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

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.[5] 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.[12] ED_{50} values were calculated by probit analysis.[20]

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.[3] 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.[5] The VI (nonconflict) 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 (Crl:(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.[20]

Acknowledgment. We are grateful to Lia Raabis for taking the ^{1}H 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.

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

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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,[2] asthma,[3] atherosclerosis,[4] and tumor-cell metastasis,[5] while inadequately controlled platelet activation and aggregation may lead to vascular

[†] Department of Cardiovascular Biochemistry.

(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.

0022-2623/91/1834-2906$02.50/0

occlusion.[6] 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.[7,8] 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[9] and those considered to be at risk.[10-14] Aspirin[9-11,15] remains the most widely studied and clinically effective platelet-aggregation inhibitor, although some trials have shown dipyridamole, sulfinpyrazone, prostacyclin, and ticlopidine[12-14] 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.[8,16] 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.[17,18] 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.[19-21] 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.[22] 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.[23]

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.[24-26] The presence of the guanidine moiety was

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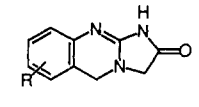

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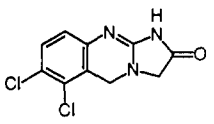
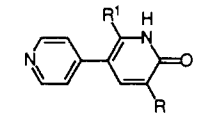
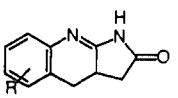


2. ANAGRELIDE



3. R^1 = H, R = NH_2, AMRINONE
4. R^1 = CH_3, R = CN, MILRINONE



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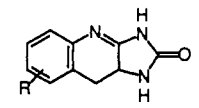

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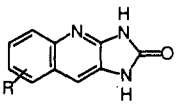

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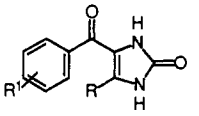

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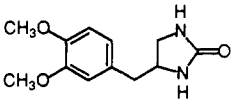
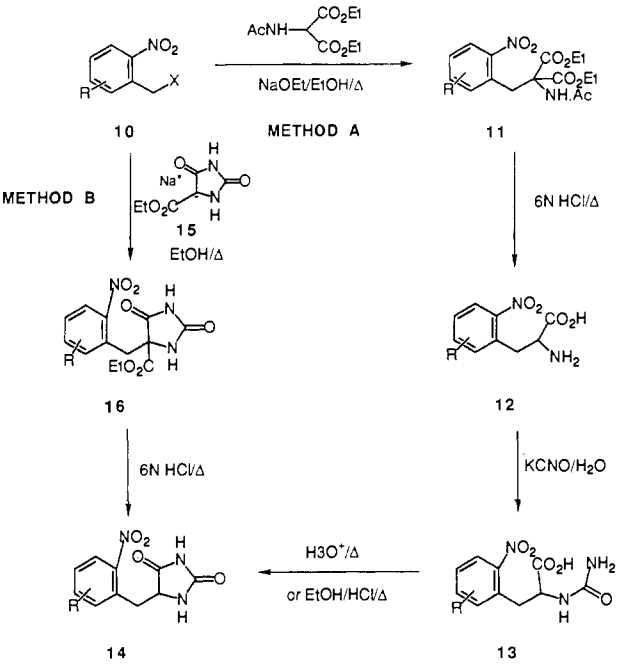


