, 52 mmol, 70%) as a thick syrup. Anal. ($C_{19}H_{27}N_2O_4Cl \cdot 0.05CH_2Cl_2 \cdot 0.25H_2O$) C, H, N, Cl.

*N*-Cyclohexyl-*N*-methyl-4-[(5-methyl-1,2,3,5-tetrahydro-2-oxoimidazo[2,1-*b*]quinazolin-7-yl)oxy]butyramide (92). A mixture of **90** (9.6 g, 25 mmol), glycine ethyl ester[61] (12.9 g, 125 mmol), *N*,*N*-diisopropylethylamine (5.4 mL, 31 mmol), and sodium iodide (0.38 g, 2.5 mmol) in absolute ethanol (50 mL) was heated at reflux for 48 h. The mixture was cooled and partitioned between ethyl acetate and saturated sodium bicarbonate (200 mL each). The organic extract was washed with saturated sodium bicarbonate (4 × 100 mL) and brine (2 × 100 mL) and then was dried, filtered, and evaporated. Chromatography of the residue over silica gel (0–2.5% methanol in dichloromethane) afforded crude **91** (12.0 g).

A solution of **91** in ethanol (100 mL) was hydrogenated over 10% Pd–C (1.0 g) at 60 psi overnight. The mixture was filtered, treated with cyanogen bromide (2.92 g, 27.5 mmol), and stirred 24 h. Addition of ammonium hydroxide did not afford a precipitate. The reaction mixture was concentrated and dissolved in ethyl acetate (3 × 100 mL). The organic layer was washed with 1 M ammonium hydroxide (3 × 100 mL) and brine (2 × 100 mL) and then was dried, filtered, and evaporated. Chromatography over silica gel (0–10% methanol in dichloromethane) followed by crystallization from dichloromethane–ether gave **92** (2.55 g, 6.4 mmol, 26%), mp 162–164 °C. Anal. ($C_{22}H_{30}N_4O_3$) C, H, N.

*N*-Cyclohexyl-*N*-methyl-4-[(1,2,3,5-tetrahydro-2-oxotriazolo[3,2-*b*]quinazolin-7-yl)oxy]butyramide (95). A mixture of **64** (12.9 g, 35 mmol) and methyl carbazate (3.78 g, 42 mmol) in methanol (100 mL) containing 1 M HCl (1 drop) was heated briefly on the steam bath. Cooling, dilution with water (50 mL), filtration, and drying afforded **93** (13.9 g, 33 mmol, 94%), mp 81–82 °C. Anal. ($C_{20}H_{28}N_4O_6 \cdot H_2O$) C, H, N. A suspension of **93** (10.5 g, 25 mmol) in ethanol (150 mL) was hydrogenated over 10% Pd–C at 60 psi. The mixture was filtered, treated with cyanogen bromide (2.92 g, 27.5 mmol), and stirred overnight. Thorough evaporation of the solution, followed by reevaporation

from methanol, gave **94** as an amorphous foam. Anal. ($C_{21}H_{32}N_5O_4Br \cdot 0.5H_2O$) C, H, N, Br. A solution of **94** (6.3 g, 12.5 mmol) and sodium methoxide (3.4 g, 62.5 mmol) in methanol (250 mL) was heated at reflux for 4 h. The solution was diluted with 0.5 M HCl (200 mL) to afford a precipitate, collected by filtration and dried to yield **95** (4.20 g, 88% from **94**), mp 144–145 °C. Anal. ($C_{20}H_{27}N_5O_3 \cdot H_2O$) C, H, N.

