

199, 171, 104, 77 ( $C_6H_5^+$ ). Anal. ( $C_{14}H_{15}N_3O_2$ ) C, H, N.

**5,6,7,8-Tetrahydro-6-(phenylmethyl)pyrido[4,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10g).** A mixture of **9g** (2.2 g, 7.7 mmol) and 3 N hydrochloric acid (40 mL) was stirred at reflux for 4 h, cooled, and gradually made basic to pH 8 with ammonium hydroxide. Solid began to appear at ca. pH 4. The solid was washed with water and dried under high vacuum to afford white crystals (1.95 g, 98%, mp 290–292 °C): IR 3160, 1735, 1700, 1655, 1520, 1150, 750  $cm^{-1}$ ; NMR  $\delta$  2.35–2.75 (m, 2  $CH_2$ ), 3.05 (s,  $CH_2$ ), 3.65 (s,  $CH_2$ ), 7.4 (s, 5 aryl *H*); MS *m/e* 257 ( $M^+$ ), 256 ( $M^+ - 1$ ), 166 ( $M^+ - C_6H_5CH_2$ ). Anal. ( $C_{14}H_{15}N_3O_2$ ) C, H, N.

**4-[(2-Fluorophenyl)methyl]amino]-5,6,7,8-tetrahydro-6-(phenylmethyl)pyrido[4,3-*d*]pyrimidin-2(1*H*)-one (11g).** A mixture of **9g** (1.0 g, 3.5 mmol) and 2-fluorobenzylamine (5 mL) was heated at 150 °C for 2 h, cooled, and filtered. The solid was washed thoroughly with ether and dried at 100 °C under high vacuum. The pale yellow solid (320 mg, 25%, mp 241–247 °C) was analytically pure: IR 3210, 1668, 1640, 1580, 1540, 1350, 1230, 755  $cm^{-1}$ ; NMR  $\delta$  2.3–2.75 (m, 2  $CH_2$ ), 3.3 (m,  $CH_2$ ), 3.75 (s,  $CH_2$ ), 4.6 (d,  $CH_2$ ), 7.15–7.55 (m, 9 aryl *H*), 10.5 (s, NH); MS *m/e* 364 ( $M^+$ ), 363 ( $M^+ - 1$ ), 320 ( $M^+ - CONH_2$ ), 255 ( $M^+ - CH_2C_6H_4F$ ), 109 ( $CH_2C_6H_4F^+$ ). Anal. ( $C_{21}H_{21}FN_4O$ ) C, H, N.

**Biology. Radioligand Binding Assays.** The determination of [ $^3H$ ]flunitrazepam binding in rat forebrain (FNZ text) was carried out essentially as described previously.<sup>10,3</sup> In this procedure, the geometrical mean of diazepam  $IC_{50}$ s was  $4.4 \pm 0.2$  nM ( $n = 13$ ). [ $^3H$ ]Flunitrazepam binding to the BZ receptor complex (designated FNZ\*) was determined as described in detail in our earlier publication.<sup>3</sup> In this procedure,  $IC_{50}$  values for **1b** ( $n = 3$ ) were  $0.37 \pm 0.04$  nM without GABA and  $0.45 \pm 0.05$  with GABA to give a GABA ratio of 0.82.<sup>3</sup> Similarly, **1a** showed an  $IC_{50}$  value of 0.5 nM without GABA and a GABA ratio of 1.10.<sup>4b</sup>

**Behavioral Tests. PTZ Drug Discrimination.** Rats were trained to discriminate the anxiogenic effects of a subconvulsant dose of pentylenetetrazole (PTZ), according to the procedure of Bennett et al.<sup>5</sup> Animals then were administered the test compound 30 min prior to the anxiogenic dose of PTZ. Antagonism of the PTZ stimulus was indicative of an anxiolytic response.<sup>12</sup>  $ED_{50}$  values were calculated by probit analysis.<sup>20</sup>

#### Antagonism of Diazepam-Induced Rotorod Deficit.

Compounds were tested for their ability to antagonize the rotorod deficit induced by diazepam (30 mg/kg ip) as described in an earlier publication.<sup>3</sup> The test compound was administered po 30 min prior to diazepam. After an additional 30 min, rats were tested for their ability to remain on a rotating rod. If the test agent antagonized the rotorod deficit typically noted with diazepam, it was identified as a potential BZ antagonist/inverse agonist.

**Cook-Davidson Behavioral Conflict Paradigm.** Potential anxiolytic activity was determined by the Cook-Davidson conflict procedure in rats as described by Bennett et al.<sup>5</sup> The VI (non-conflict) portion of this test also provided information as to the potential sedation/muscle relaxation. Rats were trained to press a lever for milk reward both in the presence (conflict) and absence (nonconflict) of shock. Compounds with anxiolytic potential increase responding during the shock component. Rats press for milk reward even if it means experiencing a mild shock. If a compound decreases nonconflict responding (no shock), it has the potential for producing sedation and/or muscle relaxation.

**Ethanol Interaction.** Drug interaction with ethanol was evaluated by using the rotorod procedure. Vehicle or test compounds were orally administered to male Wistar (CrI:WI)BR rats (130–160 g) 30 min prior to ethanol. Rats were then administered various doses of ethanol ip 30 min before testing on the rotorod. Rotorod performance was assessed with a conventional rotorod apparatus modified by placing a section of polyethylene material over the drum. The rat was placed on the drum, which rotated at a speed of 16 rpm, and was required to remain on the drum for 30 s. Each animal was allowed up to three trials to reach the criterion. Any animal not meeting the criterion was considered to have neurological deficit. The  $ED_{50}$  values for ethanol alone, or in combination with various test drug doses, were estimated. The  $ED_{50}$  values were calculated by probit analysis.<sup>20</sup>

**Acknowledgment.** We are grateful to Lia Raabis for taking the  $^1H$  NMR spectra, to Magda Brzechffa for the mass spectral analysis and to Natalie Cahoon and Michael Hatolski for UV and IR data. Microanalyses were performed by Robertson Laboratory Inc., Madison, NJ.

(20) Finney, D. J. *Statistical Method in Biological Assay*, 3rd ed.; MacMillan: New York, 1978; pp 350–370.

(21) Daly, J. W. Adenosine Receptors: Targets for Future Drugs. *J. Med. Chem.* 1982, 25, 197–207.

## 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones—Inhibitors of Blood Platelet cAMP Phosphodiesterase and Induced Aggregation<sup>1</sup>

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A series of 1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one derivatives was synthesized and evaluated as inhibitors of cAMP hydrolysis by a crude human platelet phosphodiesterase preparation and as inhibitors of ADP- and collagen-induced aggregation of rabbit blood platelets. The parent structure **7a**, demonstrated potent inhibitory activity that was enhanced by the introduction of alkyl, alkoxy, or halogen substituents at the 5-, 6-, 7-, and 8-positions. Methylation at N-1 or N-3 produced weaker inhibitors of cAMP PDE and platelet aggregation. 1,3,9,9a-Tetrahydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones (**6**) were found to be equipotent with their fully oxidized congeners (**7**). On the basis of platelet inhibitory properties in vitro, efficacy at preventing thrombus formation in animal models of thrombosis, and a favorable hemodynamic profile, 1,3-dihydro-7,8-dimethyl-2*H*-imidazo[4,5-*b*]quinolin-2-one (**7o**, BMY 20844) was selected for advancement into toxicological evaluation and clinical trial. An efficient synthesis of **7o** is described.

Blood platelets normally circulate as quiescent disk-shaped cells found in the outermost layer of blood where they are poised to fulfill their role in hemostasis. Platelet involvement has been implicated in a number of disease states including migraine,<sup>2</sup> asthma,<sup>3</sup> atherosclerosis,<sup>4</sup> and

tumor-cell metastasis,<sup>5</sup> while inadequately controlled platelet activation and aggregation may lead to vascular

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(1) Presented in part at the 193rd National Meeting of the American Chemical Society, Denver, CO, April 5–10, 1987; Meanwell, N. A.; Roth, G. R.; Smith, E. C. R.; Wedding, D. L.; Wright, J. J. Inhibitors of Blood Platelet Aggregation and cAMP Phosphodiesterase; MEDI 16.

occlusion.<sup>6</sup> Thrombotic and thromboembolic events, manifest as myocardial infarction and stroke, remain the leading cause of death and disability in the United States. Inhibitors of blood platelet aggregation have been explored clinically for the management of a variety of arterial vascular disorders and in surgical procedures that inflict vascular damage or introduce artificial surfaces into the vascular tree.<sup>7,8</sup> A number of these studies have clearly demonstrated clinical benefit while others have shown only beneficial trends that failed to reach statistical significance. Recent clinical trials with antiplatelet therapy have demonstrated a significant reduction in the incidence of occlusive vascular events in both healthy individuals<sup>9</sup> and those considered to be at risk.<sup>10-14</sup> Aspirin<sup>9-11,15</sup> remains the most widely studied and clinically effective platelet-aggregation inhibitor, although some trials have shown dipyridamole, sulfinpyrazole, prostacyclin, and ticlopidine<sup>12-14</sup> to be of clinical value. However, all of these agents suffer from deficiencies such that none satisfies the requirements demanded of the ideal antiplatelet drug.<sup>8,16</sup>

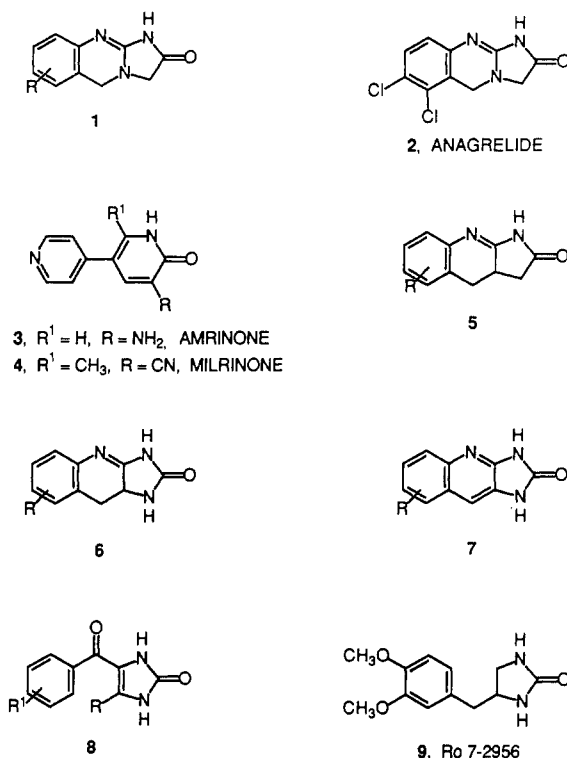
This provides an impetus for developing more powerful and effective platelet-aggregation inhibitors with fewer side effects that would allow the clinician to exercise more precise control over platelet function.

