period. Pigs were alloted to treatment groups one day prior to the challenge with *A. pleuropneumoniae* inoculum.

**Inoculum.** *A. pleuropneumoniae* strain B316HM was incubated on enriched Brain heart infusion (BHI) agar plates overnight in 5–10% $CO_2$ at 37 °C. The bacteria were rinsed from the plates with saline, diluted, and standardized by OD so that $10^9$ cfu/mL of bacteria were present in the inoculum.

**Challenge Procedure.** Pigs were challenged by intranasal administration of the inoculum. Three 1-mL increments were administered per nostril using a 50-mL Roux Automatic syringe fitted with a small teat cannula.

**Treatment.** All im-dosed pigs were treated according to the experimental design within 1 h of challenge and in the order of challenge. Intramuscular (im) administration of either a 1% or a 2.5% solution of the test compound in sterile water was made in the ham with a 20-gauge needle. Oral gavage dosing of 0.5% solutions in sterile water was via a gastric tube and all medication was rinsed from the tube with water before it was removed. All medication was administered only once (SID × 1).

**Evaluation Parameters.** All pigs were evaluated daily during the experiment for body temperature, depression, and dyspnea. At necropsy, the lungs of all pigs were scored for lung pathology. *As with the P. haemolytica model, lung pathology as reflected in the lung lesion scores and mortality are considered the key indicators of disease severity.* A sample of lung was also cultured.

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**Supplementary Material Available:** Proton magnetic resonance ($^1$H NMR) and high-resolution mass spectral (HRMS) data for all compounds included in Table I (**2a**–**9c**) (15 pages). Ordering information is given on any current masthead page.

# 3,4-Dihydroquinolin-2(1*H*)-ones as Combined Inhibitors of Thromboxane $A_2$ Synthase and cAMP Phosphodiesterase[1]

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A series of 1*H*-imidazol-1-yl- and 3-pyridyl-substituted 3,4-dihydroquinolin-2(1*H*)-ones was designed and synthesized as combined inhibitors of thromboxane ($TXA_2$) synthase and cAMP phosphodiesterase (PDE) in human blood platelets. A number of structures, e.g. **4b**, **7a**, **7e**, **13a**, and **21**–**25**, were superior to dazoxiben **26** as inhibitors of $TXA_2$ synthase in in vitro ADP-induced aggregation experiments with human blood platelets. The $TXA_2$ synthase inhibitory activity was confirmed by measurement of the prostanoid metabolites derived from $^{14}$C-labeled arachidonic acid. Three compounds (**7a**, **7e**, and **25**) demonstrated in vitro inhibition of human platelet cAMP PDE at micromolar concentrations in conjunction with their $TXA_2$ synthase inhibitory activity. Synergistic enhancement of antiaggregatory and antithrombotic actions was expected when simultaneous stimulation of adenylate cyclase (through increased $PGI_2$ production) and inhibition of platelet cAMP PDE were possible from the same compound. Ex vivo and in vivo experiments were conducted in rats and mice, respectively, to evaluate the effects of compounds **7e** and **23** on platelet aggregation and thrombotic events within these animals. Compound **7e**, which has a comparable level of $TXA_2$ synthase ($IC_{50}$ 1.2 μM) and human platelet cAMP PDE ($IC_{50}$ 6.4 μM) inhibitory activities, was found to be orally bioavailable with a long duration of action and offered effective protection against mortality in a collagen–epinephrine-induced pulmonary thromboembolism model in mice. Significant blood pressure and heart rate effects were observed for several compounds, e.g. **7e**, **9e**, **13a**, **13d**, **18**, **20**, **21**, and **23**, when dosed orally in conscious spontaneously hypertensive rats.

## Introduction

During the past several years, investigations directed toward the inhibition of blood platelet aggregation have involved a number of approaches which include adenylate cyclase stimulation, cAMP phosphodiesterase inhibition, thromboxane ($TXA_2$) synthase inhibition, or $TXA_2$ receptor antagonism.[2-4] As evidenced by the number of pathways considered, the problem of effective in vivo control of thrombosis and hemostasis is a multidimensional challenge. An approach which would address more than one of these pathways simultaneously may prove more effective than a unilateral approach in the inhibition of platelet aggregation.

In the arachidonic acid metabolic cascade, in vitro inhibition of $TXA_2$ production in human platelet rich plasma, in the presence of prostacyclin ($PGI_2$) synthase, has been shown to redirect the balance of prostaglandin synthesis in favor of the production of $PGI_2$.[5] The an-

