Inhibitors of Blood Platelet cAMP Phosphodiesterase. 3. 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one Derivatives with Enhanced Aqueous Solubility[1,2]

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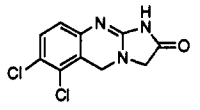
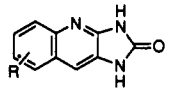
Two series of 1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one derivatives incorporating an additional site for acid salt formation were synthesized and evaluated as inhibitors of human blood platelet cAMP phosphodiesterase (PDE) and ADP-induced platelet aggregation. The objective of this study was to identify compounds that blended potent biological activity with a satisfactory level of aqueous solubility. From a series of 7-aminoimidazo[4,5-*b*]quinolin-2-ones, biological and physical properties were optimally combined in the 1-piperidinyl derivative **11c**. However, this compound offered no significant advantage over earlier studied compounds as an antithrombotic agent in an animal model of small vessel thrombosis. A series of 7-alkoxy alkanoic piperazinamide derivatives, in which the additional basic nitrogen atom was remote from the heterocyclic nucleus and accommodated in a secondary binding region of the cAMP PDE enzyme, demonstrated greater intrinsic cAMP PDE inhibitory activity. Structural modifications of this series focused on variation of the piperazine substituent and side-chain length. The lipophilicity of the N-substituent influenced biological potency and aqueous solubility, with substituents of seven carbon atoms or less generally providing acceptable solubility properties. The *N*-(cyclohexylmethyl)piperazinamide **21h** was identified from this series of compounds as a potent inhibitor of platelet cAMP PDE, IC_{50} = 0.4 nM, and ADP-induced platelet aggregation, IC_{50} = 0.51 μM after a 3-min exposure and 0.1 μM after a 15-min exposure of platelet-rich plasma to the drug. Evaluation of **21h** and representative analogues in vivo using a rabbit model of small vessel thrombosis revealed significantly greater antithrombotic efficacy compared to that of previously studied compounds with similar intrinsic biological activity measured in vitro but inferior aqueous solubility.

Introduction

For some time, we have been engaged in an effort to develop 1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one derivatives (1), potent and selective inhibitors of blood platelet low K_m, cGMP-inhibited cAMP phosphodiesterase (PDE), as agents for the prevention and treatment of thrombotic and thromboembolic phenomena.[2,3] Many derivatives of 1 have been identified as highly potent inhibitors of platelet function in vitro and several have demonstrated efficacy in animal models of thrombosis. Some of the more interesting compounds to emerge from this initiative are BMY 20844[4,5] (2), BMY 21259[2] (3), and BMY 21638[5,6] (4). However, a recurring problem associated with this structural class has been the generally poor and variable oral bioavailability. This is most probably a consequence of the physical properties of 1, which are generally isolated as high-melting solids with limited solubility in common organic solvents, water, and, more importantly, physiological media. However, the structural features of 1 that are responsible for these undesirable properties are also of paramount importance for high-affinity interaction with the active site of the cAMP PDE isozyme.[7] In an effort to provide compounds with en-



Anagrelide



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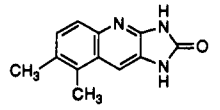

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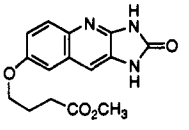

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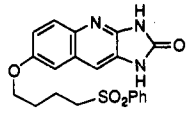

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(1) Presented in part at the 201st National Meeting of the American Chemical Society, Atlanta, GA, April 21-26, 1991: Meanwell, N. A.; Rosenfeld, M. J.; Wright, J. J.; Fleming, J. S.; Gillespie, E.; Seiler, S. M.; Brassard, C. L. Imidazo[4,5-*b*]-quinolin-2-one Derivatives with Enhanced Aqueous Solubility: Potent Inhibitors of Platelet Low K_m cAMP Phosphodiesterase and Induced Aggregation; MEDI 60.

(2) For part 2 see: Meanwell, N. A.; Pearce, B. C.; Roth, H. R.; Smith, E. C. R.; Wedding, D. L.; Wright, J. J. K.; Buchanan, J. O.; Baryla, U. M.; Fleming, J. S.; Gamberdella, M.; Gillespie, E.; Hayes, D. C.; Seiler, S. M.; Stanton, H. C.; Zavoico, G. B. Inhibitors of Blood Platelet cAMP Phosphodiesterase. 2. Structure-Activity Relationships Associated with Imidazo-[4,5-*b*]quinolin-2-ones Substituted with Functionalized Side Chains. *J. Med. Chem.*, preceding paper in this issue.

(3) Meanwell, N. A.; Roth, H. R.; Smith, E. C. R.; Wedding, D. L.; Wright, J. J. K.; Fleming, J. S.; Gillespie, E. 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones—Inhibitors of Blood Platelet cAMP Phosphodiesterase and Induced Aggregation. *J. Med. Chem.* **1991**, *34*, 2906-2916.

(4) Buchanan, J. O.; Fleming, J. S.; Cornish, B. T.; Baryla, U. M.; Gillespie, E.; Stanton, H. C.; Seiler, S. M.; Keely, S. L. Pharmacology of a Potent, New Antithrombotic Agent, 1,3-Dihydro-7,8-dimethyl-2*H*-imidazo[4,5-*b*]quinolin-2-one (BMY 20844). *Thromb. Res.* **1989**, *56*, 333-346.

(5) Seiler, S. M.; Gillespie, E.; Arnold, A. J.; Brassard, C. L.; Meanwell, N. A.; Fleming, J. S. Imidazoquinoline Derivatives: Potent Inhibitors of Platelet cAMP Phosphodiesterase which Elevate cAMP Levels and Activate Protein Kinase in Platelets. *Thromb. Res.* **1991**, *62*, 31-42.

(6) Fleming, J. S.; Buchanan, J. O.; Baryla, U. M.; Seiler, S. M.; Meanwell, N. A.; Gillespie, E.; Keely, S. L. BMY 21638, A New, Potent Broad-Spectrum Inhibitor of Blood Platelet Aggregation and Experimental Thrombosis. *Thromb. Haemostasis* **1989**, *62*, 409.

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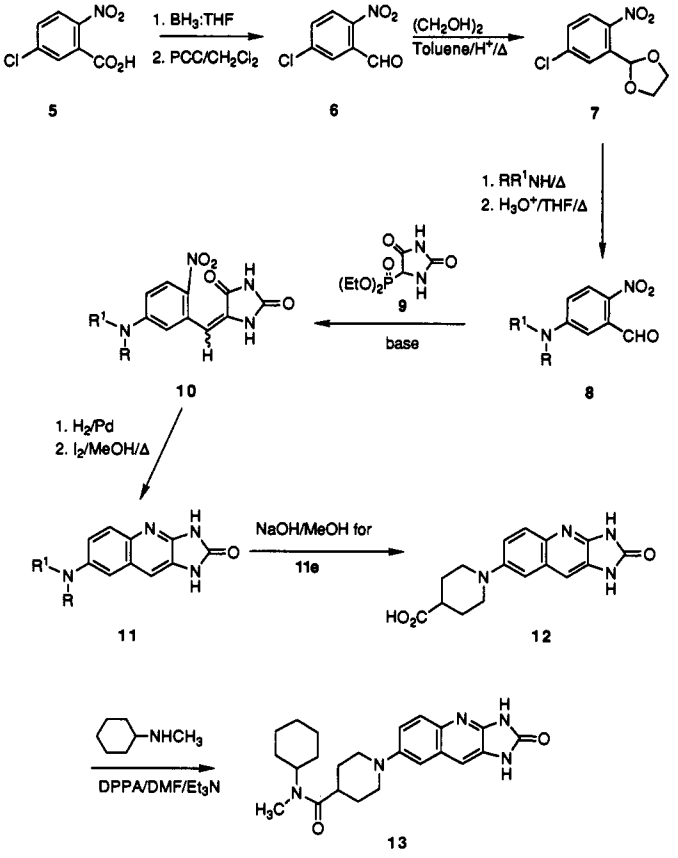
hanced solubility and more predictable oral absorption, we explored several different avenues for modifying the physical properties of 1. Although a prodrug approach, involving derivatization of N-1 and/or N-3 of **3** and **4**, provided some improvement in oral bioavailability,[8] a molecule with increased aqueous solubility would also provide a drug with broader potential. In this report we describe successful efforts to identify derivatives of 1 that combine a high degree of biological activity with enhanced aqueous solubility.