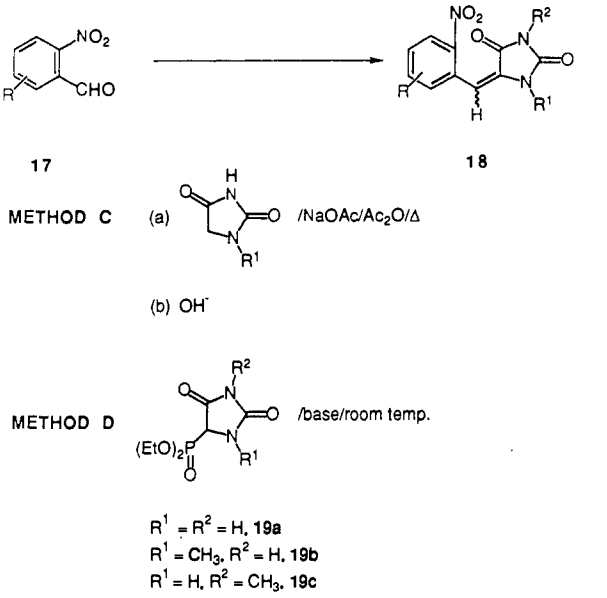
9. Ro 7-2956

not an essential prerequisite for biological activity since tetrahydropyrrolo[2,3-*b*]quinolin-2-one derivatives (**5**) possessed antiaggregatory properties.[27] 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_m cAMP PDE,[28] and Ro 7-2956 (**9**), another cAMP PDE inhibitor.[29] A survey of the literature revealed

Scheme I



Scheme II



R^1 = R^2 = H, **19a**
R^1 = CH_3, R^2 = H, **19b**
R^1 = H, R^2 = CH_3, **19c**

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

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reported to exhibit biological activity. An attempt to prepare 6 (R = H) according to the method of Connors[30] 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.[30] 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[34] (**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.[24a] 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[35] in CH_2Cl_2, and subsequent reduction with BH_3–THF complex in THF at 50 °C.[24a] 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,[36] with hydantoin or 1-methylhydantonin in hot acetic anhydride containing

Scheme III

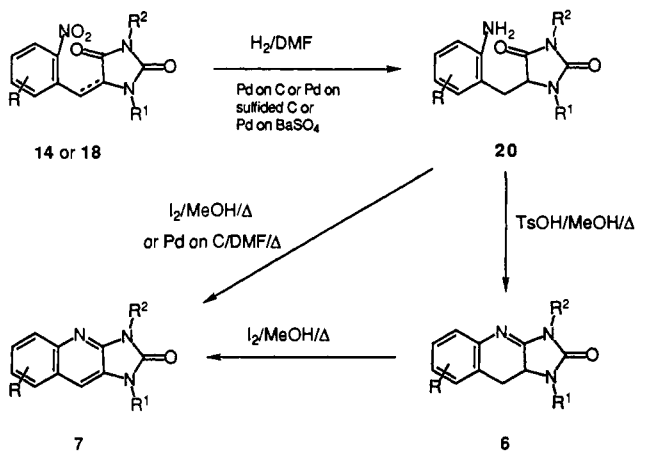
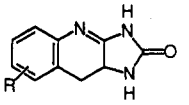


Table I. Structure and Physical Properties of 1,3,9,9a-Tetrahydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones (**6**)



6

compd	R^1	R^2	R^3	mp, °C	mol form. (elem anal.)
6e	OAc	H	H	288–290	$C_{12}H_{11}N_3O_3$
6f	OH	H	H	>330	$C_{10}H_9N_3O_2$
6k	H	CH_3	H	220–225 dec	$C_{11}H_{11}N_3O\cdot HCl\cdot 0.55H_2O$
6o	CH_3	CH_3	H	>320	$C_{12}H_{13}N_3O\cdot HCl$
6x	CH_3	CH_3	CH_3	340–345 dec	$C_{12}H_{13}N_3O\cdot HCl$

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).[37] 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**;[38] 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.[39] 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

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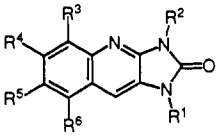
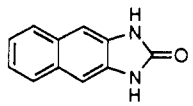
Table II. Structure and Physical Properties of 1,3-Dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-ones (7a–7ad)