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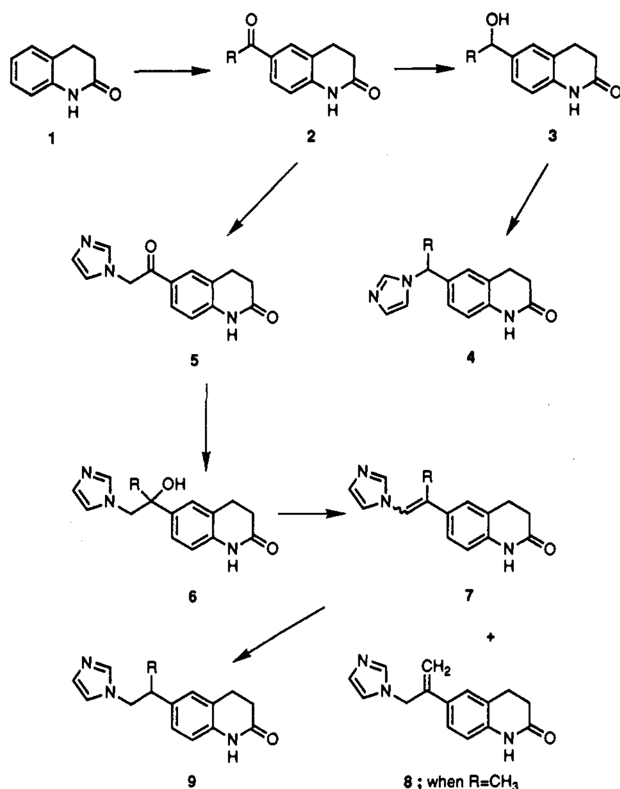
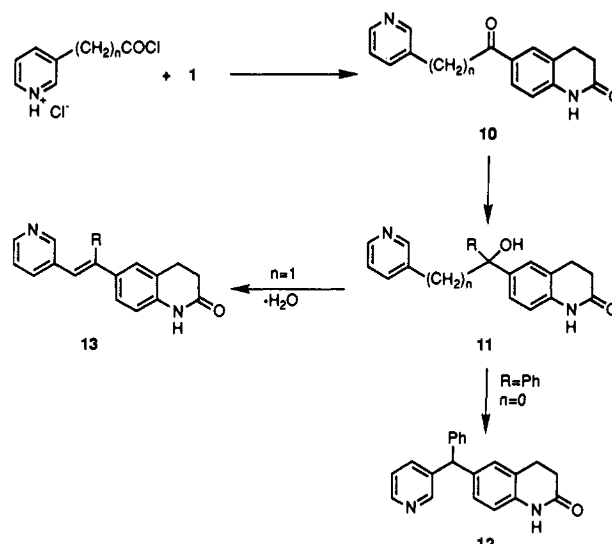
**Scheme I**




**Scheme II**




tiaggregatory effect of an increased level of $PGI_2$ production is believed to originate from stimulation of adenylate cyclase activity which leads to an enhanced level of platelet cAMP. While accumulation of intracellular cAMP is the primary mechanism for the inhibition of platelet aggregation, a competing degradation of cAMP occurs through enzymatic hydrolysis by phosphodiesterases. An agent which would inhibit the degradation of cAMP during simultaneous stimulation of adenylate cyclase might be expected to show synergistic inhibition of platelet aggregation. The validity of this premise was demonstrated earlier by Jorgensen,[6] J. B. Smith,[7,8] E. F. Smith,[9] and Heptinstall[10,11] in in vitro and in vivo aggregation studies where separate agents of adenylate cyclase stimulation and phosphodiesterase inhibition were challenged in the presence of each other.

In an effort to expand the therapeutic usefulness of $TXA_2$ synthase inhibitors as antithrombotic agents, we explored the feasibility of designing compounds with added phosphodiesterase inhibitory activity. The minimal structural parameters necessary for a selective $TXA_2$ synthase inhibitor have been described earlier[12] and consist of a sterically unhindered 1*H*-imidazol-1-yl or 3-pyridinyl nitrogen separated from a carboxylic acid terminus by 8–10 Å. The carbostyril heterocycle, as well as its dihydro derivative, is found as a fragment in a number of reported cAMP PDE inhibitors.[3a] Using the carboxamide portion of the dihydrocarbostyril heterocycle as an isosteric replacement for the carboxylic terminus, we saw an opportunity to design compounds which may exhibit both $TXA_2$ synthase and cAMP PDE inhibitory activity. In this paper we describe the synthesis and evaluation of a series of 6-substituted 3,4-dihydroquinolin-2(1*H*)-ones as compounds which may combine $TXA_2$ synthase inhibition with cAMP phosphodiesterase inhibition.

## Chemistry

Schemes I–III illustrate the preparation of the 3,4-dihydroquinolin-2(1*H*)-one compounds. In Scheme I, Friedel–Crafts acylation of 3,4-dihydroquinolin-2(1*H*)-one (**1**) with an appropriate acid chloride furnished ketones **2a–d**. The (1*H*-imidazol-1-yl)methyl-substituted compounds **4a–c** were prepared in a two-step sequence: reduction of ketone **2** to carbinol **3**, followed by treatment with freshly prepared thionylbis(imidazole). The corresponding homologous series **9a–e** was prepared in a three-step sequence from ketone **5** (available from imidazole displacement of chloro ketone **2d**): Grignard addition or sodium borohydride reduction to give carbinol **6**, followed by acid-catalyzed dehydration and catalytic hydrogenation of the resulting isomeric olefin mixture **7**. Although in most cases the isomeric mixture was inseparable, dehydration of **6a** ($R = CH_3$) afforded two characterizable products **7a** (R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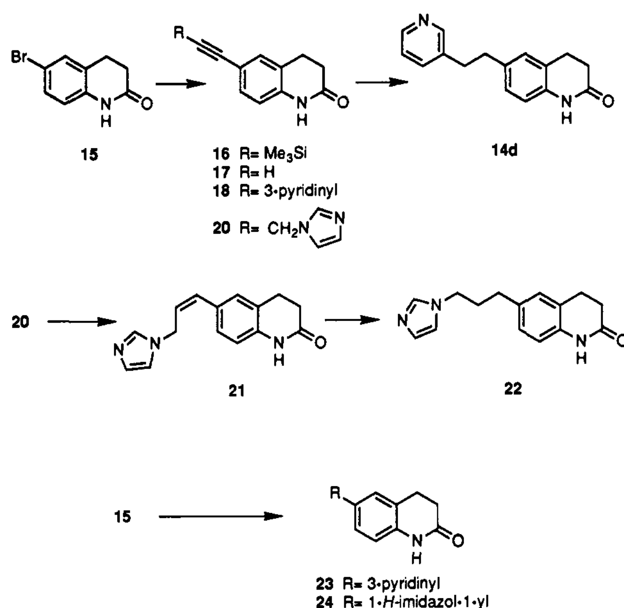
Scheme III




= $CH_3$) and 8, while 6e (R = H) provided 7e exclusively as the *E* isomer.