Chemistry

The most logical approach to increase the aqueous solubility of imidazo[4,5-*b*]quinolin-2-one derivatives appeared to be by introduction of a second site for salt formation within the molecule. Initial studies focused on the introduction of a substituted basic nitrogen atom bound directly to the heterocyclic ring nucleus and the 7-position was considered to be an appropriate site for substitution based on the seminal SAR studies reported earlier.[2,3] 7-Aminoimidazo[4,5-*b*]quinolin-2-one derivatives were obtained by the procedures outlined in Schemes I and II. Reduction of the commercially available benzoic acid **5** with BH_3–THF complex followed by oxidation of the alcohol with PCC[9] provided aldehyde **6**, which was derivatized with ethylene glycol to give acetal **7** as shown in Scheme I. Heating **7** with an excess of an amine was followed by hydrolytic removal of the protecting group to provide aldehyde **8**. Attempts to effect displacement of the chlorine atom in earlier intermediates were not successful. Tricyclic ring construction, following the previously developed protocol,[3] began with a Wadsworth–Emmons-type reaction of aldehyde **8** with phosphonate **9**[10] to give adduct **10**, generally isolated as a mixture of geometrical isomers. Exhaustive catalytic hydrogenation of **10** followed by cyclization with concomitant oxidation, induced by I_2 in MeOH at reflux, provided the target compounds **11**. The isonipecotate ester **11e** was hydrolyzed with aqueous alkali to give acid **12**, which was coupled with *N*-methylcyclohexylamine, using DPPA[11] in DMF, to furnish amide **13**.

Scheme I



Heating **7** with an excess of piperazine provided amine **14**, which was derivatized with the appropriate acyl halide and then deprotected to furnish aldehyde **15**, as outlined in Scheme II. Elaboration of **15** to the imidazo[4,5-*b*]-quinolin-2-one heterocycle system followed the standard ring-forming sequence to provide the target compounds **16**. Combination of **7** with 1-phenylpiperazine followed by acid-catalyzed unmasking of the aldehyde provided **17** which was transformed into the target compound **18** in conventional fashion.

The 7-amino derivatives prepared by the methods outlined in Schemes I and II are presented in Table I along with associated physical properties.

Introduction of a basic amine moiety several atoms remote from the heterocyclic nucleus, where it would be accommodated in a secondary binding region of the enzyme,[12,13] was also explored as an approach to identify compounds with good aqueous solubility. Coupling of acids **19**[2] with various diamines typically proceeded with high efficiency using DPPA[2,11] in DMF to furnish amides **21**, generally isolated as the hydrochloride derivatives (Scheme III). However, this procedure failed to provide amines **21an–ap** and **21ar**, and an alternative approach that entailed heating esters **20**[2] with an excess of amine at 200 °C was employed. Dissolution of ^{t}Boc derivative **21a** in CF_3CO_2H followed by evaporation of the excess acid

(7) (a) Rakhit, S.; Marciniak, G.; Leclerc, G.; Schwarz, J. Computer Assisted Pharmacophore Search in a Series of Nonsteroidal Cardiotonics. *Eur. J. Med. Chem.* **1986**, *21*, 511–515. (b) Davis, A.; Warrington, B. H.; Vinter, J. G. Strategic Approaches to Drug Design. II. Modelling Studies on Phosphodiesterase Substrates and Inhibitors. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 97–120. (c) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A.; McPhail, A. T. Cardiotonic Agents. 8. Selective Inhibitors of Adenosine 3′,5′-Cyclic Phosphate Phosphodiesterase III. Elaboration of a Five Point Model for Positive Inotropic Activity. *J. Med. Chem.* **1987**, *30*, 1963–1972. (d) Erhardt, P. W.; Hagedorn, A. A., III; Sabio, M. Cardiotonic Agents. 3. A Topographical Model of the Cardiac cAMP Phosphodiesterase Receptor. *Mol. Pharmacol.* **1988**, *33*, 1–13. (e) Erhardt, P. W.; Chou, Y-L. A Topographical Model for the cAMP Phosphodiesterase III Active Site. *Life Sci.* **1991**, *49*, 553–568.

(8) Meanwell, N. A.; Fleming, J. S.; Buchanan, J. O.; Russell, J. W., unpublished studies.

(9) Corey, E. J.; Suggs, J. W. Pyridinium Chlorochromate. An Efficient Reagent for Oxidation of Primary and Secondary Alcohols to Carbonyl Compounds. *Tetrahedron Lett.* **1975**, *31*, 2647–2650.

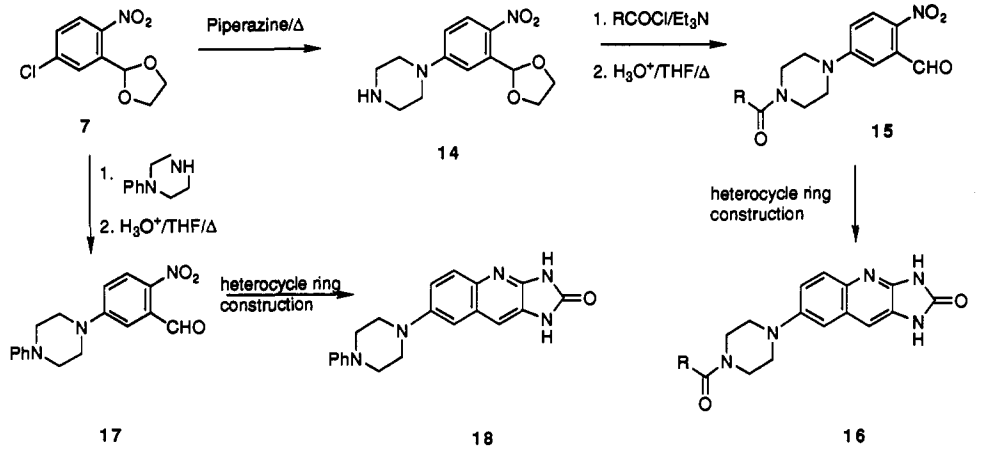
(10) Meanwell, N. A.; Roth, H. R.; Smith, E. C. R.; Wedding, D. L.; Wright, J. J. K. Diethyl 2,4-Dioxoimidazolidine-5-phosphonate: A Wadsworth–Emmons Reagent for the Mild and Efficient Preparation of C-5 Unsaturated Hydantoins. *J. Org. Chem.* **1991**, *56*, 6897–6904.

(11) Shioiri, Y.; Ninomiya, K.; Yamada, S. Diphenylphosphoryl Azide. A New Convenient Reagent for a Modified Curtius Reaction and for the Peptide Synthesis. *J. Am. Chem. Soc.* **1972**, *94*, 6203–6205.

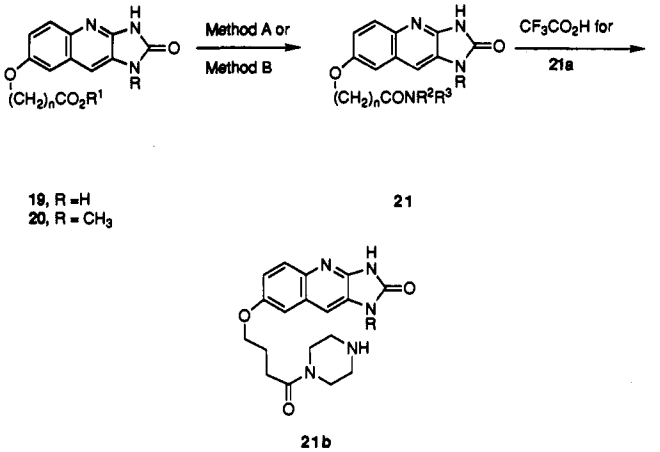
(12) Jones, G. H.; Venuti, M. C.; Alvarez, R.; Bruno, J. J.; Berks, A. H.; Prince, A. Inhibitors of Cyclic AMP Phosphodiesterase. 1. Analogues of Cilostamide and Anagrelide. *J. Med. Chem.* **1987**, *30*, 295–303.

(13) Venuti, M. C.; Jones, G. H.; Alvarez, R.; Bruno, J. J. 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). *J. Med. Chem.* **1987**, *70*, 303–318.

Scheme II

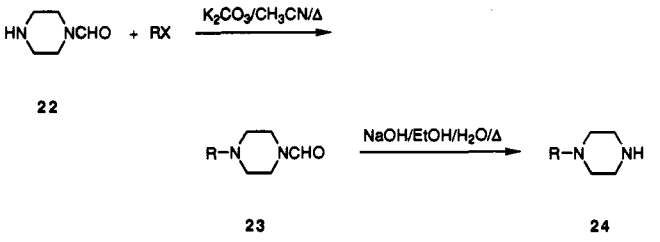


Scheme III[a]



[a] Method A: $R^2R^3NH/DPPA/DMF/Et_3N$/cat. DMAP for R = H. Method B: R^2R^3NH/200 °C for R = CH_3.

Scheme IV

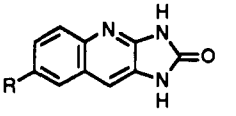


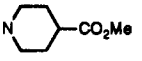
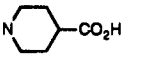
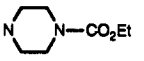
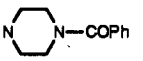
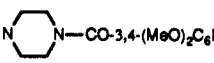
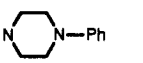
at reduced pressure provided the unsubstituted piperazinamide **21b**, isolated as its bis-trifluoroacetate salt. The amines required for this aspect of the study were obtained either from commercial sources or from 1-piperazinecarboxaldehyde (**22**) by way of alkylation, to give **23**, and deformylation, using aqueous hydroxide in EtOH, to provide piperazines **24** (Scheme IV).[14] For comparison purposes, the two imidazo[2,1-*b*]quinazolin-2-ones **26a** and **26b** were prepared by coupling acid **25**[13] with the appropriate piperazine, using DPPA in DMF, as depicted in Scheme V.