compd	R^1	R^2	R^3	R^4	R^5	R^6	mp, °C	mol form. (elem anal.)
7a	H	H	H	H	H	H	358–361	$C_{10}H_7N_3O\cdot HCl\cdot 0.1H_2O$
7b	H	H	H	H	F	H	>360	$C_{10}H_6FN_3O\cdot 0.42H_2O$[a]
7c	H	H	H	H	Cl	H	>360	$C_{10}H_6ClN_3\cdot 0.1H_2O$
7d	H	H	H	H	CH_3	H	>360	$C_{11}H_9N_3O\cdot HCl$
7e	H	H	H	H	OAc	H	>330	$C_{12}H_9N_3O_3$
7f	H	H	H	H	OH	H	>400	$C_{10}H_7N_3O_2\cdot 0.1CH_3OH\cdot 0.1H_2O$
7g	H	H	H	H	OCH_3	H	>360	$C_{11}H_9N_3O_2$
7h	H	H	H	H	OEt	H	>320	$C_{12}H_{11}N_3O_2$
7i	H	H	H	H	O^{i}Pr	H	>320	$C_{13}H_{13}N_3O_2$
7j	H	H	H	H	H	Cl	>360	$C_{10}H_6ClN_3O\cdot 0.1H_2O$
7k	H	H	H	H	H	CH_3	350–355 dec	$C_{11}H_9N_3O\cdot HCl\cdot 0.35H_2O$
7l	H	H	H	H	H	OCH_3	>300	$C_{11}H_9N_3O_2\cdot 0.25H_2O$
7m	H	H	CH_3	H	H	H	340–343	$C_{11}H_9N_3O$
7n	H	H	OCH_3	H	H	H	351–354 dec	$C_{11}H_9N_3O_2$
7o	H	H	H	H	CH_3	CH_3	>360	$C_{12}H_{11}N_3O$
7p	H	H	H	CH_3	CH_3	H	>360	$C_{12}H_{11}N_3O\cdot HCl\cdot 0.15H_2O$
7q	H	H	H	CH_3	Cl	H	>360	$C_{11}H_8ClN_3O_2$
7r	H	H	H	H	CH_3	Cl	>360	$C_{11}H_8ClN_3O\cdot 0.2H_2O$
7s	H	H	H	OCH_3	OCH_3	H	>360	$C_{12}H_{11}N_3O_3$
7t	H	H	H	CH_3	Cl	CH_3	>300	$C_{12}H_{10}ClN_3O$
7u	H	H	H	CH_3	Br	CH_3	>300	$C_{12}H_{10}BrN_3O$
7v	H	H	H	OCH_3	OCH_3	OCH_3	>320	$C_{13}H_{13}N_3O_4$
7w	CH_3	H	H	H	H	H	309–311	$C_{11}H_9N_3O$
7x	CH_3	H	H	H	H	CH_3	340–341 dec	$C_{12}H_{11}N_3O\cdot HCl\cdot 0.1H_2O$
7y	CH_3	H	H	H	CH_3	H	>320	$C_{12}H_{11}N_3O\cdot 0.04H_2O$
7z	CH_3	H	H	H	OCH_3	H	>310	$C_{12}H_{11}N_3O\cdot 0.02H_2O$
7aa	CH_3	H	H	H	CH_3	CH_3	>300	$C_{13}H_{13}N_3O$
7ab	H	CH_3	H	H	OCH_3	H	296–297	$C_{12}H_{11}N_3O_2$
7ac	H	CH_3	H	H	CH_3	CH_3	274–277	$C_{13}H_{13}N_3O\cdot CH_3SO_3H$
7ad	CH_3	CH_3	H	H	CH_3	CH_3	354–357 dec	$C_{14}H_{15}N_3O\cdot HCl$

[a] 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_2 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_2CO_3 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**[40] was prepared from commercially available 2,3-diaminonaphthalene by stirring with 1,1′-carbonyldiimidazole in THF overnight at room temperature.



21

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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.[20] The IC_{50}'s for prevention of [^{3}H]cAMP and [^{3}H]cGMP hydrolysis were determined from dose–response curves and are reported in Table III. In this assay, anagrelide (**2**) displayed IC_{50}'s of $5.4 \pm 1.4 \times 10^{-8}$ M vs cAMP and $34 \pm 7 \times 10^{-6}$ 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.[41] Dose–response curves were obtained and effective concentration (EC_{50}) 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_{50}'s are listed in Table III. Anagrelide was employed as a positive reference agent in these evaluations and displayed EC_{50}'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.[41]

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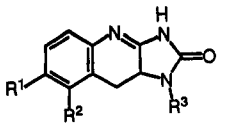
Table III. Biological Evaluation of Imidazo[4,5-b]quinolin-2-one Derivatives 6 and 7 and Related Compounds



compd	inhibn of human platelet phosphodiesterase: IC_{50}, μM		EC_{50}, μM: inhibn of platelet aggregation in rabbit PRP		inhibn of ADP-induced[a] platelet aggregation in human PRP	rat ex vivo vs ADP: ED_{50}, mg/kg
	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[b]
7v	2.0	NT	0.91	0.47		NT[c]
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

[a] 5.86 μM ADP. [b] 33% inhibition at 10 mg/kg. [c] NT = not tested.