The tetrahydroimidazo[2,1-b]quinazolin-2-ones (1) were discovered by Bristol Laboratories to be potent inhibitors of in vitro blood platelet aggregation induced by a wide variety of physiologically relevant stimuli, including ADP, collagen and thrombin, and anagrelide (2) emerged from this series as a clinical candidate.<sup>17,18</sup> However, clinical development of anagrelide was suspended with the observation of a drug-induced thrombocytopenia upon multiple dosing during phase 1 evaluation. We were therefore prompted to initiate a search for potent, broad-spectrum inhibitors of blood platelet aggregation that would not induce thrombocytopenia. Anagrelide appears to modulate platelet function by inhibiting cAMP phosphodiesterase, thereby increasing intracellular cAMP levels.<sup>19-21</sup> We were encouraged that thrombocytopenia was not related to mechanism of action as a result of observations made during clinical evaluation of amrinone (3) and milrinone (4), cardiotonic agents that are selective inhibitors of low  $K_m$  cAMP PDE.<sup>22</sup> Although 4 is the more potent cardiac stimulant and enzyme inhibitor, it did not produce the thrombocytopenia in man associated with 3, suggesting that the side effect was not related to inhibition of low  $K_m$  cAMP PDE.<sup>23</sup>

The approach adopted to design target structures for biological evaluation employed the tetrahydroimidazo[2,1-b]quinazolin-2-ones (1) as a template. An analysis of the structure-activity relationship (SAR) data available at the inception of this work indicated the importance of the secondary amide functionality and its orientation, a relatively planar topography, and an intact tricyclic ring system.<sup>24-26</sup> The presence of the guanidine moiety was

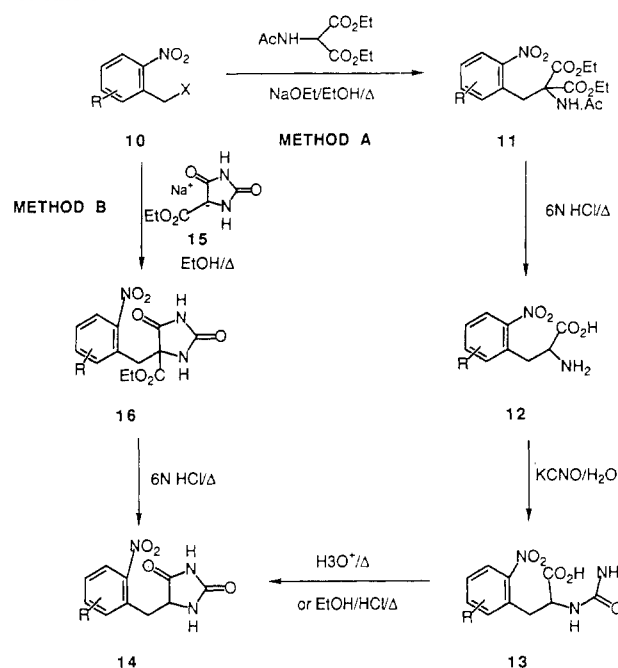
- (2) Hanington, E. The Platelet and Migraine. *Headache* 1986, 26, 411-415.
- (3) Morley, J.; Page, C. P.; Sanjar, S. Platelets in Asthma. *Lancet* 1985, ii, 726-727.
- (4) (a) Ross, R.; Glomset, J. A. The Pathogenesis of Atherosclerosis (First of Two Parts). *N. Engl. J. Med.* 1976, 295, 369-377. (b) Ross, R.; Glomset, J. A. The Pathogenesis of Atherosclerosis (Second of Two Parts). *N. Engl. J. Med.* 1976, 295, 420-425. (c) Ross, R. The Pathogenesis of Atherosclerosis: An Update. *N. Engl. J. Med.* 1986, 314, 488-500.
- (5) Honn, K. V.; Busse, W. D.; Sloane, B. F. Prostacyclin and Thromboxanes. Implications for their Role in Tumor Cell Metastasis. *Biochem. Pharmacol.* 1983, 32, 1-11.
- (6) Fuster, V.; Badimon, L.; Adams, P. C.; Turitto, V.; Cheseboro, J. Drugs Interfering with Platelet Functions: Mechanisms and Clinical Relevance. *Thrombosis and Haemostasis*; Verstraete, M., Vermeylen, J., Lijnen, H. R., Arnout, J., Eds.; International Society on Thrombosis and Haemostasis and Leuven University Press: Leuven, 1987; pp 349-418.
- (7) Gallus, A. S. The Use of Antithrombotic Drugs in Artery Disease. *Clin. Hematol.* 1986, 15, 509-559.
- (8) Harker, L. A. Antiplatelet Drugs in the Management of Patients with Thrombotic Disorders. *Semin. Thromb. Hemostas* 1986, 12, 134-155.
- (9) Steering Committee of the Physician's Health Study Research Group. Final Report on the Aspirin Component of the Ongoing Physicians' Health Study. *N. Engl. J. Med.* 1989, 321, 129-135.
- (10) Stroke Prevention in Atrial Fibrillation Study Group Investigators. Preliminary Report of the Stroke Prevention in Atrial Fibrillation Study. *N. Engl. J. Med.* 1990, 322, 863-868.
- (11) Antiplatelet Trialists' Collaboration. Secondary Prevention of Vascular Disease by Prolonged Antiplatelet Treatment. *Br. Med. J.* 1988, 296, 320-331.
- (12) Gent, M.; Blakely, J. A.; Easton, J. D.; Ellis, D. J.; Hachinski, V. C.; Harbison, J. W.; Panak, E.; Roberts, R. S.; Sicurella, J.; Turpie, A. G. G.; the CATS Group. The Canadian American Ticlopidine Study (CATS) in Thromboembolic Stroke. *Lancet* 1989, 1, 1215-1220.
- (13) Hass, W. K.; Easton, J. D.; Adams, H. P., Jr.; Pryse-Phillips, W.; Molony, B. A.; Anderson, S.; Kamm, B. A Randomized Trial Comparing Ticlopidine Hydrochloride with Aspirin for the Prevention of Stroke in High-Risk Patients. *N. Engl. J. Med.* 1989, 321, 501-507.
- (14) Balsano, F.; Rizzon, P.; Violi, F.; Scrutinio, D.; Cimminiello, C.; Aguglia, F.; Pasotti, C.; Rudelli, G.; the Studio della Ticlopidina nell'Angina Instabile Group. Antiplatelet Treatment with Ticlopidine in Unstable Angina. *Circulation* 1990, 82, 17-26.
- (15) Silver, M. J.; DiMinno, G. Aspirin as an Antithrombotic Agent. *Prog. Drug. Res.* 1989, 33, 43-62.

- (16) Harker, L. A.; Fuster, V. Pharmacology of Platelet Inhibitors. *J. Am. Coll. Cardiol.* 1986, 8, 21B-32B.
- (17) Meanwell, N. A.; Seiler, S. M. Inhibitors of Platelet cAMP Phosphodiesterase. *Drugs Future* 1990, 15, 369-390.
- (18) Meanwell, N. A.; Seiler, S. M. Platelet Activation and Drugs that Modify Platelet Function. *Drugs and the Delivery of Oxygen to Tissues*; Fleming, J. S., Ed.; CRC Press Inc.: Boca Raton, FL, 1989; Chapter 5.
- (19) Tang, S. S.; Frojmovic, M. M. Inhibition of Platelet Function by Antithrombotic Agents which Selectively Inhibit Low  $K_m$  Cyclic 3',5'-Adenosine Monophosphate Phosphodiesterase. *J. Lab. Clin. Med.* 1980, 95, 241-257.
- (20) Gillespie, E. Anagrelide: A Potent and Selective Inhibitor of Platelet Cyclic AMP Phosphodiesterase Enzyme Activity. *Biochem. Pharmacol.* 1988, 37, 2866-2868.
- (21) Seiler, S. M.; Arnold, A. J.; Grove, R. I.; Fifer, C. A.; Keely, S. L., Jr.; Stanton, H. C. Effects of Anagrelide on Platelet cAMP Levels, cAMP-Dependent Protein Kinase and Thrombin-Induced  $Ca^{++}$  Fluxes. *J. Pharmacol. Exp. Ther.* 1987, 243, 767-774.
- (22) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. Cardiotonic Agents. 1. 4,5-Dihydro-6-[4-(1H-imidazol-1-yl)phenyl]-3(2H)-pyridazinones: Novel Positive Inotropic Agents for the Treatment of Congestive Heart Failure. *J. Med. Chem.* 1984, 27, 1099-1101.
- (23) Baim, D. S.; McDowell, A. V.; Cherniles, J.; Monrad, E. S.; Parker, J. A.; Eddson, J.; Braunwald, E.; Grossman, W. Evaluation of a New Bipyridine Inotropic Agent—Milrinone—in Patients with Severe Congestive Heart Failure. *N. Engl. J. Med.* 1983, 309, 748-756.
- (24) (a) Beverung, W. N.; Partyka, R. A. Optionally Substituted 1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-2-ones and 6(H)-1,2,3,4-tetrahydropyrimido[2,1-b]quinazolin-2-ones. U.S. Patent 3,932,407, 1976. (b) Beverung, W. N.; Partyka, R. A., unpublished data.

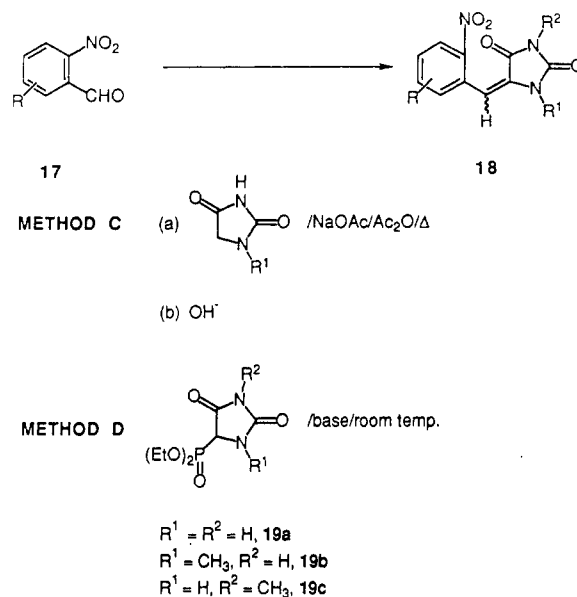


not an essential prerequisite for biological activity since tetrahydropyrrolo[2,3-*b*]quinolin-2-one derivatives (5) possessed antiaggregatory properties.<sup>27</sup> An appreciation of these factors led to the identification of imidazo[4,5-*b*]quinolin-2-ones 6 and 7 as suitable synthetic objectives. In addition to preserving the relative disposition of the salient structural features of 1, compounds 6 and 7 incorporate structural elements present in 1,3-dihydro-2*H*-imidazol-2-ones 8, a class of cardiotonic agent reported to inhibit low *K<sub>m</sub>* cAMP PDE,<sup>28</sup> and Ro 7-2956 (9), another cAMP PDE inhibitor.<sup>29</sup> A survey of the literature revealed

## Scheme I



## Scheme II



that although the parent structures 6<sup>30</sup> and 7<sup>31</sup> (R = H) were known, few other examples of this structural class had been described,<sup>32</sup> and except for the presence as the chromophore of the siderophore azotobactin,<sup>33</sup> none were

- (25) (a) Ishikawa, F.; Kosasayama, A.; Yamaguchi, H.; Watanabe, Y.; Saegusa, J.; Shibamura, S.; Sakuma, K.; Ashida, S.-i.; Abiko, Y. Cyclic Guanidines. 14. Imidazo[1,2-*a*]thienopyrimidin-2-one Derivatives as Blood Platelet Aggregation Inhibitors. *J. Med. Chem.* 1981, 24, 376-382. (b) Ishikawa, F.; Watanabe, Y. Cyclic Guanidines. VIII. Synthesis of Imidazo-1,3- and -2,4-benzodiazepine Derivatives as Blood Platelet Aggregation Inhibitors. *Chem. Pharm. Bull.* 1980, 28, 1307-1310. (c) Ishikawa, F.; Watanabe, Y.; Saegusa, J. Cyclic Guanidines. IX. Synthesis of 2-Amino-3,4-dihydroquinazolines as Blood Platelet Aggregation Inhibitors. *Chem. Pharm. Bull.* 1980, 28, 1357-1364.
- (26) Kienzle, F.; Kaiser, A.; Chodnekar, M. S. 1,5-Dihydroimidazoquinazolines as Blood Platelet Aggregation Inhibitors. *Eur. J. Med. Chem.* 1982, 17, 547-556.
- (27) Partyka, R. A.; Holava, H. M.; Beverung, W. N. Hypotensive Agents. U.S. Patent 3,974,165, 1976.
- (28) (a) Schnettler, R. A.; Dage, R. C.; Grisar, J. M. 4-Aryl-1,3-dihydro-2*H*-imidazol-2-ones, a New Class of Cardiotonic Agents. *J. Med. Chem.* 1982, 25, 1477-1481. (b) Kariya, T.; Wille, L. J.; Dage, R. C. Biochemical Studies on the Cardiotonic Activity of MDL 17,043. *J. Cardiovasc. Pharmacol.* 1982, 4, 509-514. (c) Kariya, T.; Wille, L. J.; Dage, R. C. Studies on the Mechanism of the Cardiotonic Activity of MDL 19,205: Effects on Several Biochemical Systems. *J. Cardiovasc. Pharmacol.* 1982, 6, 50-55.
- (29) Dalton, C.; Quinn, J. B.; Burghardt, C. R.; Sheppard, H. Investigation of the Mechanism of Action of the Lipolytic Agent 4-(3,4-Dimethoxybenzyl)-2-imidazolidinone (Ro 7-2956). *J. Pharmacol. Exp. Ther.* 1970, 173, 270-276.