The compounds prepared as this part of the study were generally isolated as their dihydrochloride salts, which proved to be hygroscopic and difficult to crystallize. This hampered purification and the target compounds were

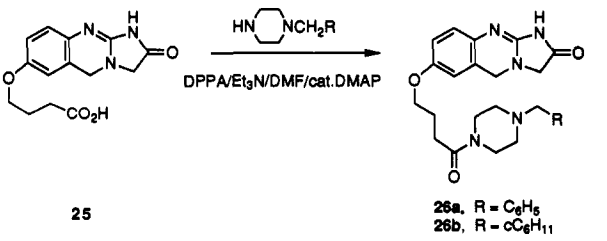
(14) Tricerri, S.; Elitropi, G.; Panto, E.; Jafolla, G.; Bianchetti, A.; Mazzi, L.; Riva, M. Non-anticholinergic Gastric Acid Secretion Inhibitors. New Piperazine and Related Derivatives. *Eur. J. Med. Chem.—Chim. Ther.* **1974**, *9*, 555–562.

Table I. Structure and Physical Properties of 7-Amino-1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one Derivatives



no.	R	mp, °C	mol formula (elem anal.)
11a	NEt_2	300–303 (dec)	$C_{14}H_{16}N_4O \cdot 0.15H_2O$
11b	1-pyrrolidinyl	>300	$C_{14}H_{14}N_4O \cdot 0.15DMF$
11c	1-piperidinyl	300 (dec)	$C_{15}H_{16}N_4O \cdot 2HCl \cdot 0.6H_2O$
11d	4-morpholinyl	>310	$C_{14}H_{14}N_4O_2 \cdot HCl \cdot 0.7H_2O$
11e		>310	$C_{17}H_{18}N_4O_3 \cdot 0.05H_2O$
12		>310	$C_{16}H_{16}N_4O_3 \cdot 0.25DMF$
13		318–321	$C_{23}H_{29}N_5O_2 \cdot 0.33DMF$
16a		>300	$C_{17}H_{19}N_5O_3$
16b		>300	$C_{21}H_{19}N_5O_2 \cdot 0.25 CH_3OH$
16c		285–287 (dec)	$C_{23}H_{23}N_5O_4 \cdot HCl \cdot 0.25H_2O$
18		350–355 (dec)	$C_{20}H_{19}N_5O \cdot 0.1H_2O$

Scheme V



generally isolated as amorphous solids after precipitation from an alcohol by the addition of ether. The target amides are compiled in Table II along with relevant analytical data.

Biological Evaluation

The target compounds were evaluated as inhibitors of cAMP hydrolysis by a human platelet sonicate as previ-

ously described.[5,15] The IC_{50}'s were determined from dose–response curves and are reported in Table III. In this assay, anagrelide was used as a control and displayed an IC_{50} of $5.4 \pm 1.4 \times 10^{-8}$ M. Platelet inhibitory activity was determined in human platelet-rich plasma (PRP) using ADP as the activating agent as previously described.[3,4] Dose–response curves were obtained, and the results are expressed as the concentration of drug necessary to prevent aggregation by 50% compared to drug-free controls. The data compiled in Table III are the result of a single determination or the average of duplicates. Anagrelide was employed as a positive reference agent in these evaluations and displayed an EC_{50} of 1.05 ± 0.3 μM vs ADP in human PRP. Aqueous solubility was determined by adding 0.5 mL of H_2O to 10 mg of test compound at room temperature and diluting up to 4-fold unless solution occurred. The results are included in Table III.

Results and Discussion

Within the series of 7-amino derivatives **11a–d**, potent biological activity and good aqueous solubility are optimally combined in piperidine **11c**, a pattern of substitution that also provided an effective blend of physical and pharmacological properties in a series of imidazo[2,1-*b*]-quinazolin-2-ones.[16] Of the relatively simple structural variants of **11c** explored, only morpholine analogue **11d** displays enhanced potency in both biological assays. However, this compound is poorly soluble in water, presumably because the weakly basic morpholino nitrogen atom is unable to support the formation of a stable hydrochloride salt.

The rigidity inherent in the piperidine ring of **11c** provided an opportunity to probe the boundaries of the cAMP PDE inhibitor pharmacophore and provide some insight into the relative location of the secondary binding region.[2,12,13] Functionalization at the 4-position of the piperidine ring of **11c** with a carbomethoxy substituent gave an ester (**11e**) 10-fold weaker in the cAMP PDE assay that exhibited improved platelet inhibitory properties, but at the expense of aqueous solubility. The corresponding acid, **12**, is equipotent with the parent compound **11c** in the cAMP PDE assay but over 30-fold less effective as an inhibitor of platelet aggregation, presumably reflecting reduced membrane permeability. This phenomenon was observed with several carboxylic acid derivatives in an earlier series.[2] A further increase in the size and lipophilicity of the 4-substituent led to an inferior cAMP PDE inhibitor, amide **13**, that does not significantly inhibit platelet aggregation at the highest concentration evaluated. This region of the pharmacophore was further explored with the series of piperazine derivatives **16a–c**. For this series, cAMP PDE inhibitory activity increases with the size of the N-substituent, but only incrementally, and this trend is reversed in the platelet aggregation assay. The N-phenylpiperazine **18** is the weakeest cAMP PDE inhibitor in this series of compounds and is an unimpressive inhibitor of platelet aggregation. Taken together, these data indicate limitations in the size of the substituent tolerated at the 7-position of the heterocycle by the cAMP PDE enzyme and suggest that the 4-position of the piperidine ring of **11c** does not approximate the secondary binding region of the enzyme. This site is known to tolerate quite large substituents[2,12,13] and the data presented here and previously[2] are consistent with the more linear bound conformation favored by Venuti.[13]

From the 7-amino series of compounds, piperidine **11c** was selected for evaluation in the biolaser model of micro vessel thrombosis, conducted in conscious rabbits.[4,17] Although **11c** provides dose-related protection in this model with 32% inhibition at 1 mg/kg po and 59% inhibition at 3 mg/kg, it offers minimal advantage over anagrelide (ED_{50} = 2 mg/kg) or BMY 20844 (2) (ED_{50} = 3 mg/kg) and the duration of effect is considerably shorter.[18]

The relatively poor level of in vitro and in vivo activity observed with the 7-amino series prompted an attempt to combine an acceptable level of aqueous solubility with the inherently high potency observed for a series of imidazo-[4,5-*b*]quinolin-2-ones substituted at the 7 position with functionalized side chains.[2] The potent platelet aggregation inhibitory properties of **3** and **4** and related compounds demonstrates the functional diversity acceptable at the side-chain terminus of this structural class. However, the incorporation of a basic nitrogen atom into the amide moiety of cAMP PDE inhibitors with this kind of a side chain has been explored only in a very cursory fashion.[19] From an examination of the data presented in Table III, it is apparent that this structural modification provides a series of compounds **21** that not only demonstrate potent biological activity but several representatives are also readily soluble in water at 10 mg/mL.

The parent piperazinamide **21b** is a potent inhibitor of cAMP PDE that is highly soluble in water but only weakly prevents ADP-induced platelet aggregation. Substitution of the piperazine N atom with alkyl groups of increasing size and lipophilicity leads to a 100-fold increase in cAMP PDE inhibitory potency across the series of compounds **21c–m**, a pattern of activity fully reflected in the platelet aggregation assay. For the cyclohexylmethyl derivative **21h**, side-chain length is optimal with the pentanoate **21aa**, which is 1 order of magnitude more potent in both biological assays than butyrate **21h** or hexanoate **21ab**. These compounds are among the most potent cAMP PDE inhibitors to emerge from this structural class, and the level of intrinsic activity is perhaps somewhat surprising in view of the trends observed for a related series of compounds.[13] Consistent with previously developed structure–activity relationships,[2,3] methylation at N-1 of the heterocycle (**21ac**) leads to a 200-fold reduction in potency. However, on the basis of observations with more simply substituted

(15) Gillespie, E. Anagrelide: A Potent and Selective Inhibitor of Platelet Cyclic AMP Phosphodiesterase Enzyme Activity. *Biochem. Pharmacol.* **1988**, *37*, 2866–2868.

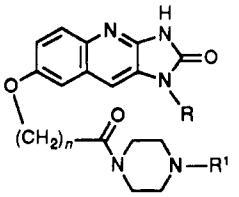
(16) Ishikawa, F.; Saegusa, J.; Inamura, K.; Sakuma, K.; Ashida, S.-I. Cyclic Guanidines. 17. Novel (N-substituted amino)-imidazo[2,1-*b*]quinazolin-2-ones: Water Soluble Platelet Aggregation Inhibitors. *J. Med. Chem.* **1985**, *28*, 1387–1395.

(17) Fleming, J. S.; Buchanan, J. O.; King, S. P.; Cornish, B. T.; Bierwagen, M. E. Use of the Biolaser in the Evaluation of Antithrombotic Agents. *Platelets and Thrombosis*; Scriabine, A., Sherry, S., Eds.; Baltimore Univ. Park Press: Baltimore, 1974; pp 247–262.