**Methylation of 1.** A suspension of **1** (6.73 g, 17.5 mmol) in methanol (250 mL) was warmed on the steam bath and was treated with sodium hydroxide (1.4 g) in water (10 mL). After 10 min, the clear solution was cooled and poured into ether (1 L) to give a precipitate of **1**·Na salt (6.28 g, 14.5 mmol, 83%), mp 184–185 °C. Anal. ($C_{21}H_{27}N_4O_3Na \cdot 1.5H_2O$) C, H, N. A mixture of the above salt and methyl iodide (1.25 mL, 20 mmol) in DMF (150 mL) was stirred at ambient temperature overnight. The reaction mixture was evaporated and chromatographed over silica gel (0–5% methanol in dichloromethane). Crystallization of the two isolated products from dichloromethane–ether afforded N-methylated analogue **96** (1.4 g, 3.5 mmol, 20%), mp 146–147 °C (anal. ($C_{22}H_{30}N_4O_3$) C, H, N), and O-methylated analogue **97** (1.25 g, 3.1 mmol, 18%), mp 164–165 °C (anal. ($C_{22}H_{30}N_4O_3$) C, H, N).

**Registry No.** 1, 94192-59-3; 4, 94193-36-9; 5a, 105695-06-5; 5a (amine), 74-89-5; 5b, 105695-07-6; 5b (amine), 124-40-3; 5c, 105695-08-7; 5c (amine), 108-91-8; 5d, 105695-09-8; 5d (amine), 5459-93-8; 5e, 105695-10-1; 5e (amine), 1195-42-2; 5f, 105695-11-2; 5f (amine), 2439-56-7; 5g, 105695-12-3; 5g (amine), 42870-65-5; 5h, 105695-13-4; 5h (amine), 40221-52-1; 5 i, 105695-14-5; 5i (amine), 100-61-8; 5j, 94193-41-6; 5j (amine), 103-67-3; 5k, 94193-42-7; 5k (amine), 103-49-1; 5l, 94193-40-5; 5l (amine), 101-83-7; 5m, 94193-43-8; 5m (amine), 14683-47-7; 6a, 94193-44-9; 6a (amine), 110-91-8; 6b, 94193-45-0; 6b (amine), 110-89-4; 6c, 94193-46-1; 6c (amine), 123-75-1; 6d, 94193-47-2; 6d (amine), 635-46-1; 6e, 94193-48-3; 6e (amine), 91-21-4; 6f, 105695-15-6; (±)-*trans*-6g, 105695-16-7; (±)-*trans*-6g (amine), 105728-23-2; 6h, 105695-17-8; 6h (amine), 6621-47-2; 7a, 94193-39-2; 7a (amine), 2842-38-8; 7b, 105695-18-9; 7b (amine), 31121-12-7; 7c, 105695-19-0; 7c (amine), 78345-58-1; 7d, 105695-20-3; 7d (amine), 6951-34-4; 7e, 105695-21-4; 7e (amine), 105728-24-3; 7f, 105695-22-5; 7f (amine), 105728-25-4; *trans*-7g, 105695-23-6; *trans*-7g (amine), 22348-44-3; 8a, 99694-61-8; 8a (amine), 40447-13-0; 8b, 99694-62-9; 8b (amine), 83808-20-2; 8c, 105695-24-7; 8c (amine), 105728-26-5; 8d, 105695-25-8; 8d (amine), 55611-82-0; 8e, 105695-26-9; 8e (amine), 105728-27-6; 9a, 94193-35-8; 9a (alcohol), 64-17-5; 9b, 105695-27-0; 9b (alcohol), 108-93-0; 10a, 105695-28-1; 10b, 105695-29-2; 10c, 94193-04-1; 10d, 94192-74-2; 10e, 94192-75-3; 10f, 94192-73-1; 10g, 94192-63-9; 10h, 94192-72-0; 10i, 94192-61-7; 10j, 94192-65-1; 10k, 94192-66-2; 10l, 94192-67-3; 10m, 94192-78-6; 10n, 105695-30-5; 10, 94192-68-4; 11b, 94192-69-5; 11c, 94192-70-8; 11d, 94192-76-4; 11e, 94192-77-5; 11f, 105695-31-6; (±)-*trans*-11g, 105814-23-1; 11h, 94192-71-9; 12a, 94192-60-6; 12b, 105695-32-7; 12c, 105695-33-8; 12d, 105695-34-9; 12e, 105695-35-0; 12f, 105695-36-1; *trans*-12g, 105695-37-2; 13a, 99694-55-0; 13:, 99694-56-1; 13c, 94192-62-8; 13d, 94192-64-0; 13e, 105695-38-3; 13f, 99694-57-2; 14a, 94193-54-1; 14b, 105695-39-4; 14c, 94193-55-2; 15a, 94193-00-7; 15b, 94193-01-8; 15c, 94193-06-3; 15d, 94219-49-5; 16, 42454-06-8; 17a, 105728-02-7; 17b, 105728-03-8; 17c, 105728-04-9; 17d, 105728-05-0; 18a, 105728-06-1; 18b, 105728-07-2; 18c, 105728-08-3; 18d, 105728-09-4; 19a, 105728-10-7; 19b, 105728-11-8; 19c, 105728-12-9; 19d, 105728-13-0; 20a, 105695-40-7; 20b, 94192-82-2; 20c, 94192-83-3; 20d, 94219-47-3; 22a, 105728-14-1; 22b, 105728-15-2; 22c, 105728-16-3; 23a, 105728-17-4; 23b, 105728-18-5; 23c, 105728-19-6; 24a, 105728-20-9; 24b, 105728-21-0; 24c, 105728-22-1; 25a, 94192-79-7; 25b, 94219-46-2; 25c, 94192-80-0; 26, 13280-60-9; 26 (diazonium tetrafluoroborate), 105728-30-1; 27, 35674-28-3; 28, 105728-31-2; 29, 105728-32-3; 30, 105728-33-4; 31,