In an analogous manner described in Scheme II, the 3-pyridyl congeners were prepared by Friedel–Crafts acylation of 1 with either nicotinyl chloride hydrochloride or 3-pyridylacetyl chloride hydrochloride to afford ketone **10a** ($n = 0$) or **10b** ($n = 1$), respectively. Addition of an appropriate Grignard reagent or sodium borohydride to ketone 10 furnished carbinol 11, which was dehydrated to olefin 13 (for $n = 1$) or reduced to compound 12 (for $n = 0$) using sodium borohydride in trifluoroacetic acid. Whereas in most cases dehydration of alcohol 6 produced an isomeric mixture of olefins, similar treatment of alcohol 11 ($n = 1$) afforded 13 exclusively as the *E* isomer.

The preparation of analogues which are bonded directly to a heterocycle or through an acetylenic linkage is displayed in Scheme III. Ethynylation of bromide 15 using the procedure described by Lau et al.[13] furnished 17, which was subsequently coupled with 3-bromopyridine under similar conditions to give disubstituted acetylene 18. Using the above described conditions, bromide 15 was coupled with *N*-propargylimidazole 19 to furnish disubstituted acetylene 20. Sequential catalytic hydrogenation of 20 afforded 21 and 22, respectively, while exhaustive atmospheric hydrogenation of 18 afforded **14d**. Diethyl(3-pyridyl)borane was coupled using tetrakis(triphenylphosphine)palladium(0) catalysis with bromide 15 according to the method described by Terashima et al.[14] to give 23. Cuprous iodide catalyzed displacement of bromide 15 with imidazole in DMF furnished 24.

## Results and Discussion

Table I exhibits the results of an in vitro evaluation of 6-substituted 3,4-dihydroquinolin-2(1*H*)-ones for their ability to inhibit human blood platelet aggregation in the presence or absence of pig aortic microsomes (PAM), as a source of $PGI_2$ synthase, when challenged by ADP. The rationale for this screen has been described in earlier work,[12] where specific inhibitors of $TXA_2$ synthase require the presence of PAM for their antiaggregative effect whereas inhibition of aggregation in the absence of PAM is taken as evidence of an alternative pathway of action, e.g. cyclooxygenase or phosphodiesterase (PDE) inhibition. The latter pathway of action is discernible by aggregation experiments performed in the presence of $PGE_1$, where $PGE_1$ effects the potentiation of the inhibition of blood platelet aggregation.[15]

Using the dihydrocarbostyril heterocycle as an isosteric replacement for a carboxylic acid with attachment of the 1*H*-imidazol-1-yl or 3-pyridinyl heterocycle at the chemically accessible 6-position, one can logically predict that a one- or preferably a two-carbon bridge between the heterocycle and dihydrocarbostyril would satisfy the optimal distance requirement of a $TXA_2$ synthase inhibitor. A number of structures were prepared and evaluated where the nature of the bonding between the heterocycle and dihydrocarbostyril varied from a direct bond to a carbon chain varying in length (one to three carbons), substitution, and saturation. A number of structures showed a respectible level of inhibition against platelet aggregation when tested in the presence of PAM. Examples were found for each manner of bonding between the heterocycle and dihydrocarbostyril which exhibited activity comparable to dazoxiben (**26**),[16] an experimental standard for $TXA_2$ synthase inhibition, including **4a** and **4c** (one-carbon bridge), **7a**, 7e, and **13a** (two-carbon bridge), 21, and 22 (three-carbon bridge), and 23–25 (direct bond).

Generally, substitution by either an imidazole or pyridine heterocycle was effective for the expression of $TXA_2$ synthase inhibition in human blood platelets in the presence of PAM, but only imidazole-substituted compounds, e.g. **7a**, 7e, and 25, were appreciably active in the absence of PAM. While side-chain substitution adjacent to the dihydrocarbostyril was tolerated for the one carbon bridged compounds (Table I; Het = I, P; X = A; $n = 0$), the inhibitory activity of the homologated series in the presence of PAM (Table I; Het = I, P; X = A; $n = 1$) became less as the size of the substituent increased. For the unsaturated two carbon bridged compounds (Table I; Het = I, P; X = B, C; $n = 0, 1$), a similar dependence upon substituent size was observed in relationship to the effect on the inhibition of platelet aggregation. Both compounds 23 and 24, in which the pyridine and imidazole heterocycles are bonded directly to the dihydrocarbostyril, showed inhibition of human blood platelet aggregation at micromolar levels in the presence of PAM.