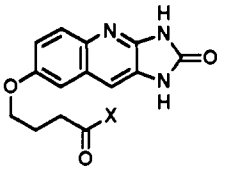
(18) Fleming, J. S.; Buchanan, J. O., unpublished studies.

(19) (a) Nishi, T.; Yamamoto, K.; Shimizu, T.; Kanbe, T.; Kimura, Y.; Nakagawa, K. Studies on 2-Oxoquinoline Derivatives as Blood Platelet Aggregation Inhibitors. I. Alkyl 4-(2-Oxo-1,2,3,4-tetrahydro-6-quinolinyloxy)butyrates and Related Compounds. *Chem. Pharm. Bull.* **1983**, *31*, 798–810. (b) Nishi, T.; Tabusa, F.; Tanaka, T.; Shimizu, T.; Kanbe, T.; Kimura, Y.; Nakagawa, K. Studies on 2-Oxoquinoline Derivatives as Blood Platelet Aggregation Inhibitors. II. 6-[3-(1-Cyclohexyl-5-tetrazolyl)propoxy]-1,2-dihydro-2-oxoquinoline and Related Compounds. *Chem. Pharm. Bull.* **1983**, *31*, 1151–1157. (c) Nishi, T.; Tabusa, F.; Tanaka, T.; Ueda, H.; Shimizu, T.; Kanbe, T.; Kimura, Y.; Nakagawa, K. Studies on 2-Oxoquinoline Derivatives as Blood Platelet Aggregation Inhibitors. III. *N*-Cyclohexyl-*N*-(2-hydroxyethyl)-4-(1,2-dihydro-2-oxo-6-quinolinyloxy)butyramide and Related Compounds. *Chem. Pharm. Bull.* **1983**, *31*, 852–860.

Table II. Structure and Physical Properties of 7-Oxy-1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one Derivatives and 7-Oxy-1,2,3,5-tetrahydro-2*H*-imidazo[2,1-*b*]quinazolin-2-one Derivatives **26a** and **26b**



no.	*n*	R	R^1	mp, °C	mol formula (elem anal.)
21a	3	H	^{t}BuOCO	246–248 (dec)	$C_{23}H_{29}N_5O_5$
21b	3	H	H	233–235	$C_{18}H_{21}N_5O_3 \cdot 2CF_3CO_2H \cdot 0.7H_2O$
21c	3	H	CH_3	286–288 (dec)	$C_{19}H_{23}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$
21d	3	H	$(CH_3)_2CHCH_2$	240–243	$C_{22}H_{29}N_5O_3 \cdot 2HCl \cdot 0.5H_2O$
21e	3	H	Et_2CHCH_2	198–201	$C_{24}H_{33}N_5O_3 \cdot 2HCl$
21f	3	H	$^n Pr_2CHCH_2$	180–185	$C_{26}H_{37}N_5O_3 \cdot 2HCl \cdot 0.1H_2O$
21g	3	H	$cC_5H_9CH_2$	244–250	$C_{24}H_{31}N_5O_3 \cdot 2HCl \cdot 0.8H_2O$
21h	3	H	$cC_6H_{11}CH_2$	258–260 (dec)	$C_{25}H_{33}N_5O_3 \cdot 2HCl \cdot 1.5H_2O$
21i	3	H	$cC_7H_{13}CH_2$	260–263 (dec)	$C_{26}H_{35}N_5O_3 \cdot 2HCl \cdot 0.5H_2O$
21j	3	H	2-pyranyl-CH_2	222–232 (dec)	$C_{24}H_{31}N_5O_4 \cdot 2HCl \cdot H_2O$
12k	3	H	cC_7H_{13}	210–225 (dec)	$C_{25}H_{33}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$
21l	3	H	$cC_6H_{11}CH_2CH_2$	indistinct	$C_{26}H_{35}N_5O_3 \cdot 2HCl$
21m	3	H	$CH_3O(CH_2)_2$	227–231	$C_{21}H_{27}N_5O_4 \cdot 2HCl \cdot 0.5H_2O$
21n	3	H	$C_6H_5CH_2$	210–216 (dec)	$C_{25}H_{27}N_5O_3 \cdot 2HCl \cdot 0.1H_2O$
21o	3	H	4-$FC_6H_4CH_2$	indistinct	$C_{25}H_{26}FN_5O_3 \cdot 2HCl \cdot 0.2H_2O \cdot 0.4Et_2O$[a]
21p	3	H	4-$ClC_6H_4CH_2$	indistinct	$C_{25}H_{26}ClN_5O_3 \cdot 2HCl \cdot 0.17H_2O \cdot 0.58Et_2O$
21q	3	H	3-$CF_3C_6H_4CH_2$	198–208	$C_{26}H_{26}F_3N_5O_3 \cdot 2HCl \cdot 0.5H_2O$
21r	3	H	4-$CF_3C_6H_4CH_2$	274–276	$C_{26}H_{26}F_3N_5O_3 \cdot HCl \cdot 0.26H_2O$[b]
21s	3	H	4-$CH_3OC_6H_4CH_2$	200–207	$C_{26}H_{29}N_5O_4 \cdot 2HCl \cdot 1.6H_2O$
21t	3	H	3,4-$(OCH_2O)C_6H_3CH_2$	210–215 (dec)	$C_{26}H_{27}N_5O_5 \cdot 2HCl \cdot 0.4H_2O$
21u	3	H	$C_6H_5CH_2CH_2$	260–263 (dec)	$C_{26}H_{29}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$[c]
21v	3	H	2-$CF_3C_6H_4CH_2CH_2$	210–215 (dec)	$C_{27}H_{26}F_3N_5O_3 \cdot 2HCl \cdot 0.5H_2O$
21w	3	H	2-thienyl-CH_2	244–245 (dec)	$C_{23}H_{25}N_5O_3S \cdot 2HCl$
21x	3	H	3-thienyl-CH_2	indistinct	$C_{23}H_{25}N_5O_3S \cdot 2HCl$
21y	3	H	$(C_6H_5)_2CH$	213–215 (dec)	$C_{31}H_{31}N_5O_3 \cdot 2HCl \cdot 0.75H_2O$[d]
21z	3	H	$(4\text{-}FC_6H_4)_2CH$	209–212 (dec)	$C_{31}H_{28}F_2N_5O_3 \cdot 2HCl \cdot 0.35H_2O$[e]
21aa	4	H	$cC_6H_{11}CH_2$	indistinct	$C_{26}H_{35}N_5O_3 \cdot 2HCl \cdot 0.8H_2O$
21ab	5	H	$cC_6H_{11}CH_2$	290–292 (dec)	$C_{27}H_{37}N_5O_3 \cdot 2HCl \cdot 0.7H_2O$[f]
21ac	3	CH_3	$cC_6H_{11}CH_2$	indistinct	$C_{26}H_{35}N_5O_3 \cdot 2HCl \cdot 0.7H_2O$[g]
21ad	4	H	$C_6H_5CH_2$	190–195 (dec)	$C_{26}H_{29}N_5O_3 \cdot 2HCl \cdot 0.1H_2O$
21ae	3	CH_3	$C_6H_5CH_2$	indistinct	$C_{26}H_{29}N_5O_3 \cdot 2HCl \cdot 0.5H_2O$[h]
21af	3	H	2-pyrimidinyl	184–187	$C_{22}H_{23}N_7O_3 \cdot 2HCl \cdot H_2O$
21ag	3	H	2-pyridinyl	195–200 (dec)	$C_{23}H_{24}N_6O_3 \cdot 2HCl \cdot H_2O$[i]
21ah	3	H	EtO_2CCH_2	indistinct	$C_{22}H_{27}N_5O_3 \cdot 2HCl \cdot H_2O$
21ai	3	H	1-pyrrolidinyl-$COCH_2$	190–200 (dec)	$C_{24}H_{30}N_6O_4 \cdot 2HCl \cdot 0.45H_2O$
21aj	3	H	C_6H_5	277–279	$C_{24}H_{25}N_5O_3 \cdot 0.15DMF$
21ak	3	H	3-$CF_3C_6H_4$	185 (dec)	$C_{25}H_{24}F_3N_5O_3$



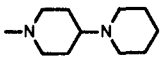
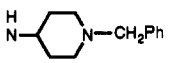
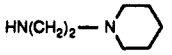
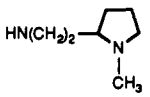
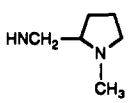
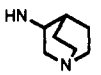
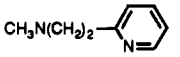
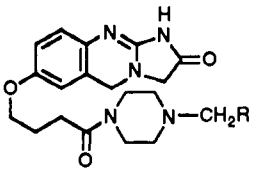
no.	X	mp, °C	mol formula (elem anal.)
21al		indistinct	$C_{24}H_{31}N_5O_3 \cdot 2HCl \cdot H_2O$
21am		202–204	$C_{26}H_{29}N_5O_3 \cdot 2HCl \cdot 0.4H_2O$
21an		indistinct	$C_{21}H_{27}N_5O_3 \cdot 2HCl$[j]
21ao		248–251	$C_{21}H_{27}N_5O_3 \cdot 0.8H_2O$[k]
21ap		233–237	$C_{21}H_{27}N_5O_3$[l]
21aq		242–245	$C_{21}H_{25}N_5O_3 \cdot 2HCl$
21ar		175–180	$C_{22}H_{23}N_5O_3 \cdot 2HCl \cdot 0.3H_2O$