- (30) Connors, T. A.; Ross, W. C. J.; Wilson, J. G. Aryl-2-halogenoalkylamines. Part XIX. Some *N,N*-Di-2-chloroethylaminophenyl- and -phenylalkyl hydantoin and Related Amino Acids. *J. Chem. Soc.* 1960, 2994-3007.
- (31) Kozak, J.; Musial, L. Condensation of Hydantoin with *o*-nitrobenzaldehyde. *Bull. Int. Acad. Pol. A* 1930, 432-438 (*Chem. Abstr.* 1931, 25, 5400).
- (32) (a) Fryer, R. I.; Earley, J. V.; Blount, J. F. Quinazolines and 1,4-Benzodiazepines. 77. Reaction of 2-Amino-1,4-Benzodiazepines with Bifunctional Acylating Agents. *J. Org. Chem.* 1977, 42, 2212-2219. (b) Reid, W.; Grabosch, J. Note on *N*-substituted 2,3-diaminopyridines and 2,3-dianilinoquinolines. *Chem. Ber.* 1956, 89, 2684-2687. (c) Musial, L. The Derivatives of Quinimidazolinone. *Roczniki. Chem.* 1955, 25, 46-56 (*Chem. Abstr.* 1953, 47, 4885f).

reported to exhibit biological activity. An attempt to prepare 6 ( $R = H$ ) according to the method of Connors<sup>30</sup> resulted in isolation of 7 ( $R = H$ ). Evaluation of 7 ( $R = H$ ) revealed it to be equipotent with 1 ( $R = H$ ) as an inhibitor of ADP- and collagen-induced aggregation of rabbit platelets in vitro and prompted a more detailed investigation into this structural class. The objective of this study was the identification of an orally effective antithrombotic agent that would provide an adequate level of protection while incurring minimal hemodynamic burden.

### Chemistry

All of the imidazo[4,5-b]quinolin-2-one derivatives described were prepared by way of an approach that involved closure of the central ring as the final synthetic transformation. Precursors 14 and 18 were assembled by the routes depicted in Schemes I and II. Method A (Scheme I) involves the stepwise elaboration of the hydantoin ring from a 2-nitrobenzyl halide precursor 10 as described by Connors.<sup>30</sup> Alkylation of diethyl acetamidomalonate by 10 provided 11, which was deprotected and decarboxylated simultaneously by exposure to 6 N HCl solution at reflux, affording substituted phenylalanine 12. Treatment of 12 with potassium cyanate furnished urea 13, which was cyclized to hydantoin 14 under acidic conditions. A more convergent approach to 14 was developed that relied upon alkylation of the sodium salt of ethyl hydantoin-5-carboxylate<sup>34</sup> (15) to provide 16 (method B, Scheme I). Acid-induced hydrolytic decarboxylation of 16 gave 14. 2-Nitrobenzyl halides 10 were obtained from either commercial sources or synthetically via bromination of 2-nitrotoluene derivatives with NBS in  $CCl_4$  at reflux and chlorination of 2-nitrobenzyl alcohols using thionyl chloride.<sup>24a</sup> 2-Nitrobenzyl alcohols, in turn, were prepared from anthranilic acids by a two-step sequence involving oxidation to a 2-nitrobenzoic acid, using pertrifluoroacetic acid<sup>35</sup> in  $CH_2Cl_2$ , and subsequent reduction with  $BH_3$ -THF complex in THF at 50 °C.<sup>24a</sup> The approaches depicted in Scheme I were employed for the preparation of all target molecules substituted with halogens.

5-Benzylidenehydantoin derivatives 18 were obtained by the two methods delineated in Scheme II. Condensation of 2-nitrobenzaldehydes 17, available from 2-nitrobenzyl alcohols by oxidation with PCC,<sup>36</sup> with hydantoin or 1-methylhydantoin in hot acetic anhydride containing

### Scheme III

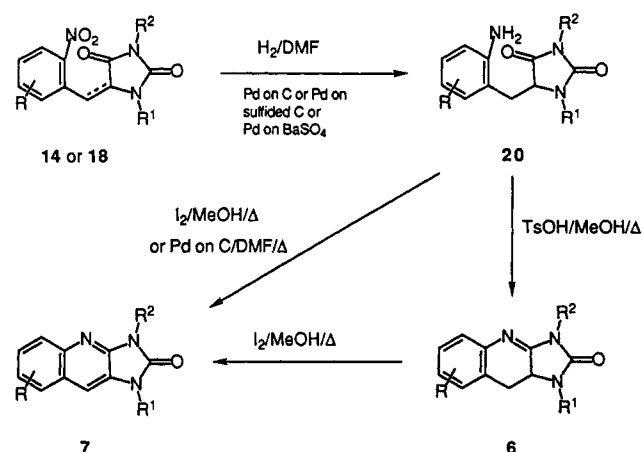


Table I. Structure and Physical Properties of 1,3,9a-Tetrahydro-2H-imidazo[4,5-b]quinolin-2-ones (6)

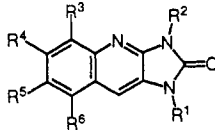
compd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	mp, °C	mol form. (elem anal.)
6e	OAc	H	H	288–290	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>
6f	OH	H	H	>330	C <sub>10</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>
6k	H	CH <sub>3</sub>	H	220–225 dec	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> O·HCl·0.55H <sub>2</sub> O
6o	CH <sub>3</sub>	CH <sub>3</sub>	H	>320	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O·HCl
6x	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	340–345 dec	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O·HCl

a molar equivalent of sodium acetate, followed by alkaline hydrolysis of the acetylated intermediate, provided adducts 18 in modest to good yield (method C, Scheme II).<sup>37</sup> However, this approach failed with 2-nitrobenzaldehydes substituted at the 6-position and prompted the development of a more effective method of executing this transformation (method D, Scheme II). Base-mediated coupling of phosphonates 19 ( $R' = R'' = H$ , 19a;<sup>38</sup>  $R' = CH_3$ ,  $R'' = H$ , 19b;  $R' = R'' = CH_3$ , 19c), readily available from the corresponding hydantonins via bromination ( $Br_2$ /AcOH/80 °C) and subsequent exposure to triethyl phosphite in AcOH, with aldehydes 17, provided adducts 18 rapidly and reliably in excellent yields under mild conditions.<sup>39</sup> Adducts 18 were frequently isolated as mixtures of geometrical isomers but this was of no consequence to the overall synthetic strategy since the olefinic bond was subsequently hydrogenated.

The synthesis of the target compounds was completed as depicted in Scheme III and was initiated by catalytic hydrogenation of 14 and 18 in DMF. 10% Palladium on charcoal was generally employed as the catalyst, with the exception of some halogen-containing substrates (14) when 5% platinum on sulfided carbon or Pd on  $BaSO_4$  was

- (33) (a) Corbin, J. L.; Karle, I. L.; Karle, J. Crystal Structure of the Chromophore from the Fluorescent Peptide Produced by Iron-Deficient *Azotobacter vinelandii*. *J. Chem. Soc., Chem. Commun.* 1970, 186–187. (b) Karle, I. L.; Karle, J. Structure of the Chromophore from the Fluorescent Peptide Produced by Iron-Deficient *Azotobacter vinelandii*. *Acta Crystallogr. Sect. B: Struct. Crystallogr. Cryst. Chem.* 1971, B27, 1891–1898. (c) Fukusawa, K.; Goto, M.; Sasaki, K.; Hirata, Y. Structure of the Yellow-Green Fluorescent Peptide Produced by Iron-Deficient *Azotobacter vinelandii* strain O. *Tetrahedron* 1972, 28, 5359–5365. (d) Demange, P.; Bateman, A.; Dell, A.; Abdallah, M. A. Structure of Azoto bacin D, a Siderophore of *Azotobacter vinelandii* strain D (CCM 289). *Biochemistry* 1988, 27, 2745–2752.
- (34) Garner, W.; Tieckelmann, H. Ethyl Hydantoin-5-carboxylates. *J. Org. Chem.* 1964, 29, 2003–2005.
- (35) (a) Emmons, W. D. Peroxytrifluoroacetic Acid. II. The Oxidation of Anilines to Nitrobenzenes. *J. Am. Chem. Soc.* 1954, 76, 3470–3474. (b) Pagano, A. S.; Emmons, W. D. 2,6-Dichloronitrobenzene. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 367–370.
- (36) 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.

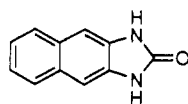
- (37) Billek, G. Condensation of Aromatic Aldehydes with Hydantoin. *Monatsch. Chem.* 1961, 92, 352–360.
- (38) Khokhlov, P. S.; Kashemirov, B. A.; Mikityuk, A. D.; Chimishkyan, A.; Strepikheev, Yu. A. Phosgenation of Amino-phosphonoacetic Esters. *Zh. Obshch. Khim.* 1984, 54, 3337–3339.
- (39) The synthesis and reactions of hydantoin-5-phosphonates 19 ( $R' = H$ ,  $R'' = H$ ) and 20, ( $R' = CH_3$ ,  $R'' = H$ ) will be the subject of a separate publication. A preparative procedure can be found in U.S. Patent 4,668,686, May 26, 1987.