For each compound in Table I, an approximate ratio of the $IC_{50}$ values for the inhibition of platelet aggregation in the presence or absence of PAM was used as an indicator of the relative contribution of $TXA_2$ synthase and possible cAMP PDE activities. Platelet aggregation experiments performed in the absence of PAM resulted, in most cases, in an attenuation of inhibitory activity. For those compounds where the inhibitory activity was only marginally reduced in the absence of PAM, cAMP PDE activity was suspected as a major component of their antiaggregatory activity. This was confirmed by evaluation of PDE activity in vitro (Table II) for a group of compounds having, for the most part, favorable antiaggregatory activity relative to dazoxiben 26 in the presence of PAM

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**Table I.** In Vitro Biological Activity of $TXA_2$ Synthase/PDE Inhibitors

Het-$(CH_2)_n$-X

$A = R_1R_2C<$

$B = R_2C{=}CR_1$

$C = -C{\equiv}C-$

| no. | het.[a] | $n$ | X | $R_1$ | $R_2$ | yield, % | mp, °C | recryst solvent | formula | anal.[b] | $IC_{50}$,[c] μM w/PAM | $IC_{50}$,[c] μM w/o PAM | ratio[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **4a** | I | 0 | A | $CH_3$ | H | 38 | 173–175 | EtOAc/MeOH | $C_{14}H_{15}N_3O$ | C, H, N | 6.2 (0.37) | 320 (0.01) | 52 |
| **4b** | I | 0 | A | Ph | H | 74 | 215–217 | EtOAc | $C_{19}H_{17}N_3O$ | C, H, N | 0.35 (6.3) | 44% | |
| **4c** | I | 0 | A | $n$-$C_5H_{11}$ | H | 57 | 137–141 | EtOAc/hexane | $C_{18}H_{23}N_3O$ | C, H, N | 2.5 (0.88) | 37% | |
| **7a** | I | 0 | B[e] | $CH_3$ | H | 40 | 183–185 | EtOAc/MeOH | $C_{15}H_{15}N_3O$ | C, H, N | 1.6 (1.4) | 13 (0.17) | 8.1 |
| **7b** | I | 0 | B[f] | Ph | H | 85 | foam | | $C_{20}H_{17}N_3O$ | MS[g] | 28 (0.07) | 270 (0.01) | 9.6 |
| **7c** | I | 0 | B[h] | $n$-$C_5H_{11}$ | H | 48 | gum | | $C_{19}H_{23}N_3O$ | MS[i] | nt | nt | |
| **7e** | I | 0 | B[e] | H | H | 57 | 191–193 | | $C_{14}H_{13}N_3O{\cdot}0.25H_2O$ | C, H, N | 1.2 (1.7) | 5.6 (0.37) | 4.7 |
| **8** | I | 1 | A | $=CH_2$ | | 13 | 147–149 | EtOAc/MeOH | $C_{15}H_{15}N_3O$ | C, H, N | 7.6 (0.29) | 74 (0.03) | 9.7 |
| **9a** | I | 1 | A | $CH_3$ | H | 96 | 140–142 | EtOAc | $C_{15}H_{17}N_3O$ | H, N; C[j] | 5.5 (0.50) | 350 (0.01) | 64 |
| **9b** | I | 1 | A | Ph | H | 77 | 87–90 | EtOH | $C_{20}H_{19}N_3O$ | C, H, N | 200 (0.01) | nt | |
| **9c** | I | 1 | A | $n$-$C_5H_{11}$ | H | 95 | gum | | $C_{19}H_{25}N_3O$ | MS[k] | 25% | nt | |
| **9e** | I | 1 | A | H | H | 80 | 137–139 | | $C_{14}H_{15}N_3O{\cdot}0.35H_2O$ | C, H, N | 8.3 (0.28) | 140 (0.01) | 17 |
| **12** | P | 0 | A | Ph | H | 66 | foam | | $C_{21}H_{18}N_2O$ | MS[l] | 3.0 (0.77) | 11% | |
| **13a** | P | 0 | B[e] | $CH_3$ | H | 50 | 163–165 | EtOAc | $C_{17}H_{16}N_2O$ | C, H, N | 2.2 (1.5) | 90 (0.04) | 41 |
| **13b** | P | 0 | B[e] | Ph | H | 20 | 202–203 | EtOAc | $C_{22}H_{18}N_2O$ | C, H, N | 170 (0.02) | nt | |
| **13c** | P | 0 | B[e] | $n$-$C_5H_{11}$ | H | 25 | 189–191 | MeOH/EtOAc | $C_{21}H_{24}N_2O{\cdot}HCl{\cdot}H_2O$ | C, H, N | 58 (0.04) | nt | |
| **13d** | P | 0 | B[e] | H | H | 62 | 215–217 | $CHCl_3/Et_2O$ | $C_{16}H_{14}N_2O{\cdot}0.4H_2O$ | C, H, N | 6.2 (0.52) | 95 (0.03) | 15 |
| **14a** | P | 1 | A | $CH_3$ | H | 100 | gum | | $C_{17}H_{18}N_2O$ | MS[m] | 35 (0.07) | nt | |
| **14c** | P | 1 | A | $n$-$C_5H_{11}$ | H | 86 | gum | | $C_{21}H_{26}N_2O$ | C, H, N | 37% | nt | |
| **14d** | P | 1 | A | H | H | 95 | 135–136 | | $C_{16}H_{16}N_2O$ | C, H, N | 8.5 (0.39) | nt | |
| **18** | P | 0 | C | | | 76 | 195–197 | | $C_{16}H_{12}N_2O$ | C, H, N | 11 (0.29) | 240 | 22 |
| **20** | I | 1 | C | | | 94 | 164–168 | | $C_{15}H_{13}N_3O$ | C, H, N | 11 (0.24) | 55 (0.05) | 5.0 |
| **21** | I | 1 | B[n] | H | H | 85 | 158–160 | EtOAc/MeOH | $C_{15}H_{15}N_3O$ | C, H, N | 1.7 (1.5) | 160 (0.01) | 94 |
| **22** | I | 2 | A | H | H | 68 | 139–141 | | $C_{15}H_{17}N_3O$ | C, H, N | 3.0 (1.0) | 49% | 167 |
| **23** | | | | | | 71 | 180–182 | | $C_{14}H_{12}N_2O$ | C, H, N | 0.54 (4.8) | 27 (0.1) | 50 |
| **24** | | | | | | 12 | 205–208 | | $C_{12}H_{11}N_3O$ | C, H, N | 2.0 (1.3) | 80 (0.03) | 40 |
| **25** | | | | | | 3 | 211–213 dec | | $C_{12}H_9N_3O$ | MS[o] | 0.38 (6.8) | 2.8 (0.93) | 7.4 |
| **26** | COOH •HCl | | | | | | 132–134[p] | | $C_{12}H_{12}N_2O_3{\cdot}HCl$ | | 2.28 ± 0.48[q] | | |