Table II (Continued)



no.	R	mp, °C	mol formula (elem anal.)
26a	C_6H_5	indistinct	$C_{25}H_{29}N_5O_3 \cdot 2HCl \cdot 0.5H_2O$
26b	cC_6H_{11}	indistinct	$C_{25}H_{35}N_5O_3 \cdot 2HCl \cdot 1.55H_2O$

[a] H: calcd, 5.46; found, 6.36. [b] H: calcd, 5.00; found, 6.44. [c] H: calcd, 5.95; found, 6.44. [d] H: calcd, 5.72; found, 6.20. [e] H: calcd, 5.02; found, 5.54. [f] N: calcd, 12.40; found, 13.23. [g] N: calcd, 12.75; found, 12.01. [h] H: calcd, 5.96; found, 6.50. [i] H: calcd, 5.40; found, 5.96, N: calcd, 16.06; found, 15.45. [j] C: calcd, 53.62; found, 54.16. [k] H: calcd, 6.99; found, 6.46. [l] C: calcd, 63.46; found, 65.50.

imidazo[4,5-*b*]quinolin-2-one derivatives,[3] the amides **21** are weaker platelet aggregation inhibitors than might be anticipated in view of the efficiency with which they prevent hydrolysis of cAMP by a platelet PDE enzyme preparation. This suggested that the membrane permeability of the drugs may be a limiting factor and that platelet inhibitory activity may develop only slowly, perhaps not unanticipated based on the charged nature of the compounds. This was indeed found to be the case in the single example examined since extending the duration of exposure of platelets to **21h** from 3 to 15 min, prior to the addition of the agonist, resulted in a marked enhancement in potency. The IC_{50} for **21h** is 0.51 μM after 3-min exposure to platelets, but this falls to 0.1 μM if platelets are exposed to the drug for 15 min. Consequently, the data reported in Table III probably underestimate of the platelet inhibitory activity of many of these compounds.

In addition to influencing biological potency, the piperazine N-substituent is also a crucial determinant of aqueous solubility. Small alkyl substituents of up to a maximum of seven carbon atoms in any of the configurations examined provide compounds that are soluble in water (as their dihydrochlorides) at a concentration of 10 mg/mL. However, aqueous solubility is exquisitely dependent on substituent lipophilicity. Thus, although the (cyclohexylmethyl)piperazine derivative **21h** is soluble in water, introduction of a single additional CH_2, either into the cyclohexyl ring (**21i**) or the tether (**21l**), leads to a precipitous reduction in solubility. The acyclic series **21c–f** behaves similarly and **21f** is not soluble in water at 10 mg/mL. Interestingly, increasing the global lipophilicity by homologation of the side chain (**21aa,ab**) or methylation at N-1 of the heterocyclic nucleus (**21ac**) does not markedly decrease aqueous solubility compared to the prototype, **21h**.

The *N*-benzylpiperazine derivative **21n** is comparable in potency in both the cAMP PDE enzyme and platelet aggregation assays to its saturated counterpart **21h**, demonstrating a tolerance for an aromatic ring in this region of the pharmacophore. Substituting the phenyl ring of **21n** with either electron-donating or -withdrawing groups (**21n–t**), extending the tether (**21u,v**), replacing the tether by an isosteric thiophene ring in either configuration (**21w,x**), or dramatically increasing lipophilicity of the terminus (**21y,z**) leads to a potency variation of less than 1 order of magnitude. The effects of side-chain homologation (**21ad**) and heterocycle methylation (**21ae**) on the biological activity of **21n** are similar but slightly less pronounced than that observed for **21h**. Within this series, aqueous solubility is also very sensitive to the lipophilicity of the piperazine substituent and is determined by the nature and location of the aryl substituent. This is most dramatically demonstrated by comparing the *p*-F- and *p*-Cl-substituted compounds **21o** and **21p**, respectively. Whereas fluoride **21o** is completely soluble in water at a concentration of 10 mg/mL, the slightly more lipophilic *p*-chloro analogue **21p** is remarkably insoluble. The *p*-(trifluoromethyl)-substituted derivative **21r** is not soluble in water, unlike the meta isomer **21q**, because it does not form a stable dihydrochloride salt.

The *N*-phenylpiperazinamides **21aj** and **21ak** exhibit potent biological activity but poor aqueous solubility due to the very weakly basic nature of the piperazine nitrogen atom, which precludes stable salt formation. A 2-pyrimidinyl piperazine (**21af**) or 2-pyridylpiperazine (**21ag**) substituent restores aqueous solubility while retaining effective biological activity. The introduction of polar functionality into the piperazine substituent is well-tolerated by the PDE enzyme, but **21ah** and **21ai** are not able to fully express their biological activity in the platelet assay during the 3-min incubation period. A similar circumstance prevails for the piperidine derivatives **21al** and **21am**. None of the secondary amides **21am–aq** or **21ar** demonstrate impressive platelet inhibitory activity although all are potent cAMP PDE inhibitors with excellent aqueous solubility.

The two imidazo[2,1-*b*]quinazolin-2-ones **26a** and **26b** show good aqueous solubility but are at least 1 order of magnitude weaker in both biological tests than their imidazo[4,5-*b*]quinolin-2-one counterparts **21n** and **21h**, respectively.

Several representatives of **21** that combine potent biological activity in vitro with acceptable aqueous solubility were selected for evaluation in the biolaser model of small vessel thrombosis, conducted in conscious rabbits.[4,17,20] The results, which are compiled in Table IV, reveal an impressive level of protection against thrombus formation in this model, which is believed to be dependent upon the release of proaggregatory substances from red blood cells. All of the compounds evaluated in this model demonstrate a distinct potency advantage over the prototype of this structural class, BMY 20844 (**2**), and anagrelide. BMY 20844 (**2**) is half-maximally effective at a dose of 5 mg/kg in this model while anagrelide is slightly more potent with an ED_{50} of approximately 3.2 mg/kg. The cyclohexylmethyl-substituted piperazinamide **21h** is 40-fold more potent than BMY 20844 (**2**) in this model with an ED_{50} of 0.074 mg/kg.[20] The intrinsically more potent homologue **21aa** is even more effective with an ED_{50} of 0.03 mg/kg, which represents a 100-fold potency advantage over BMY 20844 (**2**), and is the most powerful, orally active anti-

(20) Fleming, J. S.; Buchanan, J. O.; Seiler, S. M.; Meanwell, N. A. Antithrombotic Activity of BMY 43351, a New Imidazoquinoline with Enhanced Aqueous Solubility. *Thromb. Res.* **1991**, *63*, 145–155.

thrombotic agent that we have identified using this model.

The pharmacological properties of **21h** (BMY 43351) have been examined in some detail and this compound has been characterized as the most effective antithrombotic agent to emerge from this series of platelet cAMP PDE inhibitors to date,[20,21] being significantly more potent than the clinically studied platelet inhibitor cilostazole.[22] The markedly enhanced potency of **21h** in the biolaser model of thrombosis, when compared to earlier studied compounds, was confirmed in a modified Folts model of large vessel thrombosis, conducted in dogs.[21] In this setting, significant protection against occlusive thrombus formation was observed at doses as low as 1 μg/kg id, 100-fold lower than a similarly effective dose of BMY 20844 (**2**).

Conclusion

In summary, we have shown that the poor aqueous solubility of 1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one derivatives can be markedly improved by the introduction of an additional basic nitrogen atom into the molecule, thereby providing a second site for acid salt formation. The more potent cAMP PDE and platelet aggregation inhibitors possess a conformationally mobile functionalized side chain appended at the 7-position of the heterocycle. In this series of compounds, **21**, a basic nitrogen atom is incorporated into the side-chain terminus where it can be accommodated in a secondary binding site thought to exist[12,13] in the cGMP-inhibited cAMP PDE isozyme.[23] The structure–activity studies presented in Table III provide further insight into the functional demands of this domain of the enzyme, which clearly demonstrates a tolerance for a charged atom. Interestingly, the more potent cAMP PDE inhibitors within the amide series **21** are those in which the amide nitrogen is incorporated into a ring, a structure–activity trend not predicted by an earlier study of structurally similar imidazo[2,1-*b*]quinazolin-2-one derivatives.[13] Compared to poorly soluble compounds with a similar degree of intrinsic activity in vitro, the increased aqueous solubility associated with the amides **21** results in enhanced bioavailability following oral administration. This is most effectively demonstrated by the high level of biological activity observed for several representatives of **21** in a rabbit model of thrombosis.