**Table II.** Structure and Physical Properties of 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones (**7a–7ad**)


compd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	mp, °C	mol form. (elem anal.)
<b>7a</b>	H	H	H	H	H	H	358–361	C <sub>10</sub> H <sub>7</sub> N <sub>3</sub> O·HCl·0.1H <sub>2</sub> O
<b>7b</b>	H	H	H	H	F	H	>360	C <sub>10</sub> H <sub>6</sub> FN <sub>3</sub> O·0.42H <sub>2</sub> O <sup>a</sup>
<b>7c</b>	H	H	H	H	Cl	H	>360	C <sub>10</sub> H <sub>6</sub> ClN <sub>3</sub> O·0.1H <sub>2</sub> O
<b>7d</b>	H	H	H	H	CH <sub>3</sub>	H	>360	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O·HCl
<b>7e</b>	H	H	H	H	OAc	H	>330	C <sub>12</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>
<b>7f</b>	H	H	H	H	OH	H	>400	C <sub>10</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub> ·0.1CH <sub>3</sub> OH·0.1H <sub>2</sub> O
<b>7g</b>	H	H	H	H	OCH <sub>3</sub>	H	>360	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>
<b>7h</b>	H	H	H	H	OE <sub>t</sub>	H	>320	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>
<b>7i</b>	H	H	H	H	O <sup>i</sup> Pr	H	>320	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>
<b>7j</b>	H	H	H	H	H	Cl	>360	C <sub>10</sub> H <sub>6</sub> ClN <sub>3</sub> O·0.1H <sub>2</sub> O
<b>7k</b>	H	H	H	H	H	CH <sub>3</sub>	350–355 dec	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O·HCl·0.35H <sub>2</sub> O
<b>7l</b>	H	H	H	H	H	OCH <sub>3</sub>	>300	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub> ·0.25H <sub>2</sub> O
<b>7m</b>	H	H	CH <sub>3</sub>	H	H	H	340–343	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O
<b>7n</b>	H	H	OCH <sub>3</sub>	H	H	H	351–354 dec	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>
<b>7o</b>	H	H	H	H	CH <sub>3</sub>	CH <sub>3</sub>	>360	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O
<b>7p</b>	H	H	H	CH <sub>3</sub>	CH <sub>3</sub>	H	>360	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O·HCl·0.15H <sub>2</sub> O
<b>7q</b>	H	H	H	CH <sub>3</sub>	Cl	H	>360	C <sub>11</sub> H <sub>8</sub> ClN <sub>3</sub> O <sub>2</sub>
<b>7r</b>	H	H	H	H	CH <sub>3</sub>	Cl	>360	C <sub>11</sub> H <sub>8</sub> ClN <sub>3</sub> O·0.2H <sub>2</sub> O
<b>7s</b>	H	H	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	>360	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>
<b>7t</b>	H	H	H	CH <sub>3</sub>	Cl	CH <sub>3</sub>	>300	C <sub>12</sub> H <sub>10</sub> ClN <sub>3</sub> O
<b>7u</b>	H	H	H	CH <sub>3</sub>	Br	CH <sub>3</sub>	>300	C <sub>12</sub> H <sub>10</sub> BrN <sub>3</sub> O
<b>7v</b>	H	H	H	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	>320	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>4</sub>
<b>7w</b>	CH <sub>3</sub>	H	H	H	H	H	309–311	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O
<b>7x</b>	CH <sub>3</sub>	H	H	H	H	CH <sub>3</sub>	340–341 dec	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O·HCl·0.1H <sub>2</sub> O
<b>7y</b>	CH <sub>3</sub>	H	H	H	CH <sub>3</sub>	H	>320	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O·0.04H <sub>2</sub> O
<b>7z</b>	CH <sub>3</sub>	H	H	H	OCH <sub>3</sub>	H	>310	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O·0.02H <sub>2</sub> O
<b>7aa</b>	CH <sub>3</sub>	H	H	H	CH <sub>3</sub>	CH <sub>3</sub>	>300	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O
<b>7ab</b>	H	CH <sub>3</sub>	H	H	OCH <sub>3</sub>	H	296–297	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>
<b>7ac</b>	H	CH <sub>3</sub>	H	H	CH <sub>3</sub>	CH <sub>3</sub>	274–277	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O·CH <sub>3</sub> SO <sub>3</sub> H
<b>7ad</b>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	CH <sub>3</sub>	CH <sub>3</sub>	354–357 dec	C <sub>14</sub> H <sub>15</sub> N <sub>3</sub> O·HCl

<sup>a</sup> N: calcd, 19.95; found, 19.24.

substituted. Racemic anilines **20** were usually not isolated. Exposure of the crude hydrogenation product to a catalytic quantity of pTsOH in hot methanol or acetonitrile provided **6** and those representatives of **6** characterized are listed in Table I. Oxidation of **6** to **7** was most effectively accomplished by treatment with a molar equivalent of iodine in either methanol or acetonitrile at reflux or in DMF at 65 °C. In practice, it proved more expedient to effect conversion of **20** to the target compounds **7** in a single operation by sequential treatment with pTsOH and I<sub>2</sub> in either methanol or acetonitrile at reflux. Alternatively, heating solutions of **20** in DMF on a steam bath in the presence of the hydrogenation catalyst promoted cyclization and oxidation. 7-Hydroxy derivative **7f** was prepared from acetate **7e** by alkaline hydrolysis and 1,3,7,8-tetramethyl congener **7ad** was obtained from **7o** by alkylation with excess iodomethane in DMF at 100 °C using pulverized K<sub>2</sub>CO<sub>3</sub> as the base. The 1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one derivatives synthesized for this study are presented in Table II. The known imidazonaphthalene **21**<sup>40</sup> was prepared from commercially available 2,3-diaminonaphthalene by stirring with 1,1'-carbonyldiimidazole in THF overnight at room temperature.

**21**

### Biological Evaluation

The target compounds **6**, **7**, and **21** were evaluated as inhibitors of a crude human platelet phosphodiesterase enzyme preparation using methods previously described.<sup>20</sup> The IC<sub>50</sub>'s for prevention of [<sup>3</sup>H]cAMP and [<sup>3</sup>H]cGMP hydrolysis were determined from dose-response curves and are reported in Table III. In this assay, anagrelide (**2**) displayed IC<sub>50</sub>'s of 5.4 ± 1.4 × 10<sup>-8</sup> M vs cAMP and 34 ± 7 × 10<sup>-6</sup> M vs cGMP. Inhibition of blood platelet aggregation was assessed in vitro in rabbit platelet-rich plasma (PRP), with ADP and collagen as the activating agents according to established protocols.<sup>41</sup> Dose-response curves were obtained and effective concentration (EC<sub>50</sub>) values determined. The results are presented in Table III and are in most cases the result of a single determination. Representative compounds were evaluated as inhibitors of ADP-induced aggregation of human platelets and the EC<sub>50</sub>'s are listed in Table III. Anagrelide was employed as a positive reference agent in these evaluations and displayed EC<sub>50</sub>'s of 1.05 ± 0.27 μM vs ADP and 0.27 ± 0.07 μM vs collagen in rabbit PRP and 1.05 ± 0.3 μM vs ADP in human PRP. A measure of oral efficacy of drug candidates was determined by ex vivo aggregometry studies. PRP was obtained from rats 2 h following oral administration of the drug. The extent of aggregation in response to an ADP challenge was compared to that of controls.<sup>41</sup>

(40) Staab, H. A. Reactive Heterocyclic Diamides of Carbon Dioxide. *Justus Liebigs Ann. Chem.* 1957, 609, 75–83.

(41) 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 (BMJ 20844). *Thromb. Res.* 1989, 56, 333–346.

Table III. Biological Evaluation of Imidazo[4,5-b]quinolin-2-one Derivatives 6 and 7 and Related Compounds

compd	EC <sub>50</sub> , μM	rat ex vivo vs ADP: ED <sub>50</sub> , mg/kg				
	inhibn of human platelet phosphodiesterase: IC <sub>50</sub> , μM		inhibn of platelet aggregation in rabbit PRP		inhibn of ADP-induced* platelet aggregation in human PRP	
	vs cAMP	vs cGMP	vs ADP	vs collagen		
7a	0.5	80	30	4.5		NT
7b	0.3	>100	3.5	0.37		18.9
7c	0.05	>100	1.8	0.56		14.9
7d	0.05	>100	0.75	0.25		12.2
7e	0.2	NT	6.2	1.03		NT
7f	0.3	NT	0.49	0.05		10
7g	0.035	>100	0.19	0.09	0.003	5
7h	0.02	>100	0.22	0.22		>10
7i	0.008	50	0.62	0.41	0.03	8.2
7j	0.3	>100	3.6	0.9		12.6
7k	0.01	>100	0.33	0.12		13.3
7l	0.15	>100	0.68	0.46	0.18	8.4
7m	0.09	NT	2.51	0.75		>10
7n	2.0	NT	4.65	1.16		NT
7o	0.01	20	0.39	0.12	0.02	3.2
7p	0.08	>100	2.0	0.4		18.3
7q	0.05	>100	2.6	1.3	0.4	6.8
7r	0.04	>100	0.4	0.4		73
7s	0.005	70	0.53	0.33		18.8
7t	0.02	60	0.44	0.36		32
7u	0.005	6	3.3	3.2		>10 <sup>b</sup>
7v	2.0	NT	0.91	0.47		NT <sup>c</sup>
7w	20.0	60	>150	>150		NT
7x	7.0	60	40	40		NT
7y	3.0	70	33	13		NT
7z	3.0	30	17	17		NT
7aa	1.5	>100	31	13		NT
7ab	3.0	NT	3.48	1.09	>140	NT
7ac	3.0	NT	5.57	0.77	>100	NT
7ad	70.0	NT	>100	>100		NT
6a	0.1	>100	0.2	0.12		NT
6b	0.03	40	0.4	0.16		>10
6c	9.0	80	79	48		NT
6d	0.3	NT	0.61	0.16		NT
6e	0.4	NT	1.48	0.74		NT
2l	7.0	>100	380	163		NT
1 R = H)	NT	NT	32	11		NT
2	0.05	34	1.05	0.27	1.05	4.9

<sup>a</sup> 5.86 μM ADP. <sup>b</sup> 33% inhibition at 10 mg/kg. <sup>c</sup> NT = not tested.

ED<sub>50</sub>'s were determined in milligrams per kilogram and are presented in Table III.

## Results and Discussion

The parent compound, 7a, is an effective inhibitor of ADP- and collagen-induced aggregation of rabbit blood platelets in vitro (Table III). The level of biological activity intrinsic to 7a is comparable to that inherent in the imidazo[2,1-b]quinazolin-2-one skeleton (1, R = H), the prototype of a number of potent cAMP PDE inhibitors that have demonstrated platelet inhibitory, cardiostimulant, and vasodilatory properties.<sup>17,18</sup> Analogous to 1 (R = H) and its congeners, 7a is a potent inhibitor of cAMP hydrolysis by a crude enzyme preparation but is 60-fold weaker when cGMP is presented as the substrate. In this assay, 7a is of comparable potency to milrinone and superior to amrinone.<sup>20</sup> Molecular modeling studies have demonstrated that a dipole associated with an acidic hydrogen atom and located ca. 5 Å away from the center of an aromatic ring, configured in a topographically relatively planar arrangement, are the essential structural features common to many potent and selective inhibitors of low K<sub>m</sub> cAMP

PDE.<sup>42-45</sup> These structural elements are readily apparent in 7a, which is virtually planar,<sup>33</sup> and prompted further study in an effort to define the structural requirements responsible for potent interaction with the PDE enzyme and to identify compounds possessing enhanced biological activity.

- (42) Rakhit, S.; Marciniak, G.; Leclerc, G.; Schwarz, J. Computer Assisted Pharmacophore Search in a Series of Non-steroidal Cardiostimulants. *Eur. J. Med. Chem.* 1986, 21, 511-515.
- (43) 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.
- (44) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A.; McPhail, A. T. Cardiostimulant 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.
- (45) Erhardt, P. W.; Hagedorn, A. A., III; Sabio, M. Cardiostimulant Agents. 3. A Topographical Model of the Cardiac cAMP Phosphodiesterase Receptor. *Mol. Pharmacol.* 1988, 33, 1-13.