ED_{50}'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, cardiotonic, and vasodilatory properties.[17,18] 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.[20] 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_m cAMP PDE.[42-45] These structural elements are readily apparent in **7a**, which is virtually planar,[33] 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.

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(45) 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.

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 μM 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 μM 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.[42-45] 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.[46,47]

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 10 000-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.[24,26] 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,[24,26,46] although positive inotropic activity is maintained.[48] This disparity may be accounted for by considering that **7m** and **7n** are urea derivatives and as such possess two relatively acidic hydrogen 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.[42-45] 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.[48]

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 that 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.[24,26,46]

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 a 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 μM (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

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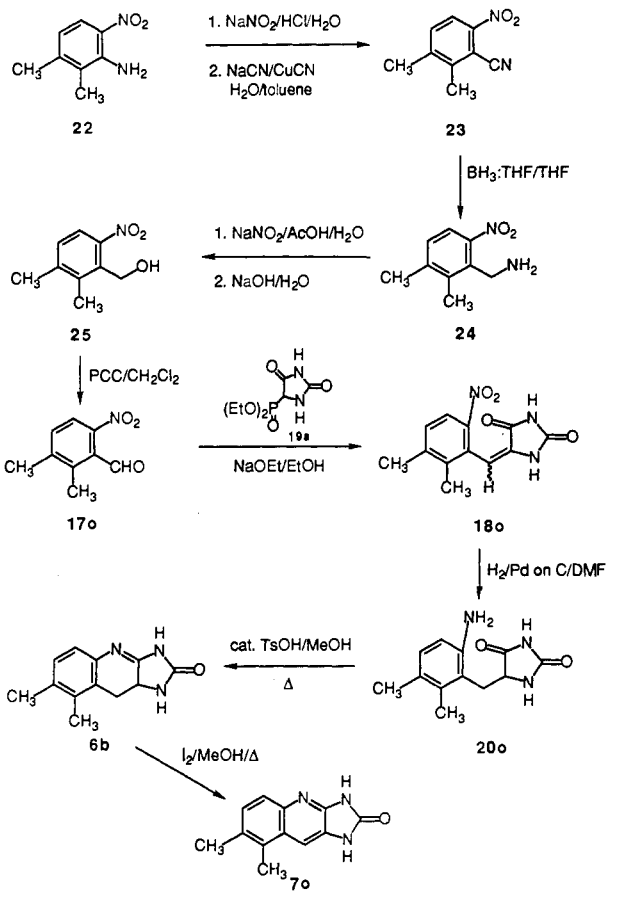
manipulating the oxidation state of the central pyridine-derived heterocycle, aromaticity and its attendant planarity can be confined to a single ring, as in **6**, or allowed to extend into the central region of the molecule, as in **7**. The partially reduced imidazo[4,5-*b*]quinolin-2-ones **6e**, **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_{50}'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_{50} of 3.2 mg/kg, which compares favorably to an ED_{50} 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. 1,3,9,9a-Tetrahydro-2*H*-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,[41] 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_m, 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.[41,49] 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**[50] 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**[51] 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_3–THF in THF provided amine **24**[24a] (95%), which was diazotized with sodium nitrite in aqueous acetic acid.[52]

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[36] in CH_2Cl_2 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.[39] 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_2 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_m cAMP PDE from a study of several different structural types.[42-45] Compounds **6** and **7** are effective inhibitors of platelet aggregation in vitro and several representatives of **7** demonstrate platelet inhibitory

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activity following oral administration to rats. 1,3-Dihydro-7,8-dimethyl-2*H*-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[49] and pharmacological[41] 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[46,47] 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 (^{1}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_6 or $CDCl_3$ as solvent with tetramethylsilane as an internal standard. Signal multiplicity was designated according to the following abbrevations: 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^{-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 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_2O and extracted with CH_2Cl (3×). After drying, the organic phase was concentrated to leave a solid which was dissolved in CH_2Cl_2. Addition of hexanes precipitated **11p** (55.93 g, 66%), mp 155–158 °C. Anal. ($C_{18}H_{24}N_2O_7$) 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_2O to precipitate **12p** (32.25 g, 81%), mp 226–228 °C dec. Anal. ($C_{11}H_{14}N_2O_4 \cdot HCl \cdot 0.25H_2O$) 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_2O (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. ($C_{12}H_{15}N_3O_5$) 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. ($C_{12}H_{13}N_3O_4$) C, H, N.