[a] I = 1*H*-imidazol-1-yl; P = 3-pyridinyl. [b] Elemental analyses were within 0.4% of theoretical values unless otherwise noted. [c] $IC_{50}$'s are screening values for in vitro inhibition of ADP-induced aggregation of human PRP in the presence and absence of pig aortal microsomes (PAM). Each $IC_{50}$ value is a one-time determination generated from a dose–response curve. The curve was derived from several ($n$ = 3–5) points of drug concentration, each point being an average of measurements ($n$ = 1–3). For weakly active compounds, percentage values indicate degree of inhibition observed when tested at the maximum concentration of $5 \times 10^{-4}$ M; nt = not tested. Value in parentheses is relative activity of $TXA_2$ synthase inhibitor compared to the activity of dazoxiben (**26**) in the presence of PAM in the same experiment. [d] Ratio $IC_{50}$ (w/o PAM)/$IC_{50}$ (w/PAM). [e] *E* isomer. [f] *E*/*Z* isomeric mixture (4:1). [g] Mass spectrum exact mass: 315.137456 (0.9 ppm error). [h] *E*/*Z* isomeric mixture (2:1). [i] Mass spectrum exact mass: 309.184092 (−0.1 ppm error). [j] C: calcd, 79.56; found, 70.15. [k] Mass spectrum exact mass: 311.199722 (0.1 ppm error). [l] Mass spectrum exact mass 314.141688 (0.7 ppm error). [m] Mass spectrum exact mass 266.142032 (0.4 ppm error). [n] *Z* isomer. [o] Mass spectrum exact mass 237.090134 (−0.3 ppm error). [p] Reported mp 138–139.5 °C.[16b] [q] Average value ± standard deviation over all determinations ($n$ = 55).

while possessing attenuated activity in the absence of PAM. Compounds **7e**, **23**, and **25** were shown to possess a micromolar level of cAMP PDE inhibitory activity in conjunction with their $TXA_2$ synthase inhibitory activity.

Several points are noteworthy from the comparison of $IC_{50}$ values shown in Table I with in vitro cAMP PDE activities presented in Table II. Based upon the ratios of $IC_{50}$ values in Table I, the inhibitory activity of compound **23** for platelet aggregation was believed to be more dependent upon $TXA_2$ synthase inhibition than was compound **7e**. A more direct comparison of the in vitro inhibition of cAMP PDE (Table II) with the in vitro inhibition of $TXA_2$ synthase (Table I), both in human blood platelets, also suggests that compound **23** is a more effective inhibitor of $TXA_2$ synthase than cAMP PDE, but not at the magnitude of differentiation inferred by consideration of Table I alone. Compound **7e** was a compound which has comparable in vitro activity toward inhibition of $TXA_2$ synthase and cAMP PDE in human blood platelets at micromolar concentrations and proved to be the most interesting of the prepared series (vide infra).

$TXA_2$ synthase inhibitory activity, as evidenced by inhibition of ADP-induced platelet aggregation in the presence of PAM, was confirmed by the direct measure-

**Table II.** In Vitro Inhibition of Human Blood Platelet cAMP Phosphodiesterase and Thromboxane Synthase

| | inhibitory activity | | |
|---|---|---|---|
| compound | PDE, μM[a] | $TXA_2$, μM[b] | ratio[c] |
| **4b** | 40 | 0.35 | 114 |
| **7e** | 6.4 | 1.2 | 5.3 |
| **9c** | 14 | 25%[b] | - |
| **23** | 4.4 | 0.54 | 8.1 |
| **24** | 16 | 2.0 | 8 |
| **25** | 4.0 | 0.38 | 10.5 |
| anagrelide (**27**) | 0.082 | - | - |
| CI-914 (**28**) | 4.7 | - | - |

[a] $IC_{50}$'s are screening values derived from a dose-response curve generated from five concentration points, each point being an average of three measurements. [b] See footnote c in Table I. [c] Ratio = $IC_{50}$ (PDE)/$IC_{50}$ ($TXA_2$).

**Table III.** Effect of **7e**, **23**, and Dazoxiben (**26**) on Inhibition of $TXA_2$ and $PGI_2$

| | | % [$^{14}$C]arachidonic acid as | |
|---|---|---|---|
| compound | concentration, μM | $TXB_2$ | 6-keto-$PGF_{1\alpha}$ |
| control[a] | | 14.9 ± 2.5 | 1.45 ± 0.6 |
| **7e**[b] | 500 | 4.3 ± 0.7 | 21.1 ± 2.8 |
| | 50 | 8.0 ± 1.5 | 11.5 ± 3.5 |
| | 5 | 12.3 ± 1.8 | 3.0 ± 0.8 |
| **23**[c] | 10 | 4.5 | 31.9 |
| | 1 | 14.8 | 7.7 |
| | 0.1 | 21.1 | 2.0 |
| dazoxiben (**26**)[d] | 50 | 1.5 | 25.2 |
| | 5 | 2.4 ± 0.0 | 23.6 ± 7.2 |
| | 0.5 | 15.4 | 8.0 |