It is apparent from the data presented in Table III that the introduction of one or more methyl, alkoxy, or halogen substituents, or combinations thereof, at the 5-, 6-, 7-, and 8-positions of **7a**, uniformly produces more potent inhibitors of agonist-induced platelet aggregation, **7b-v**. The most powerful inhibitors of ADP-induced platelet activation are **7g**, **7h**, **7k**, **7o**, and **7r**, with  $EC_{50}$ 's of 0.4  $\mu$ M or less, while **7d**, **7f**, **7i**, **7l**, **7s**, and **7t** are half-maximally effective at submicromolar concentrations. This compares with anagrelide, which displays an  $EC_{50}$  of 1.1  $\mu$ M vs ADP-induced aggregation of rabbit platelets. With the exceptions of **7n** and **7v**, these structural modifications are associated with an increased capacity to inhibit cAMP cleavage by the crude human platelet enzyme preparation. The most effective inhibitors of the enzyme, **7s** and **7u**, are 100-fold more potent than the prototype **7a**. The high level of biological activity observed for the 7-alkoxy-substituted compounds **7g**, **7h**, **7i**, and **7s** may reflect the hydrogen-bonding potential of this substituent, which is located in a region of the pharmacophore where many potent low  $K_m$  cAMP PDE inhibitors present hydrogen-bond-accepting functionality.<sup>42-45</sup> 7-Methoxy derivative **7g** is the most powerful platelet-aggregation inhibitor identified within this series. Increasing the lipophilicity of this substituent by homologation (**7h** and **7i**) led to a reduction in platelet inhibitory activity but, in contrast, enhanced activity in the enzyme assay. Although these trends are small, they are consistent with the notion of a lipophilic window that modulates drug delivery to the interior of the platelet.<sup>46,47</sup>

Substitution at the 6-, 7-, and/or 8-positions of **7a** also enhances the apparent selectivity for inhibition of cAMP hydrolysis compared to cGMP degradation. Compounds **7a-d**, **7g-l**, and **7o-u** significantly attenuate cGMP cleavage only when drug concentrations are at least 300-fold higher than that required to half-maximally inhibit cAMP hydrolysis. **7k** and **7s** demonstrate over 10000-fold selectivity in this respect. However, although the crude platelet enzyme preparation is of physiological relevance, quantitative evaluation of the absolute isozyme specificity exhibited by these compounds is not possible with this assay.

By aligning N-3 of **7** with N-1 of **1**, the 6-, 7-, and 8-positions of **7** correspond to the 8-, 7-, and 6-positions of **1**, respectively, and SAR with respect to substitution at these sites is qualitatively similar for the two series.<sup>24,26</sup> However, some divergence is apparent when the effects of substitution at C-5 of **7** are compared with similar modifications at the analogous position (C-9) of **1**. The C-5 methyl and methoxyl derivatives (**7m** and **7n**) are effective inhibitors of platelet aggregation and cAMP PDE but, in contrast, C-9 substituted derivatives of **1** are only weakly active or inactive in these assays,<sup>24,26,46</sup> although positive inotropic activity is maintained.<sup>48</sup> This disparity may be accounted for by considering that **7m** and **7n** are urea derivatives and as such possess two relatively acidic hy-

drogen atoms associated with the dipole that is crucial for interaction with the low  $K_m$  cAMP PDE isozyme. Presumably, either of these hydrogen atoms could participate in binding to the elements within the enzyme that are thought to recognize the cyclic phosphate moiety of the substrate.<sup>42-45</sup> If **7m** and **7n** present the N-1 H to the enzyme, the relationship between this atom and the C-5 substituent would be analogous to that between the N-3 H and the C-8 substituent in **7k** and **7l**. Only the quinoline nitrogen atom (N-4) distinguishes the two edges to **7** and some measure of the contribution of this atom to biological activity can be obtained by comparison of the relative potencies of **7k** with **7l** and **7m** with **7n**. The C-5 substituted compounds **7m** and **7n** are 2-6-fold weaker platelet-aggregation inhibitors and are 20-30-fold less effective inhibitors of cAMP PDE than the C-8-substituted analogues **7k** and **7l**. In both cases, a methyl substituent is associated with more potent biological activity and to a similar extent compared to a methoxyl. It is not clear if the reduced potency associated with **7m** and **7n** is the result of removal of a beneficial effect, the introduction of a deleterious interaction, or some combination of the two associated with the relative location of the quinoline nitrogen atom. Replacement of N-4 of **7a** by a C-H, to give **21**, results in a similar diminution in biological activity, suggesting that this nitrogen atom plays a functionally relevant role.<sup>48</sup>

Methylation at N-1 produced inhibitors of platelet function (**7w-7aa**) weaker by at least 1 order of magnitude than identically substituted N-1 H analogues. This is associated with a similar reduction in cAMP PDE inhibitory activity, although the effects on cGMP hydrolysis are much less pronounced. As a consequence, the N-1 methylated compounds **7w-7aa** exhibit an altered selectivity index with respect to inhibition of cyclic nucleotide cleavage, with ratios of the order of only 20-30-fold in favor of preventing cAMP hydrolysis. However, once again this data must be interpreted with some caution due to the presence of more than a single enzyme in the crude platelet preparation. The deleterious effects on biological activity associated with methylation at N-1 of **7** parallels observations made with C-3-methylated derivatives of **1** and has been interpreted to be the result of unfavorable steric interactions between the inhibitor and the enzyme.<sup>24,26,46</sup>

Methylation at N-3 (**7ab** and **7ac**) is associated with an 85-300-fold reduction in platelet PDE inhibitory activity when compared to the analogous N-H derivatives (**7g** and **7o** as well as **7i**, **7l**, and **7q**, respectively). This was accompanied by a much less dramatic decline in platelet-inhibitory properties, which is somewhat surprising in light of the SAR discussed above and not readily explained. However, in marked contrast to **7g** and **7o**, as well as **7i**, **7l**, and **7q**, **7ab** and **7ac** are quite ineffective inhibitors of ADP-induced aggregation of human blood platelets, with  $EC_{50}$ 's in excess of 100  $\mu$ M (Table III). These observations may reflect species-dependent differences between the platelet PDE's or be the result of interference with other regulatory pathways in rabbit platelets.

The single N-1,N-3-dimethylated derivative (**7ad**) is devoid of significant biological activity in the assays in which it was evaluated and demonstrates a requirement for at least one acidic hydrogen associated with the urea moiety.

The presence of a quinoline ring in **7** provides an opportunity to simultaneously vary electronic and stereochemical parameters that only subtly alter the relative spatial disposition of the structural elements identified as being essential for expression of biological activity. By

- (46) 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, 30, 303-318.
- (47) 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.
- (48) Bell, A. S.; Campbell, S. F.; Roberts, D. A.; Ruddock, K. S. 7-Heteroaryl-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-(1*H*)-one Derivatives with Cardiac Stimulant Activity. *J. Med. Chem.* 1989, 32, 2042-2049.

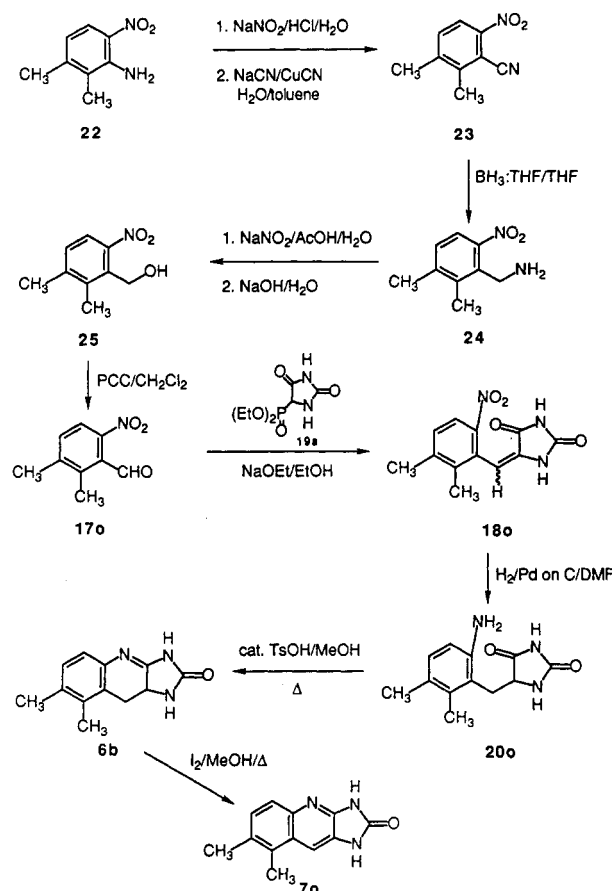


manipulating the oxidation state of the central pyridine-derived heterocycle, aromaticity and its attendant planarity can be confined to a single ring, as in 6, or allowed to extend into the central region of the molecule, as in 7. The partially reduced imidazo[4,5-*b*]quinolin-2-ones 6e, 6f, 6k, 6o, and 6x exhibit biological activity at a level very close to their fully oxidized congeners 7o, 7c, 7f, 7k, and 7x, respectively. For example, 6o is practically indistinguishable from 7o as an inhibitor of platelet aggregation and cAMP PDE. SAR within this short series of compounds parallels that for the quinoline derivatives 7. These observations suggest that the absolute planarity associated with 7 is not essential for potent cAMP PDE- and platelet-inhibitory activity and that electronic distribution within the aromatic region is of little importance.

A number of the compounds listed in Table I were evaluated for inhibitory effects on ADP-induced aggregation of rat platelets, measured *ex vivo* 2 h following oral administration of the drug. Six compounds, 7f, 7g, 7i, 7l, 7o, and 7q, demonstrated ED<sub>50</sub>'s below 10 mg/kg in this protocol and emerged as candidates for further study. 7,8-Dimethylated derivative 7o exhibited the greatest activity in this procedure with an EC<sub>50</sub> of 3.2 mg/kg, which compares favorably to an ED<sub>50</sub> of 4.9 mg/kg recorded for anagrelide. However, there is no apparent correlation between the activities of these agents measured *in vitro* and *ex vivo*, which presumably reflects differences in absorption, pharmacokinetics, and metabolism of the individual agents. 1,3,9a-Tetrahydro-2H-imidazo[4,5-*b*]quinolin-2-one (6b) studied for *ex vivo* platelet inhibitory activity is much less active than its oxidized analogue (7o).

Of those representatives of 7 profiled for hemodynamic effects in anesthetized ferrets,<sup>41</sup> all elicited some increase in heart rate and right ventricular contractile force and a reduction in blood pressure, dependent upon the dose (data not shown). This is consistent with an inhibition of the low *K<sub>m</sub>*, cGMP-inhibited, cAMP PDE in the myocardium and in vascular smooth muscle. In selecting compounds for further advancement, particular emphasis was placed on those agents that provided effective protection against experimentally induced thrombosis in animal models at doses associated with minimal hemodynamic modification. As a consequence, 7o (BMY 20844) was selected for toxicological evaluation as a prelude to clinical studies. The biochemical properties, pharmacological profile, and antithrombotic activity of BMY 20844 (7o) have been described in detail.<sup>41,49</sup> In order to provide significant quantities of 7o to facilitate development of this compound, the synthetic approach depicted in Scheme IV was developed. Diazotization of the commercially available aniline 22<sup>50</sup> was efficiently accomplished with sodium nitrite in aqueous HCl at 5 °C, followed by warming to 20 °C, filtering, and recooling to 5 °C. Addition of a mixture of NaCN and CuCN afforded benzonitrile 23<sup>51</sup> in 91% overall yield. Attempts to reduce nitrile 23 directly to aldehyde 26 in high yield were not successful, necessitating the adoption of a multistep procedure. Reduction of 23 with BH<sub>3</sub>-THF in THF provided amine 24<sup>24a</sup> (95%), which was diazotized with sodium nitrite in aqueous acetic acid.<sup>52</sup>

Scheme IV



After alkaline hydrolysis of the crude mixture of alcohol 25 and its acetate, 25 was obtained in 93% yield. Oxidation of 25 with PCC<sup>36</sup> in CH<sub>2</sub>Cl<sub>2</sub> furnished aldehyde 17o (mp 66–68 °C) in 93% yield. Coupling of 17o with phosphonate 19a provided adduct 18o in 86% yield as a single geometrical isomer identified as having the *Z* configuration.<sup>39</sup> A second crop of product amounted to 12% as a 1:1 mixture of *E* and *Z* isomers. Exhaustive catalytic hydrogenation over 10% Pd on C in DMF provided racemic aniline 20o (mp >360 °C) in quantitative yield. Cyclization and oxidation of 20o was accomplished by treatment with I<sub>2</sub> in methanol at reflux to give product 17o in 65% yield.