1,3-Dihydro-6,7-dimethyl-2*H*-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^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.42 (3 H, s, CH_3), 2.47 (3 H, s, CH_3), 7.78 (3 H, s, aromatic *H*), 9.60 (2 H, bs, H^+, N*H*), 11.60 (1 H, bs, N*H*CO). Anal. ($C_{12}H_{11}N_3O \cdot HCl \cdot 0.5H_2O$) 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[34] (**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_2Cl_2. The combined extracts were dried over sodium sulfate and concentrated in vacuo to afford a solid which was dissolved in CH_2Cl_2. Addition of hexane precipitated **16q** (12.35 g, 43%): mp 176–178 °C; IR (KBr) 3305, 1750, 1715, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.08 (3 H, t, J = 7 Hz, OCH_2CH_3), 2.28 (3 H, s, aryl CH_3), 3.56 (2 H, s, benzylic CH_2), 4.10 (2 H, q, J = 7 Hz, OCH_2), 7.46 (1 H, s, aromatic *H* meta to NO_2), 7.99 (1 H, s, aromatic *H* ortho to NO_2), 8.44 (1 H, bs, N*H*), 11.16 (1 H, bs, N*H*); MS *m/z* 356, 358 (MH^+). Anal. ($C_{14}H_{14}ClN_3O_6$) 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. ($C_{11}H_{10}ClN_3O_4$) C, H, N.

1,3-Dihydro-7-chloro-6-methyl-2*H*-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^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.76 (3 H, s, CH_3), 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^+). Anal. ($C_{11}H_{18}ClN_3O$) C, H, N, Cl.

(*E*)-5-[(2-Nitro-5-methoxyphenyl)methylene]-2,4-imidazolidinedione (18q). A mixture of 5-methoxy-2-nitrobenzaldehyde[53] (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_2Cl_2 (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 **18g** (8.00 g, 55%), mp 294–295 °C dec. Anal. ($C_{11}H_9N_3O_5$) C, H, N.

7-Methoxy-1,3-dihydro-2*H*-imidazo[4,5-*b*]quinolin-2-one (7g). A solution of **18g** (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^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.79 (3 H, s, OCH_3), 7.10 (1 H, dd, J = 9 Hz, J' = 3 Hz, aromatic *H* ortho to OCH_3), 7.28 (1 H, d,

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J = 3 Hz, aromatic H ortho to OCH_3), 7.48 (1 H, s, aromatic ortho to NHCO), 7.65 (1 H, d, J = 9 Hz, aromatic H meta to OCH_3), 10.90 (1 H, bs, NH), 11.32 (1 H, bs, NH); MS m/z 215 (M^+). Anal. ($C_{11}H_9N_3O_2$) 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 K_2CO_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 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.38 (3 H, s, aryl CH_3), 2.46 (3 H, s, aryl-CH_3), 3.38 (3 H, s, NCH_3), 3.42 (3 H, s, 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 NHCO), 9.79 (1 H, bs, H^+); MS m/z 242 (MH^+). Anal. ($C_{14}H_{15}N_3O$·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 Et_3N (13.1 g, 18.0 mL, 130 mmol) in CH_3CN (300 mL) maintained at 0 °C. The ice bath was removed and the mixture stirred at room temperature for 1 h before adding Et_3N (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 H_2O (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. ($C_{12}H_9N_3O_6$) 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 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25 (3 H, s, CH_3CO), 2.69 (1 H, t, J = 15 Hz, benzylic CH), 3.03 (1 H, dd, J = 15 Hz, J' = 7 Hz, benzylic CH), 4.29 (1 H, dd, J = 15 Hz, J' = 7 Hz, 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. ($C_{12}H_{11}N_3O_3$) 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% Na_2CO_3 solution was added until pH = 7, followed by excess 10% $Na_2S_2O_3$ 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 H_2O (4:1) to afford pure **7e** (1.20 g): mp >330 °C; IR (KBr) 3150, 3050, 1755, 1720, 1695, 1455, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.33 (3 H, s, $COCH_3$), 7.29 (1 H, dd, J = 7 Hz, J' = 3 Hz, aromatic H ortho to CH_3COO), 7.61 (1 H, s, aromatic H ortho to NHCO), 7.64 (1 H, d, J = 2 Hz, aromatic H ortho to CH_3COO), 7.81 (1 H, d, J = 7 Hz, aromatic H meta to CH_3COO), 11.10 (1 H, bs, NH), 11.55 (1 H, bs, NH); MS m/z 244 (MH^+). Anal. ($C_{12}H_9N_3O_3$) 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 H_2O (2:1) to give 1.27 g of **7f**: mp >400 °C; IR (KBr) 3200, 2700, 1730, 1625, 1435 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.79, 2.92 (s, DMF), 3.28 (s, CH_3OH), 3.76 (bs, H_2O), 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 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. ($C_{10}H_7N_3O_2$·0.1CH_3OH·0.1H_2O) 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 CH_2Cl_2 (3 × 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.[51] mp 109.5–110.5 °C).