[a] Control experiment consisted of [$^{14}$C]arachidonic acid induced aggregation, in the absence of a drug, of washed and resuspended human blood platelets in platelet-poor plasma in the presence of pig aortal microsomes. Values for $TXB_2$ and 6-keto-$PGF_{1\alpha}$ percentages are derived from an average of measurements ($n = 4$) ± standard deviation (SD). [b] Each percentage value is in average of measurements ($n = 2$) ± variation in measurements. [c] Each percentage value is a single measurement. [d] Percentage values for 5 μM drug concentration are an average of measurements ($n = 3$) ± SD; all other values are single measurements.

ment of $TXB_2$ (the stable metabolite of $TXA_2$) and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of $PGI_2$) generated from radiolabeled arachidonic acid by (washed and resuspended) human blood platelets in the presence of PAM in platelet-poor plasma (Table III). Compounds **7e** and **23**, when tested at 500 and 10 μM concentrations, respectively, had a similar effect on the distribution of prostanoid metabolites as compared to the selective $TXA_2$ synthase inhibitor dazoxiben **26** at 5 μM. $TXA_2$ synthase inhibition is demonstrated by a reduced production of thromboxane with *a concomitant increase* in prostacyclin production, and both compounds **7e** and **23** display this profile in a concentration-dependent manner. The effect of several compounds on serum $TXB_2$ levels in rats after a dose of 50 mg/kg po was determined by radioimmunoassay (Table IV). All the compounds in Table IV produced an inhibition of $TXB_2$ greater than or equal to dazoxiben **26** at the same dose after 2 h (3 h for **7e**).

In order to investigate manifestations of the $TXA_2$ synthase inhibitory activity in vivo, the most potent in vitro compounds **4b**, **7e**, and **23** were evaluated in mice in a collagen-epinephrine-induced thromboembolism model. This model of disseminated intravascular coagulation[17] has been used to evaluate the bioavailability of $TXA_2$ synthase inhibitors, although cAMP PDE inhibitors are also active

(17) Di Minno, G.; Silver, M. J. Mouse Antithrombotic Assay: A Simple Method for the Evaluation of Antithrombotic Agents *In Vivo*. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 57-60.

**Table IV.** Effect of $TXA_2$ Synthase Inhibitors on Serum $TXB_2$ Level in Rats at 50 mg/kg po[a]

| compound | time, h | inhibition, % |
|---|---|---|
| **21** | 2 | 89 ± 5 |
| **23** | 2 | 94 ± 4 |
| **24** | 2 | 77 ± 14 |
| dazoxiben (**26**) | 2 | 74 ± 12 |
| dazmegrel (**29**) | 2 | 97 ± 1 |
| **7e** | 3 | 97 ± 1 |
| dazoxiben (**26**) | 3 | 42 ± 10 |

[a] Control group, $n = 4$; test group, $n = 2$.

**Table V.** Protection against Mortality in a Pulmonary Thromboembolism Model in Mice[a]

| | % of animals surviving 24 h: dose, mg/kg po[b,c] | | | | | | |
|---|---|---|---|---|---|---|---|
| compound | 1 | 3 | 6 | 9 | 10 | 12 | 30 |
| **4b** | 20 | 20 | | | 30-40 | | |
| **7e** | 10-40[d] | 20-50[d] | 50 | 50, 90[e] | 90 | 70 | |
| **23** | 20 | 40 | | | 40 | | 50 |
| dazoxiben (**26**) | | 20 | | | 40 | | 50 |
| dazmegrel (**29**) | 30 | 30 | | 50 | | 60 | |

[a] Mortality induced by injection of collagen extract and epinephrine (see the Experimental Section). [b] In each experiment the test material vehicle, (carboxymethyl)cellulose (CMC) was tested alone as a positive control. Survival was 10% after 24 h for the positive control group in each case; $n = 10$ for each group. Test materials administered three hours prior to induced mortality. [c] For 50-60% survival, $p < 0.10$; for 70-90% survival, $p < 0.05$. [d] Values measured in four separate experiments. [e] Values measured in two separate experiments.

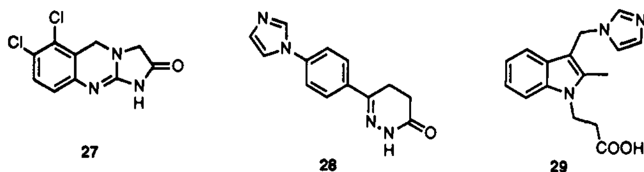
**Table VI.** A Time Course Study of the Protection against Mortality in a Pulmonary Thromboembolism Model in Mice[a,b]

| | % of animals surviving 24 h: hour of dosing | | | | |
|---|---|---|---|---|---|
| compound | -1 | -3 | -5 | -7 | -18 |
| positive control[c] | 10 | 20 | 10 | 10 | 10 |
| **7e** | 20 | 50 | 20 | 60[d] | 0 |
| dazoxiben (**26**) | 50[d] | 40 | 20 | 20 | 10 |
| dazmegrel (**29**) | 20 | 11[e] | 20 | 0 | 10 |

[a] Mortality induced by injection of collagen and epinephrine (see the Experimental Section). [b] Dose (10 mg/kg) of compound in (carboxymethyl)cellulose (CMC); $n = 10$. [c] CMC, the test material vehicle, was tested alone as a positive control. [d] $p < 0.1$. [e] Nine animals tested.