## Conclusion

In summary, the design and synthesis of a new class of selective and potent inhibitors of platelet cAMP PDE has been described. Potent biological activity was observed with the prototype 7a and enhanced by introduction of relatively simple substituents at the 5-, 6-, 7-, and/or 8-positions. Partially reduced compounds 6, in which aromaticity is limited to a single ring were found to be equipotent with their fully oxidized congeners 7. The structure-activity relationships observed for 7 generally parallel those recorded for imidazo[2,1-*b*]quinazolin-2-ones (1) and are consistent with the pharmacophore developed for inhibitors of low *K<sub>m</sub>* cAMP PDE from a study of several different structural types.<sup>42–45</sup> Compounds 6 and 7 are effective inhibitors of platelet aggregation *in vitro* and several representatives of 7 demonstrate platelet inhibitory

(49) 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, 21–42.

(50) Aldrich Chemical Co.

(51) Fischer, A.; Greig, C. C. Formation of Adducts in the Nitration of 2,3- and 3,4-Dimethylbenzonitriles, and their Rearomatization. A 1,3 Nitro shift. *Can. J. Chem.* 1974, 52, 1231–1240.

(52) Kornblum, N.; Iffland, D. C. The Selective Replacement of the Aromatic Primary Amino Group by Hydrogen in Aromatic-Aliphatic Diamines. *J. Am. Chem. Soc.* 1949, 71, 2137–2143.



activity following oral administration to rats. 1,3-Dihydro-7,8-dimethyl-2H-imidazo[4,5-*b*]quinolin-2-one (**7o**, BMY 20844) was selected from this series as a candidate for clinical assessment and has been studied in some detail at the biochemical<sup>49</sup> and pharmacological<sup>41</sup> level.

Some of the most powerful inhibitors of platelet function identified within this series (**7**) are substituted at the 7-position with an alkoxy moiety, which may act as a hydrogen-bond acceptor. The effect on biological activity of elaboration of this substituent to incorporate functional groups several atoms remote from the heterocycle that are capable of interacting with a secondary binding site within the enzyme<sup>46,47</sup> will be the subject of future publications.

## Experimental Section

Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance (<sup>1</sup>H NMR) spectra were recorded either on a Perkin Elmer R32 90-MHz CW spectrometer or a Bruker AM 300-MHz FT instrument. All spectra were recorded with DMSO-*d*<sub>6</sub> or CDCl<sub>3</sub> as solvent with tetramethylsilane as an internal standard. Signal multiplicity was designated according to the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet. Infrared (IR) spectra were obtained with a Nicolet MX1 FT spectrometer, scanning from 4000 to 400 cm<sup>-1</sup> and calibrated to the 1601 cm<sup>-1</sup> absorption of a polystyrene film. Mass spectral data were obtained on a Finnigan Model 4500 GC/MS by using electron or chemical-ionization procedures. Analytical samples were dried in vacuo at 78 °C or in the presence of phosphorus pentoxide at room temperature for at least 12 h. Elemental analyses were provided by Bristol-Myers' Analytical Chemistry Department and C, H, and N values are within ±0.4 of calculated values.

**Ethyl DL-N-Acetyl-α-(ethoxycarbonyl)-4,5-dimethyl-2-nitrophenylalaninate (11p).** Sodium (5.07 g, 0.22 g atom) was dissolved in EtOH (1 L) and diethyl acetamidomalonate (47.86 g, 0.22 mol) added. After 5 min, 3,4-dimethyl-6-nitrobenzyl chloride (44.00 g, 0.22 mol) was added and the mixture heated to reflux for 90 min. The solvent was evaporated; the residue was diluted with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). After drying, the organic phase was concentrated to leave a solid which was dissolved in CH<sub>2</sub>Cl<sub>2</sub>. Addition of hexanes precipitated **11p** (55.93 g, 66%), mp 155–158 °C. Anal. (C<sub>18</sub>H<sub>24</sub>N<sub>2</sub>O<sub>7</sub>) C, H, N.

**DL-4,5-Dimethyl-2-nitrophenylalanine Hydrochloride (12p).** A mixture of **11p** (54.93 g, 0.15 mol), concentrated HCl solution (250 mL), and water (250 mL) was heated at reflux for 21 h. The mixture was concentrated in vacuo; the residue was dissolved in hot MeOH (900 mL), filtered, and diluted with Et<sub>2</sub>O to precipitate **12p** (32.25 g, 81%), mp 226–228 °C dec. Anal. (C<sub>11</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>·HCl·0.25H<sub>2</sub>O) C, H, N.

**DL-N-(Aminocarbonyl)-4,5-dimethyl-2-nitrophenylalanine (13p).** Potassium cyanate (35.00 g, 0.43 mol) was added portionwise to a solution of **12p** (30.00 g, 0.11 mol) in H<sub>2</sub>O (250 mL). The mixture was heated on a steam bath for 30 min, cooled, and acidified with dilute HCl solution. Filtration gave **13p** (30.00 g, 97%) as a grey solid, mp 214–216 °C dec. Anal. (C<sub>12</sub>H<sub>15</sub>N<sub>3</sub>O<sub>5</sub>) C, H, N.

**5-[(4,5-Dimethyl-2-nitrophenyl)methyl]-2,4-imidazolidinedione (14p).** A mixture of **13p** (29.00 g, 106 mmol), and 10% HCl in EtOH (400 mL) was heated at reflux for 18 h. The mixture was cooled; a brown solid was filtered off and combined with the residue from the mother liquor after concentration. Recrystallization from EtOH afforded **14p** (20.30 g, 72%), mp 248–249 °C dec. Anal. (C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>) C, H, N.

**1,3-Dihydro-6,7-dimethyl-2H-imidazo[4,5-*b*]quinolin-2-one Hydrochloride (7p).** A solution of **14p** (3.00 g, 11.4 mmol) in DMF (100 mL) was hydrogenated over 10% palladium on charcoal (0.3 g) until hydrogen uptake ceased. The reaction mixture was heated on a steam bath for 3 h before filtering through Celite. Evaporation of the solvent afforded an oil which was dissolved in boiling methanol (150 mL) and treated with iodine (2.00 g, 7.5 mmol) added in two equal portions over 15 min. After a further 15 min at reflux, the solvent was evaporated and a solution of sodium carbonate (9.00 g) and sodium thiosulfate (9.00 g) in water (180 mL) added. A pale yellow solid was filtered off and dissolved

in a 10% solution of HCl gas in methanol. The solvent was evaporated and the residue recrystallized from methanol to give **7p** (1.04 g, 47%): mp >360 °C; IR (KBr) 3470, 2940, 1738, 1625 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.42 (3 H, s, CH<sub>3</sub>), 2.47 (3 H, s, CH<sub>3</sub>), 7.78 (3 H, s, aromatic H), 9.60 (2 H, bs, H<sup>+</sup>, NH), 11.60 (1 H, bs, NHCO). Anal. (C<sub>12</sub>H<sub>11</sub>N<sub>3</sub>O·HCl·0.5H<sub>2</sub>O) C, H, N.

**Ethyl 4-[(5-Chloro-4-methyl-2-nitrophenyl)methyl]-2,5-dioxoimidazolidine-4-carboxylate (16q).** Ethyl 2,5-dioxoimidazolidine-4-carboxylate sodium salt<sup>34</sup> (15; 15.50 g, 80 mmol) was added to a solution of 1-chloro-5-(chloromethyl)-2-methyl-4-nitrobenzene (17.57 g, 80 mmol) in ethanol (250 mL) and the mixture heated to reflux under an atmosphere of argon. After 16 h, the solvent was evaporated; the residue was diluted with water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined extracts were dried over sodium sulfate and concentrated in vacuo to afford a solid which was dissolved in CH<sub>2</sub>Cl<sub>2</sub>. Addition of hexane precipitated **16q** (12.35 g, 43%): mp 176–178 °C; IR (KBr) 3305, 1750, 1715, 1530 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 1.08 (3 H, t, J = 7 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 2.28 (3 H, s, aryl CH<sub>3</sub>), 3.56 (2 H, s, benzylic CH<sub>2</sub>), 4.10 (2 H, q, J = 7 Hz, OCH<sub>2</sub>), 7.46 (1 H, s, aromatic H meta to NO<sub>2</sub>), 7.99 (1 H, s, aromatic H ortho to NO<sub>2</sub>), 8.44 (1 H, bs, NH), 11.16 (1 H, bs, NH); MS *m/z* 356, 358 (MH<sup>+</sup>). Anal. (C<sub>14</sub>H<sub>14</sub>ClN<sub>3</sub>O<sub>6</sub>) C, H, N.

**5-[(5-Chloro-4-methyl-2-nitrophenyl)methyl]-2,4-imidazolidinedione (14q).** A mixture of **16q** (11.85 g, 33 mmol), concentrated HCl solution (175 mL), and water (175 mL) was heated at reflux for 2 h. After cooling, the precipitate was filtered off, washed with water, and dried in vacuo at 78 °C to afford **14q** (8.76 g, 95%), mp 211–214 °C. Anal. (C<sub>11</sub>H<sub>10</sub>ClN<sub>3</sub>O<sub>4</sub>) C, H, N.

**1,3-Dihydro-7-chloro-6-methyl-2H-imidazo[4,5-*b*]quinolin-2-one (7q).** A solution of **14q** (2.00 g, 7 mmol) in DMF (30 mL) was hydrogenated over 5% platinum on sulfided carbon (0.4 g) at 55 psi until hydrogen uptake ceased. The mixture was heated on a steam bath for 2 h and concentrated in vacuo and the residue treated with hot (90 °C) DMF. Filtration through Celite and evaporation of the solvent afforded a solid which was washed with ether and suspended in boiling methanol. Filtration gave **7q** (1.40 g, 84%): mp >360 °C; IR (KBr) 3225, 1725, 1535 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 2.76 (3 H, s, CH<sub>3</sub>), 7.92 (1 H, s, aromatic H), 8.14 (1 H, s, aromatic H), 8.38 (1 H, s, aromatic H); MS *m/z* 233, 235 (M<sup>+</sup>). Anal. (C<sub>11</sub>H<sub>10</sub>ClN<sub>3</sub>O) C, H, N, Cl.

**(E)-5-[(2-Nitro-5-methoxyphenyl)methylene]-2,4-imidazolidinedione (18q).** A mixture of 5-methoxy-2-nitrobenzaldehyde<sup>53</sup> (10.00 g, 55 mmol), imidazolidine-2,4-dione (5.52 g, 55 mmol), fused sodium acetate (4.53 g, 55 mmol), and acetic anhydride (75 mL) was heated under reflux for 1 h. The mixture was cooled and water (30 mL) added. After the commencement of an exothermic reaction, the mixture was diluted with water (270 mL) added portionwise over 15 min. The reaction mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 100 mL), and the combined extracts were dried over sodium sulfate and concentrated to afford an oil which was dissolved in methanol (150 mL) and treated with 4 N sodium hydroxide solution (150 mL). The reaction mixture was stirred for 1 h and acidified to pH = 2 with 2 N HCl solution and a tan precipitate filtered off, washed with water, and dried in air. This was suspended in methanol and filtered to give **18q** (8.00 g, 55%), mp 294–295 °C dec. Anal. (C<sub>11</sub>H<sub>9</sub>N<sub>3</sub>O<sub>5</sub>) C, H, N.