Experimental Section

Melting points were recorded on a Thomas-Hoover capillary apparatus and are uncorrected. Proton (^{1}H NMR) and carbon (^{13}C) magnetic resonance spectra were recorded on a Bruker AM FT instrument operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C or a Perkin-Elmer R32 90-MHz CW spectrometer. All spectra were recorded using tetramethylsilane as an internal standard, and signal multiplicity is designated according to the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Infrared (IR) spectra were obtained using a Perkin-Elmer 1800 FT IR, scanning from 4000 to 400 cm^{-1} and calibrated to the 1601 cm^{-1} absorption of a polystyrene film. Mass spectral data were obtained on a Finnigan Model 4500 GC/MS using chemical ionization (isobutane) procedures. Fast atom bombardment (FAB) mass spectra were obtained on a Kratos MS 25 spectrometer using *m*-nitrobenzyl alcohol (NOBA) as the matrix. Analytical samples were dried in vacuo at 78 °C or in the presence of P_2O_5 at room temperature for at least 12 h. Elemental analyses were provided by Bristol-Myers Squibb's Analytical Chemistry Department or Oneida

Table III. Biological Activity and Aqueous Solubility of 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one Derivatives and the 1,2,3,5-Tetrahydro-2*H*-imidazo[2,1-*b*]quinazolin-2-one Derivatives **26a** and **26b**

no.	inhibn of human platelet cAMP PDE, IC_{50},[a] nM	inhibn of ADP-induced aggregation of human platelets; EC_{50},[b] μM	aqueous solubility, mg/mL
11a	70	1.42	>10
11b	50	2.03	<10
11c	30	0.90	>10
11d	20	0.25	<10
11e	300	0.28	<10
12	20	30.25	<10
13	300	>37.0	<10
16a	700	3.52	<10
16b	600	6.82	<10
16c	300	37.94	<10
18	5000	8.06	<10
21a	0.3	0.27	<10
21b	40	53.68	>10
21c	7	8.90	>20
21d	1	2.43	>10
21e	1	0.43	>10
21f	0.4	0.68	<10
21g	0.5	1.30	>10
21h	0.4	0.51[c]	>10
21i	0.2	0.29	<10
21j	0.7	2.02	>10
21k	0.9	0.75	>10
21l	0.2	0.054	<10
21m	7	20.79	>10
21n	0.7	0.17	>10
21o	2	0.53	>10
21p	2	0.17	<10
21q	9	0.92	~2
21r	0.4	2.11	<10
21s	3	0.52	>10
21t	0.9	0.33	10
21u	2	0.19	<10
21v	0.3	0.11	<10
21w	0.7	0.38	>10
21x	0.7	0.38	>10
21y	3	0.062	<10
21z	0.3	0.13	<10
21aa	0.03	0.051	>10
21ab	0.6	0.71	>10
21ac	80	>58.1	>10
21ad	0.08	0.047	>10
21ae	30	>59.1	>10
21af	7	1.22	>10
21ag	5	0.67	>10
21ah	2	2.44	>10
21ai	6	>56.5	>10
21aj	2	0.05	<10
21ak	0.6	0.72	<10
21al	5	11.35	>10
21am	0.7	3.7	>20
21an	4	4.5	>20
21ao	20	79.8	>10[d]
21ap	40	6.3	>10[d]
21aq	40	12.4	>10
21ar	4.5	12.4	>10
26a	8	4.15	>5
26b	6	7.23	>5

[a] IC_{50} values were obtained from plots of cAMP hydrolysis versus concentration of test compound in the assay and the data presented are the average of duplicate determinations. BMY 20844 (**2**), IC_{50} 1.3 ± 0.6 × 10^{-8} M,[5] was used as a positive control. [b] Data shown are the result of a single or the average of duplicate determinations with anagrelide, IC_{50} = 1.05 ± 0.3 μM, used as a reference agent. Standard incubation time of PRP with drug prior to the addition of ADP was 3 min. [c] IC_{50} after 15 min of incubation of the drug with PRP prior to the addition of ADP is 0.1 μM. [d] Solubility determined in 0.25 N HCl solution.

(21) Meanwell, N. A.; Fleming, J. S.; Russell, J. W.; Seiler, S. M. BMY 43351. *Drugs Future* **1992**, *17*, 15–17.
(22) For a comprehensive review of the pre-clinical profile and early clinical studies of cilostazole, see: *Arzneim. Forsch.* **1985**, *35*, 1117–1208.
(23) Macphee, C. H.; Harrison, S. A.; Beavo, J. A. Immunological Identification of the Major Platelet Low-Km cAMP Phosphodiesterase: Probable Target for Anti-thrombotic Agents. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6660–6663.

Table IV. Inhibition of Laser-Induced Thrombus Formation in the Microvasculature of the Rabbit Ear Chamber

no.	mean thrombus area, $\mu M^2 \pm$ SE, (% inhibition of thrombus formation) at a dose of 0.1 mg/kg po[a]
21h	72 ± 9 (54)
21k	79 ± 13 (49)
21n	(50)[b]
21o	83 ± 14 (46)
21q	78 ± 14 (49)
21s	81 ± 10 (48)
21w	79 ± 13 (49)
21aa	(>50)[c]
21ag	82 ± 12 (47)

[a] Experiments were performed as previously described.[17,20] The mean thrombus area from 10 trials conducted 2 h after dosing of the test compound was compared with the mean thrombus area from 10 trials performed in the same rabbit prior to administration of the drug. Predose control mean thrombus area ± SE = 155 ± 2 μM^2. The results shown are an average from experiments conducted in at least three different rabbits. [b] Extrapolated value: 41% inhibition at 0.03 mg/kg, 64% inhibition at 0.3 mg/kg. [c] IC_{50} 0.03 mg/kg.

Research Services (Whitesboro, NY).

2-(5-Chloro-2-nitrophenyl)-1,3-dioxolane (7). A solution of BH_3–THF complex (25.8 g, 0.30 mol) in THF (300 mL) was added dropwise to a stirred solution of **5** (50.0 g, 0.24 mol) in dry THF (400 mL) maintained at 0 °C under a N_2 atmosphere. After completion of the addition, the mixture was heated at 50 °C for 96 h before being cooled in an ice bath and quenched by adding 10% HCl solution, dropwise. The mixture was heated at reflux for 30 min, the THF removed in vacuo, and the residue extracted with CH_2Cl_2 (3 × 350 mL). The combined extracts were washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated to give a solid which was recrystallized from $CHCl_3$/hexanes to give 5-chloro-2-nitrobenzenemethanol (41.0 g, 91%), mp 79–81 °C. Anal. ($C_7H_6ClNO_3$) C, H, N.

A solution of this alcohol (20.0 g, 0.11 mol) in dry CH_2Cl_2 (150 mL) was added to a vigorously stirred solution of PCC[9] (43.2 g, 0.2 mol) in dry CH_2Cl_2 (200 mL). After stirring overnight, Et_2O (1.3 L) was added, the organic phase decanted, and the residue washed with Et_2O. The ethereal layer was filtered through a plug of silica gel (6 in. deep) and the solvent evaporated to leave **6** (17.70 g, 89%), mp 76–77 °C (lit.[24] mp 78 °C).

A mixture of **6** (18.3 g, 0.1 mol), ethylene glycol (7.00 g, 0.11 mol), pTsOH (0.5 g), and benzene was heated at reflux under a Dean–Stark trap for 24 h. The solvent was evaporated; the residue was dissolved in CH_2Cl_2 and filtered through a plug of silica gel to give **7**[25] (21.8 g, 97%). Anal. ($C_9H_8ClNO_4$) C, H, N.

2-Nitro-5-(1-piperidinyl)benzaldehyde (8c). A mixture of **7** (21.13 g, 0.09 mol) and piperidine (39.6 g, 0.46 mol) was heated at reflux in an oil bath for 3 h. The excess piperidine was evaporated and the residue partitioned between H_2O and CH_2Cl_2. The organic layer was separated, the H_2O layer was extracted with CH_2Cl_2, and the combined extracts were dried and concentrated to afford a dark oil (26.1 g, 100%). This was dissolved in THF (1 L), then H_2O (315 mL) and 3 N HCl solution (130 mL) were added, and the mixture was heated at reflux for 45 min. The mixture was cooled in an ice bath and filtered to give **8c** (19.2 g, 87%). An analytical sample, purified by chromatography on silica gel using CH_2Cl_2 as eluant and subsequent recrystallization from CH_3CN, had mp 102–103 °C: IR (KBr) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.71 (6 H, bs, CH_2 of piperidine ring), 3.49 (4 H, bs, NCH_2), 6.88 (1 H, dd, J = 9 Hz, J' = 3 Hz, aromatic *H* para to CHO), 7.02 (1 H, d, J = 3 Hz, aromatic *H* ortho to CHO), 8.01 (1 H, d, J = 9 Hz, aromatic *H* ortho to NO_2), 10.45 (1 H, s, CHO). Anal. ($C_{12}H_{14}N_2O_3$) C, H, N.