in this model. Whereas compounds **4b** and **23** showed modest protection (30-40% survival) at 10 mg/kg po (Table V), **7e** showed a consistently high degree of protection (70-90% survival) at 10-12 mg/kg po. Since **7e** and **23** have comparable in vitro activities (Table II), the superior in vivo activity of **7e** is presumably a result of better bioavailability. Protection by dazoxiben **26**, a selective $TXA_2$ synthase inhibitor, did not exceed 50% at doses up to 30 mg/kg po whereas the more efficacious dazmegrel **29** initially afforded 60% protection at 12 mg/kg po. For comparison, the standard PDE inhibitors anagrelide **27** (40% survival after 24 h; 9 mg/kg po), CI-914 **28** (50% survival after 24 h; 20 mg/kg po), aminophylline (0% survival after 24 h; 50 mg/kg po), and isobutylmethylxanthine (10% survival after 24 h; 50 mg/kg po) gave varying degrees of protection. In a time course study, compound **7e** dosed at 10 mg/kg po retained good activity through 7 hours before challenge (Table VI), while dazoxiben **26** and dazmegrel **29** provided limited protection.

In Table VII, an evaluation of **7e**, **23**, and dazoxiben **26** was made using ex vivo platelet aggregation experiments in rats in order to further assess the utility of a drug with mixed action against thrombotic events. The data in Table VII shows the superiority of **7e** over **23** and dazoxiben **26** in ex vivo inhibition of blood platelet aggregation in rats.




The in vivo superiority of **7e** over **23**, presumably due to better bioavailability, is suggested in this table. At the first time point (3 h) **7e** and **23** show comparable activity although the duration of action of **7e** exceeds that of **23**. Comparisons of **7e** with **23** at the 5-h time point and dazoxiben **26** at the 8-h time point are inconclusive due to the lack of acceptable standard errors. Nevertheless, the level of inhibition afforded by **7e** toward ex vivo thrombin-induced platelet aggregation in rats is superior to that of dazoxiben **26** even when tested orally at twice the dosage (**7e** at 50 mg/kg vs **26** at 100 mg/kg) and constant throughout the 8-h time course of the experiment.

Representative compounds from Table I were evaluated for effects on blood pressure in the conscious spontaneously hypertensive rat (SHR). Phosphodiesterase inhibitors generally show activity in this model, although antihypertensive activity has been described previously for two series of "selective" $TXA_2$ synthase inhibitors.[18] As shown in Table VIII, significant antihypertensive activity was found for compounds **7e**, **13a**, **18**, **21**, and **23** at a dose of 10 mg/kg po, whereas significant increases in heart rate without a corresponding decrease in blood pressure were observed for compounds **9e** and **13d**. Generally, activity in this assay was found in those compounds most active in inhibiting platelet aggregation *in the absence* of PAM (cf. Table I), e.g. **7e**, **13a**, **21**, and **23**, as would be expected for compounds manifesting a significant level of cAMP PDE inhibitory activity. Compounds inhibiting platelet aggregation only in the presence of PAM (specific $TXA_2$ synthase inhibition, e.g. **14d** and **22**) or showing weak antiaggregatory activity (e.g. **7b**, **9e**) were inactive. The antihypertensive activity thus appears to be correlated with PDE activity as revealed by the ability to inhibit ADP-induced platelet aggregation in the absence of a source of $PGI_2$ rather than some other structural feature present in the molecules. The implied phosphodiesterase inhibitory activity of **7e**, **23**, and **25** was confirmed in an in vitro assay against human platelet cAMP PDE (vide supra), these compounds being approximately equipotent to CI-914 **28**. The most active antihypertensive compound **7e** retained significant and prolonged duration of action to 8 h at 1 mg/kg po (Table IX).

## Conclusion

Examples such as **7e**, **23**, **24**, and **25** have shown that it was possible to design agents which display both $TXA_2$ synthase and cAMP PDE inhibitory activities within the same structure. Compound **7e**, which incorporates a comparable level of both activities in vitro, has been shown to be a potent antithrombotic and antihypertensive with a long duration of action.

(18) (a) Wright, W. B., Jr.; Press, J. B.; Chan, P. S.; Marsico, J. W.; Haug, M. F.; Lucas, J.; Tauber, J.; Tomcufcik, A. S. Thromboxane Synthetase Inhibitors and Antihypertensive Agents. 1. *N*-[(1*H*-Imidazole-1-yl)alkyl]aryl Amides and *N*-[1*H*-1,2,4-Triazol-1-yl)alkyl]aryl Amides. *J. Med. Chem.* **1986**, *29*, 523–530. (b) Press, J. B.; Wright, W. B., Jr.; Chan, P. S.; Marsico, J. W.; Haug, M. F.; Tauber, J.; Tomcufcik, A. S. Thromboxane Synthetase Inhibitors and Antihypertensive Agents. 2. *N*-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-isoindole-1,3-(2*H*)-diones and *N*-[(1*H*-1,2,4-Triazol-1-yl)alkyl]-1*H*-isoindole-1,3-(2*H*)-diones as Unique Unique Antihypertensive Agents. *J. Med. Chem.* **1986**, *29*, 816–819.

**Table VII.** Ex Vivo Inhibition of Blood Platelet Aggregation in Rats with **7e**, **23**, and Dazoxiben (**26**)

| compound | dosage, mg/kg po | time of sacrifice, h | inhibition,[a,b] % |
|---|---|---|---|
| 7e | 50 | 3 | 94.7 ± 0.8 |
| | | 5 | 95.9 ± 0.8 |
| | | 8 | 96.5 ± 0.0 |
| 23 | 50 | 3 | 94.7 ± 0.8 |
| | | 5 | 53.2 ± 43.0 |
| | | 8 | 4.7 ± 2.5 |
| dazoxiben (26) | 100 | 3 | 23.4 ± 10.8 |
| | | 5 | 24.6 ± 14.0 |
| | | 8 | 50.9 ± 49.6 |

[a] Inhibition percentage is an average ($n$ = 2) of values obtained from individual animals tested in the presence of PAM with thrombin as the inducer of aggregation; control group, $n$ = 6. [b] Value ± variation of measurement.