**7-Methoxy-1,3-dihydro-2H-imidazo[4,5-*b*]quinolin-2-one (7g).** A solution of **18q** (4.50 g, 17 mmol) in DMF (120 mL) was hydrogenated over 10% palladium on charcoal (0.45 g) at 60 psi in a Parr hydrogenation apparatus. After 42 h, the mixture was filtered through Celite and the solvent evaporated to leave a brown solid. Methanol (150 mL) was added, the mixture heated to reflux, and iodine (3.65 g, 14 mmol) introduced portionwise over 15 min. The reaction mixture was heated at reflux for 45 min, cooled, and concentrated to ~20 mL, before adding a solution of sodium thiosulfate (10.00 g) and sodium carbonate (10.00 g) in water (200 mL). The precipitate was filtered off, suspended in hot (80 °C) water (200 mL), and filtered. Recrystallization from aqueous DMF afforded **7g** (1.61 g, 43%): mp >360 °C; IR (KBr) 1730 (>C=O) cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 3.79 (3 H, s, OCH<sub>3</sub>), 7.10 (1 H, dd, J = 9 Hz, J' = 3 Hz, aromatic H ortho to OCH<sub>3</sub>), 7.28 (1 H, d,

(53) Galun, A.; Markus, A.; Kampf, A. Ethers of Indoxyllic Acid. *J. Heterocycl. Chem.* 1979, 16, 221–224.

$J = 3$  Hz, aromatic  $H$  ortho to  $\text{OCH}_3$ ), 7.48 (1 H, s, aromatic ortho to  $\text{NHCO}$ ), 7.65 (1 H, d,  $J = 9$  Hz, aromatic  $H$  meta to  $\text{OCH}_3$ ), 10.90 (1 H, bs, NH), 11.32 (1 H, bs, NH); MS  $m/z$  215 ( $M^+$ ). Anal. ( $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_2$ ) C, H, N.

**1,3-Dihydro-1,3,7,8-tetramethyl-2H-imidazo[4,5-b]quinolin-2-one Hydrochloride (7ad).** A mixture of **7o** (1.00 g, 47 mmol), pulverized  $\text{K}_2\text{CO}_3$  (1.62 g, 11.7 mmol), iodomethane (3.33 g, 23 mmol), and DMF (15 mL) was stirred at 110 °C. After 45 min, the mixture was poured onto water and the white precipitate filtered off and dried in air. Dissolution in a hot solution of MeOH containing 10% HCl gas, followed by cooling, afforded **7ad** (0.45 g, 73%): mp 354–357 °C dec; IR (KBr) 1750, 1680, 1630, 1540  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.38 (3 H, s, aryl  $\text{CH}_3$ ), 2.46 (3 H, s, aryl- $\text{CH}_3$ ), 3.38 (3 H, s,  $\text{NCH}_3$ ), 3.42 (3 H, s,  $\text{NCH}_3$ ), 7.36 (1 H, d,  $J = 8$  Hz, aromatic  $H$ ), 7.73 (1 H, d,  $J = 8$  Hz, aromatic  $H$ ), 7.82 (1 H, s, aromatic  $H$  ortho to  $\text{NHCO}$ ), 9.79 (1 H, bs,  $H^+$ ); MS  $m/z$  242 ( $MH^+$ ). Anal. ( $\text{C}_{14}\text{H}_{15}\text{N}_3\text{O}\cdot\text{HCl}$ ) C, H, N.

**3-[(2,4-Dioxoimidazolidin-5-ylidene)methyl]-4-nitrophenyl Acetate (18e).** Acetyl chloride (10.12 g, 9.2 mL, 130 mmol) was added to a solution of 5-hydroxy-2-nitrobenzaldehyde (20.05 g, 120 mmol) and  $\text{Et}_3\text{N}$  (13.1 g, 18.0 mL, 130 mmol) in  $\text{CH}_3\text{CN}$  (300 mL) maintained at 0 °C. The ice bath was removed and the mixture stirred at room temperature for 1 h before adding  $\text{Et}_3\text{N}$  (14.58 g, 20.0 mL, 140 mmol) and **19a** (33.06 g, 140 mmol). After stirring for 30 min, the solvent was evaporated, the residue diluted with a mixture of EtOH and  $\text{H}_2\text{O}$  (3:7), and the EtOH removed in vacuo. A yellow precipitate was filtered off and dried in air to give **18e** (34.40 g, 98%). An analytical sample was recrystallized from aqueous EtOH, mp 195–209 °C. Anal. ( $\text{C}_{12}\text{H}_9\text{N}_3\text{O}_6$ ) C, H, N.

**2,3,9,9a-Tetrahydro-2-oxoimidazo[4,5-b]quinolin-7-yl Acetate (6e).** A solution of **18e** (32.0 g, 110 mmol) in DMF (500 mL) was hydrogenated over 10% Pd on C (3.20 g) at 500 psi in a Parr high-pressure hydrogenation apparatus. After 40 h, the mixture was filtered through Celite and concentrated in vacuo and the residual solid suspended in MeOH. Filtration gave a white solid (20.1 g) which was suspended in MeOH (150 mL) and pTsOH (0.06 g) added. The mixture was heated at reflux for 2 h and cooled and the precipitate collected to give **6e** (18.01 g, 66%). An analytical sample was prepared by suspending 2.00 g in hot MeOH (75 mL) and filtering off **6e** (1.78 g): mp 288–290 °C; IR (KBr) 3250, 1755, 1735, 1680, 1640, 1500  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.25 (3 H, s,  $\text{CH}_3\text{CO}$ ), 2.69 (1 H, t,  $J = 15$  Hz, benzylic  $\text{CH}$ ), 3.03 (1 H, dd,  $J = 15$  Hz,  $J' = 7$  Hz, benzylic  $\text{CH}$ ), 4.29 (1 H, bs,  $J = 15$  Hz,  $J' = 7$  Hz,  $\text{CHNHCO}$ ), 7.00 (3 H, m, aromatic  $H$ ), 7.70 (1 H, s, NH), 10.81 (1 H, bs, NH); MS  $m/z$  246 ( $MH^+$ ). Anal. ( $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_3$ ) C, H, N.

**2,3-Dihydro-2-oxo-1H-imidazo[4,5-b]quinolin-7-yl Acetate (7e).** A mixture of **6e** (12.16 g, 50 mmol) and DMF (150 mL) was heated to 65 °C and iodine (12.69 g, 50 mmol) added portionwise. After 30 min, the mixture was cooled; 10%  $\text{Na}_2\text{CO}_3$  solution was added until pH = 7, followed by excess 10%  $\text{Na}_2\text{S}_2\text{O}_3$  solution and water (100 mL). A white solid was filtered off and dried in air to leave **7e** (10.40 g, 86%). An analytical sample was prepared by recrystallizing 2.0 g from a mixture of DMF and  $\text{H}_2\text{O}$  (4:1) to afford pure **7e** (1.20 g): mp >330 °C; IR (KBr) 3150, 3050, 1755, 1720, 1695, 1455, 1200  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.33 (3 H, s,  $\text{COCH}_3$ ), 7.29 (1 H, dd,  $J = 7$  Hz,  $J' = 3$  Hz, aromatic  $H$  ortho to  $\text{CH}_3\text{COO}$ ), 7.61 (1 H, s, aromatic  $H$  ortho to  $\text{NHCO}$ ), 7.64 (1 H, d,  $J = 2$  Hz, aromatic  $H$  ortho to  $\text{CH}_3\text{COO}$ ), 7.81 (1 H, d,  $J = 7$  Hz, aromatic  $H$  meta to  $\text{CH}_3\text{COO}$ ), 11.10 (1 H, bs, NH), 11.55 (1 H, bs, NH); MS  $m/z$  244 ( $MH^+$ ). Anal. ( $\text{C}_{12}\text{H}_9\text{N}_3\text{O}_3$ ) C, H, N.

**1,3-Dihydro-7-hydroxy-2H-imidazo[4,5-b]quinolin-2-one (7f).** A mixture of **7e** (8.30 g, 34 mmol), 4 N NaOH solution (12.8 mL, 51 mmol), water (120 mL), and MeOH (40 mL) was stirred at room temperature for 1 h. The mixture was acidified to pH = 5 with glacial AcOH; the precipitate was filtered off and washed with water and methanol to leave a gray solid (7.02 g). A 2.1-g sample was recrystallized from a mixture of DMF and  $\text{H}_2\text{O}$  (2:1) to give 1.27 g of **7f**: mp >400 °C; IR (KBr) 3200, 2700, 1730, 1625, 1435  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.79, 2.92 (s, DMF), 3.28 (s,  $\text{CH}_2\text{OH}$ ), 3.76 (bs,  $\text{H}_2\text{O}$ ), 7.19 (1 H, dd,  $J = 9$  Hz,  $J' = 2$  Hz, aromatic  $H$  ortho to OH), 7.56 (1 H, s, aromatic  $H$  ortho to  $\text{NHCO}$ ), 7.77 (1 H, d,  $J = 9$  Hz), aromatic  $H$  meta to OH), 9.66 (1 H, bs, OH), 11.05 (1 H, bs, NH), 11.47 (1 H, bs, NH); MS  $m/z$

202 ( $MH^+$ ). Anal. ( $\text{C}_{10}\text{H}_7\text{N}_3\text{O}_2\cdot 0.1\text{CH}_3\text{OH}\cdot 0.1\text{H}_2\text{O}$ ) C, H, N.

**2,3-Dimethyl-6-nitrobenzonitrile (23).** A mixture of **22** (100.0 g, 0.6 mol) and concentrated HCl (150 mL) was ground in a mortar and then rinsed into a three-neck, 3-L round-bottomed flask with water (1200 mL). The stirred mixture was cooled to 5 °C and a solution of sodium nitrite (45.00 g, 0.65 mol) in water (300 mL) introduced dropwise over 1 h. After completing the addition, the mixture was allowed to warm to 20 °C and filtered to remove any insoluble material. The filtrate was cooled to 5 °C and added dropwise over 2 h to a solution of copper(I) cyanide (64.20 g, 0.72 mol) and sodium cyanide (88.0 g, 0.3 mol) in water (480 mL), maintained at 5 °C, to which toluene (225 mL) had been added. The mixture was warmed to room temperature and allowed to stand overnight before being diluted with diethyl ether (1500 mL). The organic phase was separated, dried over sodium sulfate and concentrated to afford a slurry. The solid was filtered off and washed with diethyl ether and hexane to give **23** (31.53 g, 30%). The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 600$  mL), the combined extracts were dried over sodium sulfate, and the solvent was evaporated to leave **23** (65.00 g, 61%): Total yield 96.53 g, 91%; mp 102–105 °C (lit.<sup>51</sup> mp 109.5–110.5 °C).

**2,3-Dimethyl-6-nitrobenzylamine (24).** A solution of  $\text{BH}_3\cdot\text{THF}$  complex (94.60 g, 1.1 mol) in THF (1100 mL) was added dropwise to a stirred solution of **23** (96.00 g, 0.55 mol) in dry THF (650 mL) maintained under an atmosphere of argon. After stirring overnight, a 10% HCl solution (1300 mL) was added dropwise and the mixture heated to reflux. After 30 min, the THF was distilled off, the residue filtered to remove insoluble material, and the filtrate made basic with concentrated  $\text{NH}_4\text{OH}$  solution (350 mL). The mixture was extracted with diethyl ether ( $2 \times 500$  mL); the combined extracts were washed with water ( $2 \times 400$  mL), dried over potassium carbonate, and concentrated to afford **24**<sup>24a</sup> (93.85 g, 95%) as an oil which was used without further purification.