2,3-Dimethyl-6-nitrobenzylamine (24). A solution of BH_3–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 NH_4OH solution (350 mL). The mixture was extracted with diethyl ether (2 × 500 mL); the combined extracts were washed with water (2 × 400 mL), dried over potassium carbonate, and concentrated to afford **24**[24a] (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 CH_2Cl_2 (3 × 500 mL), and the combined extracts were dried over $MgSO_4$ 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 CH_2Cl_2 (3 × 700 mL). The combined extracts were dried over $MgSO_4$ 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. ($C_9H_{11}NO_3$) C, H, N.

2,3-Dimethyl-6-nitrobenzaldehyde (17o). A solution of **25** (34.88 g, 0.192 mol) in HPLC grade CH_2Cl_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 × 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. ($C_9H_9NO_3$) 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, (>C=O), 1675 (>C=C<), 1520, 1340 (NO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.20 (3 H, s, CH_3), 2.37 (3 H, s, 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. ($C_{12}H_{11}N_3O_4$) 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 cm^{-1}; ^1H NMR (DMSO-d_6) δ 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 (M^+). Anal. ($C_{13}H_{13}N_3O_4 \cdot 0.1H_2O$) 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) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.04 (3 H, s, CH_3), 2.10 (3 H, s, CH_3), 2.65–3.15 (2 H, m, benzylic *H*), 4.17 (1 H, bs, C*H*CO), 4.55 (2 H, b s, 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 (M^+). Anal. ($C_{12}H_{15}N_3O_2 \cdot 3H_2O$) C, H, N, H_2O.

7,8-Dimethyl-1,3,9,9a-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) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.20 (3 H, s, CH_3), 2.27 (3 H, s, 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, C*H*CO), 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, N*H*); MS m/z 215 (M^+). Anal. ($C_{12}H_{13}N_3O \cdot HCl$) 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 $Na_2S_2O_3$ (21.00 g) and Na_2CO_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) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.41 (3 H, s, CH_3), 2.48 (3 H, s, 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 (M^+). Anal. ($C_{12}H_{11}N_3O$) C, H, N.

Phosphodiesterase Activity. Phosphodiesterase activity was determined as described.[20,54] Diluted platelet sonicate was preincubated with drug at five different concentrations in 0.3 mL of 5 mM $MgCl_2$, 3.75 mM mercaptoethanol, and 40 mM Tris-HCl, pH 8.0, for 5 min at 30 °C. [^3H]cAMP (0.15 μM, ca. 2–3 × 10^5 dpm) or [^3H]cGMP (0.25 μM, ca. 4.4 × 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.[41] Platelet-rich plasma (PRP) was separated by centrifugation from citrated (3.8%) rabbit blood. ADP in a final concentration of 29.3 μM 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 μL added to the PRP (0.9 mL) would yield the desired test concentration. The aggregometer method of Born,[55] as modified by Mustard et al.,[56] 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 EC_{50} values determined. For human platelet studies, 5.86 μM 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.[41] 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 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.

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

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