5-[[2-Nitro-5-(1-piperidinyl)phenyl]methylene]-2,4-imidazolidinedione (10c). Sodium (0.60 g, 0.026 g-atom) was dissolved in punctilious EtOH (200 mL) and phosphonate **9**[10] (6.00 g, 25 mmol) added. After 10 min, **8c** (5.00 g, 21 mmol) was added and the mixture stirred at room temperature for 5 h. Filtration gave **10c** (6.17 g, 92%) as a 3:1 mixture of *Z*:*E* geometrical isomers: mp 273–276 °C; IR (KBr) 1775, 1725 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.62 (6 H, bs), 3.49 (4 H, bs), 6.67 and 6.78 (1 H, 2 s, ratio 3:1, respectively, olefinic *H*), 6.80–7.00 (2 H, m), 7.90–8.10 (1 H, 2 d, J = 9 Hz); MS m/z 317 (MH^+). Anal. ($C_{15}H_{16}N_4O_4$) C, H, N.

1,3-Dihydro-7-(1-piperidinyl)-2*H*-imidazo[4,5-*b*]quinolin-2-one Dihydrochloride (11c). A solution of **10c** (5.00 g, 16 mmol) in MeOH (250 mL) and 5.7 N HCl in EtOH (6 mL) was hydrogenated over 10% Pd on C (0.8 g) at 50 psi in a Parr hydrogenation apparatus. After 24 h, the mixture was filtered through Celite, the solvent evaporated, and the residue dissolved in MeOH (100 mL). The mixture was heated to reflux, I_2 (2.20 g, 9 mmol) introduced portionwise, and reflux continued for 30 min. The solution was concentrated to ca. 50 mL, diluted with 10% Na_2CO_3 and 10% $Na_2S_2O_3$ solutions, and stirred for 1 h before filtration to give a solid. This was dissolved in DMF, precipitated with H_2O, and then dissolved in MeOH containing an excess of dry HCl gas. Addition of EtOAc gave **11c** (2.40 g, 44%): mp 300 °C dec; IR (KBr) 1725 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.50–2.40 (6 H, bs, CH_2 of piperidine ring), 3.60 (4 H, bs, NCH_2), 7.00 (3 H, bs, $NH + H^+$), 7.63 (1 H, s, aromatic *H* ortho to NHCO), 7.94 (1 H, d, J = 9 Hz), 8.03 (1 H, dd, J = 9 Hz, J' = 2 Hz), 8.44 (1 H, s), 11.31 (1 H, s, NH). Anal. ($C_{15}H_{16}N_4O \cdot 2HCl \cdot 0.6H_2O$) C, H, N.

Methyl 1-(2,3-Dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)-4-piperidinecarboxylate (11e). A solution of ethyl 1-[3-[(2,4-dioxoimidazolidin-5-ylidene)methyl]-4-nitrophenyl]-4-piperidinecarboxylate (35.80 g, 92 mmol) in DMF (500 mL) was hydrogenated over 10% Pd on C (3.60 g) at 200 psi in a Parr reactor. After 42 h, the mixture was filtered through Celite, the solvent evaporated and the residue diluted with a mixture of MeOH and Et_2O (1:1). A solid was filtered off and suspended in MeOH (500 mL) containing pTsOH (1.50 g), and the mixture heated at reflux. After 18 h, the mixture was cooled and filtered to give a solid (19.50 g) which was suspended in MeOH (500 mL) and heated to reflux. I_2 (13.70 g, 54 mmol) was added portionwise, and the mixture heated at reflux for 2.5 h and then cooled. 10% Na_2CO_3 solution was added until the pH = 7 followed by 10% $Na_2S_2O_3$ solution and the mixture filtered to give **11e** (19.40 g, 65%). An analytical sample, recrystallized from DMF/H_2O, had mp >310 °C: ^1H NMR (DMSO-d_6) δ 1.70–1.94 (4 H, m), 2.50–2.90 (3 H, m), 3.64 (3 H, s, CO_2CH_3), 3.70 (2 H, m, NCH_2), 7.21 (1 H, bs), 7.30 (1 H, bs), 7.45 (1 H, bs), 7.61 (1 H, bs), 10.89 (1 H, bs, NH), 11.29 (1 H, bs, NH). Anal. ($C_{17}H_{16}N_4O_3 \cdot 0.05H_2O$) C, H, N, H_2O.

1-(2,3-Dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)-4-piperidinecarboxylic Acid (12). A mixture of **11e** (3.40 g, 10 mmol), 50% MeOH/H_2O (50 mL), and 4 N NaOH (5 mL, 20 mmol) was stirred at room temperature for 1 h before being acidified to pH = 5 with 2 N HCl solution. The precipitate was collected, washed sequentially with H_2O and MeOH, dried, and suspended in hot DMF. The solid was filtered, suspended in hot MeOH, and filtered to give **11e** (1.86 g, 60%): mp >310 °C; ^1H NMR (DMSO-d_6) δ 1.70–1.97 (4 H, m), 2.43 (1 H, m, $CHCO_2H$), 2.76–2.83 (2 H, m, NCH_2), 3.68–3.72 (2 H, m, NCH_2), 7.20 (1 H, s), 7.32 (1 H, d, J = 9 Hz), 7.44 (1 H, s), 7.62 (1 H, d, J = 9 Hz). Anal. ($C_{16}H_{16}N_4O_3 \cdot 0.25DMF$) C, H, N.

***N*-Cyclohexyl-1-(2,3-dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)-*N*-methyl-4-piperidinecarboxamide (13).** A mixture of **11e** (3.12 g, 10 mmol), *N*-methylcyclohexylamine (1.36 g, 12 mmol), Et_3N (2.12 g, 2.91 mL, 21 mmol), DPPA[11] (2.59 mL, 12 mmol), and DMF (200 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (200 mL) and stirred for 72 h before being filtered to give **13** (2.09 g, 51%). Recrystallization from DMF gave analytically pure **13** (1.02 g): mp 318–321 °C; ^1H NMR (DMSO-d_6) δ 1.09–1.72 (14 H, m), 2.69 and 2.87 (3 H, s, NCH_3), 2.74 and 2.89 (s, DMF), 2.77–2.83 (3 H, m, $CHCO_2H$ and NCH_2), 3.71 and 4.25 (1 H, m, NH), 3.77–3.80 (2 H, m, NCH_2), 7.20 (1 H, s), 7.31 (1 H, d, J = 9 Hz), 7.44 (1 H, s), 7.62 (1 H, d, J = 9 Hz), 7.96 (0.3 H, s, DMF), 10.88 (1 H, s, NH), 11.28 (1 H, s, NH). Anal. ($C_{23}H_{29}N_5O_2 \cdot 0.33DMF$) C, H, N.

(24) Alford, E. J.; Schofield, K. Cinnolines. Part XXVIII. The Nature of the $C_{(3)}$-position. Part I. The Neber-Bossel Synthesis of 3-Hydroxycinnoline. *J. Chem. Soc.* **1952**, 2102–2108.
(25) O'Brien, D. E.; Weinstock, L. T.; Cheng, C. C. Synthesis of 10-Deazariboflavin and Related 2,4-Dioxopyrido[4,5-*b*]-quinolines. *J. Heterocycl. Chem.* **1970**, *7*, 99–105.

2-(2-Nitro-5-piperazinylphenyl)-1,3-dioxolane (14). A mixture of 7 (7.00 g, 38 mmol) and piperazine (8.20 g, 95 mmol) was heated in an oil bath at 130 °C for 90 min. The mixture was diluted with H_2O and 10% Na_2CO_3 solution and extracted with CH_2Cl_2. The combined extracts were dried over $MgSO_4$ and concentrated to give 14 (8.30 g, 97%), which was generally used without further purification. An analytical sample was prepared by dissolution in CH_2Cl_2 and addition of 10% HCl in EtOH solution. The solid was filtered off and dried to give 14 as its HCl salt: mp 178–180 °C; IR (KBr) 1500, 1320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.67 (4 H, bs, NCH_2), 3.43 (4 H, bs, NCH_2), 4.06 (4 H, bs, OCH_2), 6.60 (1 H, s, OCHO), 6.79 (1 H, d, J = 8 Hz, aromatic H meta to NO_2), 7.22 (1 H, s, aromatic H meta to NO_2), 8.02 (1 H, d, J = 8 Hz, aromatic H ortho to NO_2); MS m/z 279 (MH^+). Anal. ($C_{13}H_{17}N_3O_4$·HCl·0.85H_2O) C, H, N.

1-(3,4-Dimethoxybenzoyl)-4-(3-formyl-4-nitrophenyl)-piperazine (15c). Compound 14 (6.63 g, 24 mmol) was added portionwise over 1 h to a stirred solution of 3,4-dimethoxybenzoyl chloride (4.76 g, 48 mmol) and pyridine (4.04 g, 48 mmol) in dry CH_2Cl_2 (200 mL). The mixture was stirred overnight, washed twice with H_2O, and concentrated. The residual gum was combined with THF (265 mL), H_2O (80 mL), and 3 N HCl solution (33 mL) and the mixture heated to reflux. After 2 h, the THF was removed in vacuo, and the residue diluted with H_2O and extracted with CH_2Cl_2 to afford a solid. This was suspended in EtOH and filtered to give 15c (7.36 g, 77%). An analytical sample, recrystallized from $CHCl_3$/EtOH, had mp 200–201 °C: IR (KBr) 1720, 1625 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.50–3.80 (8 H, m), 3.79 (3 H, s, OCH_3), 3.81 (3 H, s, OCH_3), 6.90–7.25 (5 H, m), 8.08 (1 H, d, J = 9 Hz), 10.34 (1 H, s, CHO). Anal. ($C_{20}H_{21}N_3O_6$) C, H, N.