**2,3-Dimethyl-6-nitrobenzenemethanol (25).** Sodium nitrite (36.50 g, 0.53 mol) in water (125 mL) was added dropwise to a stirred mixture of **24** (63.50 g, 0.35 mol), acetic acid (165 mL), and water (165 mL) cooled in an ice bath. After completing the addition, the mixture was stirred for 10 min, warmed to room temperature, and stirred a further 10 min before being diluted with water (1000 mL). The mixture was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 500$  mL), and the combined extracts were dried over  $\text{MgSO}_4$  and concentrated to afford an oil which was dissolved in methanol (400 mL). NaOH (1 N, 400 mL) was added dropwise over 20 min. The methanol was removed under reduced pressure, and the residue diluted with water (1200 mL) and extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 700$  mL). The combined extracts were dried over  $\text{MgSO}_4$  and the solvent evaporated to afford **25** (59.80 g, 93%) as brown solid which was used without further purification. An analytical sample was prepared by recrystallization from hexane/diethyl ether to afford pure material, mp 48–51 °C. Anal. ( $\text{C}_9\text{H}_{11}\text{NO}_3$ ) C, H, N.

**2,3-Dimethyl-6-nitrobenzaldehyde (17o).** A solution of **25** (34.88 g, 0.192 mol) in HPLC grade  $\text{CH}_2\text{Cl}_2$  (150 mL) was added to a stirred mixture of pyridinium chlorochromate (62.20 g, 0.288 mol) in HPLC-grade dichloromethane (250 mL). The mixture was stirred vigorously for 4 h and diluted with diethyl ether (500 mL) and the organic layer decanted. The tarry residue was washed with diethyl ether (500 mL) and the combined organic solution filtered through a plug of silica gel ( $6 \times 1.5$  in.). Evaporation of the solvent afforded **17o** (32.08 g, 93%). An analytical sample was prepared by recrystallizing from diisopropyl ether and had mp 66–68 °C. Anal. ( $\text{C}_9\text{H}_9\text{NO}_3$ ) C, H, N.

**5-[(2,3-Dimethyl-6-nitrophenyl)methylene]-2,3-imidazolidinedione (18o).** Sodium (0.41 g, 0.018 g-atom) was dissolved in EtOH (40 mL) and **19a** (4.21 g, 18 mmol) added. After 5 min, solid **17o** (2.66 g, 15 mmol) was added in one portion and the mixture stirred at room temperature for 90 min. The mixture was diluted with water and filtered and the solid washed with water and air-dried to give (*Z*)-**18o** (3.35 g, 86%). An analytical sample was prepared by recrystallization from methanol; mp 293–295 °C; IR (KBr) 1775, 1725, ( $>\text{C}=\text{O}$ ), 1675 ( $>\text{C}=\text{C}<$ ), 1520, 1340 ( $\text{NO}_2$ )  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.20 (3 H, s,  $\text{CH}_3$ ), 2.37 (3 H, s,  $\text{CH}_3$ ), 6.62 (1 H, s, olefinic  $H$ ), 7.39 (1 H, d,  $J = 9$  Hz, aromatic  $H$ ), 7.82 (1 H, d,  $J = 9$  Hz, aromatic  $H$ ); MS  $m/z$  262 ( $MH^+$ ). Anal. ( $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_4$ ) C, H, N.

After standing overnight, a second crop was collected from the aqueous layer (0.5 g, 12%; mp 267–270 °C dec), a 1:1 mixture of *E* and *Z* isomers of **18o**: IR (KBr) 1775, 1725, 1665, 1520, 1380  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.20 (6 H, s), 2.33 (3 H, s), 2.37 (3 H, s), 6.45 (1 H, s, olefinic *H* trans to C=O), 6.62 (1 H, s, olefinic *H* cis to C=O), 7.31 (1 H, d,  $J$  = 8 Hz), 7.38 (1 H, d,  $J$  = 8 Hz), 7.73 (1 H, d,  $J$  = 8 Hz), 7.81 (1 H, d,  $J$  = 8 Hz); MS  $m/z$  275 ( $\text{M}^+$ ). Anal. ( $\text{C}_{13}\text{H}_{13}\text{N}_3\text{O}_4 \cdot 0.1\text{H}_2\text{O}$ ) C, H, N.

**5-[(6-Amino-2,3-dimethylphenyl)methyl]-2,4-imidazolidinedione Hydrate (20o).** A solution of **18o** (2.40 g, 9.2 mmol) in DMF (40 mL) was hydrogenated over 10% Pd on C (0.24 g) at 60 psi in a Parr hydrogenation apparatus. After 18 h, the mixture was filtered through Celite and the solvent evaporated in vacuo at 40 °C to give **20o** (2.04 g, 100%) as a khaki solid: mp >360 °C; IR (KBr) 3250 (N-H), 1730 (>C=O), 1700 (>C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.04 (3 H, s,  $\text{CH}_3$ ), 2.10 (3 H, s,  $\text{CH}_3$ ), 2.65–3.15 (2 H, m, benzylic *H*), 4.17 (1 H, bs,  $\text{CHCO}$ ), 4.55 (2 H, b s,  $\text{NH}_2$ ), 6.48 (1 H, d,  $J$  = 7 Hz, aromatic *H*), 6.74 (1 H, d,  $J$  = 7 Hz, aromatic *H*); MS  $m/z$  234 ( $\text{M}^+$ ). Anal. ( $\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ ) C, H, N,  $\text{H}_2\text{O}$ .

**7,8-Dimethyl-1,3,9a-tetrahydro-2*H*-imidazo[4,5-*b*]quinolin-2-one Hydrochloride (6o).** A mixture of **20o** (2.52 g, 10 mmol), *p*-toluenesulfonic acid (spatula tip), and methanol (50 mL) was heated at reflux under an atmosphere of argon for 1 h. The mixture was cooled; a gray solid was filtered off and dissolved in 10% HCl in methanol solution with warming. Addition of ether afforded **6o** (1.68 g, 87%): mp >320 °C; IR (KBr) 1760 (w), 1700 (s)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.20 (3 H, s,  $\text{CH}_3$ ), 2.27 (3 H, s,  $\text{CH}_3$ ), 2.80 (1 H, t,  $J$  = 14 Hz, benzylic *H*), 3.34 (1 H, dd,  $J$  = 14 Hz,  $J'$  = 8 Hz, benzylic *H*), 4.84 (1 H, dd,  $J$  = 14 Hz,  $J'$  = 8 Hz,  $\text{CHCO}$ ), 7.18 (1 H, d,  $J$  = 8 Hz, aromatic *H*), 7.31 (1 H, d,  $J$  = 8 Hz, aromatic *H*), 9.22 (2 H, s,  $\text{NH}$ ); MS  $m/z$  215 ( $\text{M}^+$ ). Anal. ( $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O} \cdot \text{HCl}$ ) C, H, N.

**1,3-Dihydro-7,8-dimethyl-2*H*-imidazo[4,5-*b*]quinolin-2-one (7o).** A solution of **18o** (19.95 g, 76 mmol) in DMF (350 mL) was hydrogenated over 10% Pd on charcoal (3 g) at 60 psi in a Parr hydrogenation apparatus. After hydrogen uptake ceased, the mixture was filtered through Celite and the solvent evaporated to leave a solid which was suspended in refluxing methanol (1 L). Iodine (19.40 g, 76 mmol) was added portionwise over 5 min and the mixture heated at reflux for 15 min before being concentrated in vacuo to ~100 mL. A solution of  $\text{Na}_2\text{S}_2\text{O}_3$  (21.00 g) and  $\text{Na}_2\text{CO}_3$  (11.00 g) in water (300 mL) was added with vigorous stirring to afford a beige precipitate which was collected, washed with water, and dried in air to give 15.70 g of **7o**. This was combined with the crude material from experiments performed on 40.00 and 41.60 g of starting material **18o**, suspended in hot (80 °C) water, filtered, suspended in refluxing methanol, and filtered. Recrystallization from dimethylacetamide afforded **7o** (53.40 g, 65%): mp >300 °C; IR (KBr) 1725 (>C=O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.41 (3 H, s,  $\text{CH}_3$ ), 2.48 (3 H, s,  $\text{CH}_3$ ), 7.31 (1 H, d,  $J$  = 8 Hz, aromatic *H*), 7.55 (1 H, d,  $J$  = 8 Hz, aromatic *H*), 7.61 (1 H, s, aromatic *H*); MS  $m/z$  213 ( $\text{M}^+$ ). Anal. ( $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}$ ) C, H, N.

**Phosphodiesterase Activity.** Phosphodiesterase activity was determined as described.<sup>20,64</sup> Diluted platelet sonicate was

preincubated with drug at five different concentrations in 0.3 mL of 5 mM  $\text{MgCl}_2$ , 3.75 mM mercaptoethanol, and 40 mM Tris-HCl, pH 8.0, for 5 min at 30 °C. [ $^3\text{H}$ ]cAMP (0.15  $\mu\text{M}$ , ca.  $2\text{--}3 \times 10^5$  dpm) or [ $^3\text{H}$ ]cGMP (0.25  $\mu\text{M}$ , ca.  $4.4 \times 10^5$  dpm) was added, and the tubes were incubated for 10 min before being immersed in boiling water for 2 min. After cooling on ice, snake venom was added to each tube and incubation conducted at 30 °C for 10 min. One milliliter of a slurry of ion-exchange resin (AGI-X2, 200–400 mesh, 1:2 v/v in methanol) was added. The tubes were centrifuged, and a portion of the clear supernatant was counted in a liquid scintillation counter. Dose-response values were obtained and activity of the test agent reported as the molar concentration providing 50% inhibition of PDE activity.

**In Vitro Inhibition of Platelet Aggregation.** Inhibition of platelet function by test compounds was performed as described.<sup>41</sup> Platelet-rich plasma (PRP) was separated by centrifugation from citrated (3.8%) rabbit blood. ADP in a final concentration of 29.3  $\mu\text{M}$  ADP for rabbit PRP or 0.05 mL of a collagen suspension was used to induce aggregation. The test compounds were dissolved in DMSO so that 5  $\mu\text{L}$  added to the PRP (0.9 mL) would yield the desired test concentration. The aggregometer method of Born,<sup>55</sup> as modified by Mustard et al.,<sup>56</sup> was employed to measure aggregation. Vehicle control trials were performed and compared with the extent of aggregation induced in PRP containing various concentrations of test compounds. Dose-response curves were obtained and  $\text{EC}_{50}$  values determined. For human platelet studies, 5.86  $\mu\text{M}$  ADP was employed to induce aggregation.

**Inhibition of Platelet Aggregation following Oral Administration.** Aggregometry was performed in vitro on PRP obtained from rats dosed with either test compound or vehicle.<sup>41</sup> Activity was determined 2 h after administration of the drug by oral gavage as a suspension in water plus a few drops of Tween 20. Drug activity was determined in  $\text{ED}_{50}$ 's (in mg/kg) calculated from results obtained from groups of 10 animals treated with various doses of test compound in comparison to results of separate controls.

**Acknowledgment.** We would like to thank C. M. Combs and R. D. Rutkowski for providing and interpreting NMR spectral data, J. G. Schmidt for mass spectral data, and C. L. Kennedy for combustion analyses. A. E. Bosza is acknowledged for preparing the manuscript.

**Supplementary Material Available:** Analytical data for intermediates **14** and **18** (4 pages). Ordering information is given on any current masthead page.

- (54) Thompson, W. J.; Brooker, G.; Appleman, M. M. Assay of Cyclic Nucleotide Phosphodiesterase and Resolution of Multiple Molecular Forms of the Enzyme. *Methods Enzymol.* 1979, 10, 69–92.
- (55) Born, G. V. R. Quantitative Investigations into the Aggregation of Blood Platelets. *J. Physiol.* 1962, 162, 67–68.
- (56) Mustard, J. F.; Hegardt, B.; Rowsell, H. C.; MacMillan, R. L. Effect of Adenosine Nucleotides on Platelet Aggregation and Clotting Time. *J. Lab. Clin. Med.* 1964, 64, 548–559.