(61) Free base prepared from hydrochloride by treatment with ammonia in chloroform followed by distillation according to: Hillmann, G. *Z. Naturforsch.* 1946, *1*, 682; *Methoden Org. Chem. (Houben-Weyl)*, 15 (I), 333.

# 1-Aryl-2-(aminomethyl)cyclopropanecarboxylic Acid Derivatives.  A New Series of Potential Antidepressants

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A series of 1-aryl-2-(aminomethyl)cyclopropanecarboxylic acid derivatives were synthesized and evaluated as potential antidepressants. Compounds with the Z configuration were synthesized from 1-aryl-2-oxo-3-oxabicyclo[3.1.0]hexane and those with the E configuration from (*E*)-1-phenyl-2-(hydroxymethyl)cyclopropanecarboxylic acid. The compounds were evaluated in animal tests designed to reveal potential antidepressant activity and the existence of undesirable side effects. Several derivatives were more active than imipramine and desipramine. On the basis of its activity in pharmacological animal tests of antidepressant activity and its potential lack of side effects, 1-phenyl-1-[(di-ethylamino)carbonyl]-2-(aminomethyl)cyclopropane hydrochloride, midalcipran (INN), was selected for further development. This compound is currently in phase III clinical evaluation.

Antidepressant drugs suffer from two main disadvantages in addition to their eventual side effects and toxicity. No antidepressant studied so far has been shown to be active in more than 60–70% of the cases of major depressive disorders and all antidepressants require 10–20 days administration (depending on the criteria used) before any therapeutic benefit is seen.[1]  Thus even if the more recent second and third generation antidepressants, such as mianserin[2] and fluoxetine,[3] have less side effects than the tricyclic antidepressants, they still suffer from these drawbacks.  In an attempt to find a new structural prototype for antidepressant therapy, we have studied a series of bifunctional cyclopropane derivatives,[4,5] some of which

**Scheme I.** Synthesis of (*Z*)-γ-Amino Acids
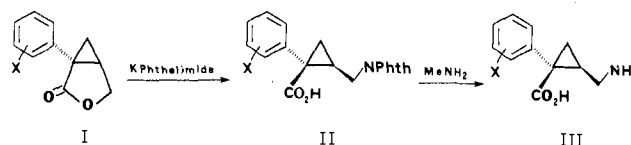


have interesting potential antidepressant profiles.

We describe here the structure–activity relationship of this series of compounds, based on variations in the phenyl ring and substitution on the amine and carboxylic acid functions.

## Chemistry

(*Z*)-γ-**Amino Carboxylic Acids and Esters (Schemes I and II).**   Amino acid III was obtained by reaction of lactone I with potassium phthalimide in di-

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