1,1-Dimethylethyl 4-[4-[(2,3-Dihydro-2-oxo-1*H*-imidazo-[4,5-*b*]quinolin-7-yl)oxy]-1-oxobutyl]-1-piperazine-carboxylate (21a). A mixture of 4-[(2,3-dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)oxy]butyric acid[2] (19, n = 3, R, R^1 = H) (2.50 g, 9 mmol), *tert*-butyl 1-piperazinecarboxylate (2.10 g, 11 mmol), DPPA (3.59 g, 2.80 mL, 13 mmol), Et_3N (1.93 g, 2.60 mL, 19 mmol), DMAP (catalytic amount), and DMF (40 mL) was stirred at room temperature overnight. The mixture was diluted with H_2O and filtered to give 21a (3.90 g, 99%). An analytical sample was prepared by recrystallization from DMF/H_2O and had mp 246–248 °C dec: IR (KBr) 1730, 1695, 1650, 1635 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.38 (9 H, s), 1.99 (2 H, quintet, J = 6 Hz), 2.51 (2 H, t, J = 6 Hz), 3.20–3.45 (8 H, m), 4.06 (2 H, t, J = 6 Hz), 7.12 (1 H, dd, J = 9 Hz, J' = 2 Hz), 7.30 (1 H, d, J = 2 Hz), 7.48 (1 H, s), 7.65 (1 H, d, J = 9 Hz), 10.93 (1 H, s, NH), 11.34 (1 H, s, NH); MS m/z 456 (MH^+). Anal. $C_{23}H_{29}N_5O_5$) C, H, N.

4-[4-[(2,3-Dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)oxy]-1-oxobutyl]piperazine (21b). A mixture of 21a (1.50 g, 3.3 mmol) and CF_3CO_2H (15 mL) was stirred at room tempertaure. After 45 min, the volatile material was evaporated and the residue triturated with Et_2O to give 21b (1.90 g, 99%): mp 233–235 °C; IR (KBr) 1750, 1695, 1635 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.01 (2 H, quintet, J = 6 Hz), 2.56 (2 H, t, J = 6 Hz), 3.00–3.25 (4 H, m), 3.60–3.75 (4 H, bs), 4.07 (2 H, t, J = 6 Hz), 7.15 (1 H, dd, J = 9 Hz, J' = 2 Hz), 7.32 (1 H, d, J = 2 Hz), 7.52 (1 H, s), 7.68 (1 H, d, J = 9 Hz), 9.02 (2 H, bs), 11.02 (1 H, s), 11.40 (1 H, bs); MS m/z 356 (MH^+). Anal. ($C_{18}H_{21}N_5O_3$·2CF_3CO_2H·0.7H_2O) C, H, N.

1-(Cyclohexylmethyl)piperazine. A mixture of cyclohexylmethyl bromide (85.40 g, 0.48 mol), 1-piperazinecarboxaldehyde (50.00 g, 0.44 mol), pulverized K_2CO_3 (72.60 g, 0.53 mol), KI (1.00 g), and CH_3CN (500 mL) was heated at reflux. After 23 h, the mixture was cooled, filtered, and concentrated. The residue was combined with 5 N NaOH solution (175 mL, 0.88 mol) and EtOH (450 mL) and the mixture heated at reflux. After 4 h, the EtOH was evaporated, and the residue diluted with H_2O and extracted with CH_2Cl_2. The combined extracts were dried over Na_2SO_4 and concentrated, and the residual oil was distilled at reduced pressure to give 1-(cyclohexylmethyl)piperazine (57.61 g, 71%), bp 104–112 °C (1.2 mmHg). An analytical sample was prepared by dissolving 2.00 g in MeOH and adding an excess of a 10% solution of dry HCl gas in MeOH. Evaporation of the solvent left a solid that was recrystallized from MeOH to give the dihydrochloride salt: mp 286–289 °C; IR (KBr) 3440, 3140, 2920, 2440, 2380, 1425 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.92 (2 H, q, J = 11 Hz), 1.00–1.35 (4 H, m), 1.40–1.95 (5 H, m), 3.05–3.90 (10 H, m), 9.93 (2 H, bs), 11.34 (1 H, bs); MS m/z 183 (MH^+ of the free base). Anal. ($C_{11}H_{22}N_2$·2HCl) C, H, N.

1-(Cyclohexylmethyl)-4-[4-[(2,3-dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)oxy]-1-oxobutyl]piperazine (21h). A mixture of 4-[(2,3-dihydro-2-oxo-1*H*-imidazo[4,5-*b*]-quinolin-7-yl)oxy]butyric acid[2] (19, n = 3, R, R^1 = H) (2.00 g, 7 mmol), *N*-(cyclohexylmethyl)piperazine (1.52 g, 8.4 mmol), Et_3N (1.54 g, 2.12 mL, 15.2 mmol), DPPA[11] (2.87 g, 2.25 mL, 10.4 mmol), DMAP (catalytic quantity), and DMF (40 mL) was stirred at room temperature overnight. The mixture was diluted with H_2O and filtered, and the solid dissolved in a 10% solution of dry HCl in MeOH. Evaporation of the solvent left a solid which was recrystallized from MeOH/Et_2O to give 21h (3.75 g, 100%): mp 258–260 °C; IR (KBr) 1740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.93 (2 H, q, J = 11 Hz, CH of cyclohexyl ring), 1.15 (3 H, m, CH of cyclohexyl ring), 1.50–1.95 (6 H, m, CH of cyclohexyl ring), 2.01 (2 H, t, J = 6 Hz, CH_2CH_2CO), 2.58 (2 H, m, CH_2CO), 2.10–2.30 (4 H, m, NCH_2), 3.33 (1 H, t, J = 12 Hz, axial $CONCH_2$), 3.44 (2 H, bs, NCH_2), 3.77 (1 H, t, J = 12 Hz, axial $CONCH_2$), 4.08 (3 H, t, J = 6 Hz, OCH_2 + equatorial $CONCH_2$), 4.39 (1 H, d, J = 12 Hz, equatorial $CONCH_2$), 7.24 (1 H, dd, J = 9 Hz, J' = 2.5 Hz, aromatic H ortho to O), 7.44 (1 H, d, J = 2.5 Hz, aromatic H ortho to O), 7.72 (1 H, s, aromatic H ortho to NHCO), 7.84 (1 H, d, J = 9 Hz, aromatic H meta to O), 11.13 (1 H, bs, NH), 11.50 (2 H, bs, NH); MS (FAB) m/z 452 (MH^+). Anal. ($C_{25}H_{33}N_5O_3$·2HCl·0.4H_2O) C, H, N.

4-[(2,3-Dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)-oxy]-*N*-[2-(1-piperidinyl)ethyl]butanamide (21an). A mixture of methyl 4-[(2,3-dihydro-2-oxo-1*H*-imidazo[4,5-*b*]quinolin-7-yl)oxy]butanoate[2] (20, n = 3, R^1 = H) (2.00 g, 6.6 mmol) and 1-(2-aminoethyl)piperidine (1.10 g, 1.23 mL, 8.6 mmol) was stirred at 200 °C under an atmosphere of N_2. Additional portions of 1-(2-aminoethyl)piperidine (1.10 g, 1.23 mL, 8.6 mmol) were added after 5 min and again after 30 min. After stirring for a further 75 min at 200 °C, the mixture was cooled and dissolved in MeOH by adding a 10% solution of dry HCl in MeOH. The solvent was evaporated, the residue triturated with a mixture of MeOH and Et_2O, and the solid collected and recrystallized from ^{i}PrOH to give 21an (2.47 g, 79%): mp indistinct (dec at 200 °C); IR (KBr) 1770, 1740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.20–1.40 (1 H, m), 1.60–1.90 (5 H, m), 1.98 (2 H, t, J = 7 Hz), 2.31 (2 H, t, J = 7 Hz, CH_2CO), 2.82 (2 H, m, NCH or ring), 3.05 (2 H, m, NCH_2), 3.30–3.60 (4 H, m, NCH_2 + NCH of ring), 4.02 (2 H, t, J = 6 Hz, OCH_2), 7.18 (1 H, dd, J = 9 Hz, J' = 2.5 Hz), 7.38 (1 H, d, J = 2.5 Hz), 7.66 (1 H, s), 7.78 (1 H, d, J = 9 Hz), 7.00–8.00 (1 H, bs, H^+), 8.44 (1 H, t, J = 5 Hz, NHCO), 10.63 (1 H, bs, NH), 11.40 (1 H, bs, NH); MS m/z 398 (MH^+). Anal. ($C_{21}H_{27}N_5O_3$·2HCl) C, H, N.

Acknowledgment. We thank C. M. Combs and R. D. Rutkowski for providing and interpreting NMR spectral data. A. E. Bosza is acknowledged for her help in preparation of the manuscript.