**Table VIII.** Antihypertensive Activity in Conscious Spontaneously Hypertensive Rats (SHR)

| compound | dose, mg/kg po | | change from control,[a] % 1 h | 2 h | 4 h |
|---|---|---|---|---|---|
| 7e | 10 | SBP[b] | -56* | -40* | -48* |
| | | HR[c] | 22* | 19* | 25* |
| 9e | 10 | SBP | -14 | -2 | -13 |
| | | HR | 17 | 34* | 12 |
| 13a | 10 | SBP | -19* | -16* | -15 |
| | | HR | 12 | 20* | 25* |
| 13d | 10 | SBP | -7 | -7 | -12 |
| | | HR | 11 | 23* | 32* |
| 18 | 10 | SBP | -13* | -6 | -5 |
| | | HR | 4 | 15* | 22* |
| 20 | 10 | SBP | -3 | -5 | -15* |
| | | HR | 9 | 11 | 11 |
| 21 | 10 | SBP | -28* | -19* | -17* |
| | | HR | 5 | 15 | 14 |
| 23 | 10 | SBP | -42* | -28* | -22* |
| | | HR | 13 | 18* | 13 |
| milrinone (30) | 10 | SBP | -65* | -54* | -47* |
| | | HR | 11 | 36* | 45* |

[a] For each time point, $n$ = 4; control group, $n$ = 8; * = $p < 0.05$. [b] SBP = systolic blood pressure, mmHg. [c] HR = heart rate, beats/min.

**Table IX.** Antihypertensive Activity of **7e** in Conscious SHR

| dose, mg/kg po | | measured change from control,[a] % 1 h | 2 h | 4 h | 8 h | 12 h |
|---|---|---|---|---|---|---|
| 10 | SBP[b] | -56* | -40* | -48* | -54* | -35* |
| | HR | 22* | 19* | 25* | 16* | 29* |
| 3 | SBP | -34* | -21* | -28* | -18* | -3 |
| | HR | 28* | 10* | 20* | 21* | 8 |
| 1 | SBP | -19* | -8* | -8* | -9* | -3 |
| | HR | 17* | 11* | 7 | -5 | 1 |
| 0.3 | SBP | -5 | 1 | -1 | -5 | -1 |
| | HR | 3 | -13* | -4 | -8 | -8 |

[a] For each time point, $n$ = 4; control group, $n$ = 8; * = $p < 0.05$. [b] SBP = systolic blood pressure, mmHg. [c] HR = heart rate, beats/min.

## Experimental Section

**Biology. (a) In Vitro Thromboxane Synthase Inhibition.** Test materials were evaluated for thromboxane synthase inhibitory activity, with or without pig aortal microsomes, by the previously described procedure.[12]

**(b) In Vitro Human Platlet cAMP Phosphodiesterase Inhibition.** Evaluation of test materials as inhibitors of human platelet cAMP phosphodiesterase was performed as described previously.[19]

(19) 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.

**(c) Measurement of Arachidonic Acid Metabolites.** Human blood was collected into sodium citrate (0.38% final concentration) and centrifuged at 200*g* for 15 min to afford platelet-rich plasma (PRP). EDTA (0.5 mL, 0.1 M) was added to the PRP (10 mL), which was again centrifuged at 2500 rpm for 15 min giving platelet-poor plasma (PPP) as the supernatant. Washed human resuspended platelets (WHRP) were obtained by gently resuspending the resultant platelet pellet in 0.5 mL 0.01 M Tris-HCl buffer (0.15 M NaCl, 0.01 M Tris, 0.001 M EDTA, 5 mM glucose) at pH 7.3. Pig aortal microsomes (PAM) were prepared by the method of Moncada and Neichi et al.[20] Preparation of the PPP–PAM mixture consisted of incubation of PPP (1.0 mL) at 37 °C with stirring (500 rpm) for 2 min followed by addition of PAM (50 $\mu$L) and incubation for another 3 min. A solution of EDTA (5 $\mu$L, 0.01 M) and $^{14}$C-labeled arachidonic acid (2.5 $\mu$L, 1.7 mM, 56.9 mCi/mmol, 0.05 mCi/mL ethanol) was added to WHRP (125 $\mu$L) with stirring (500 rpm) at 37 °C. The WHRP mixture was incubated for 20 min before an aliquot (112 $\mu$L) was transferred into the previously prepared PPP–PAM mixture. The reaction was terminated by the addition of an equal volume of ethanol 2 min after the addition of the WHRP to the PPP–PAM mixture, except for the data presented in Table III where incubation was for at least 15 min at ambient temperature.

After termination of the reaction, the solution was allowed to sit on ice for at least 30 min and an aliquot (0.75 mL) of the supernatant was diluted with water (3 mL). After preparation of a C-18 reverse-phase chromatography column (Bond-Elut, Analytichem International) by successively washing with methanol (1 mL) and water (1 mL), the diluted plasma sample (3 mL) was applied and eluted with water (1 mL) under vacuum at a flow rate which was ≤ 1 mL/min. The water eluate was discarded, and the column was further eluted with methyl formate (1 mL). The eluate was collected in an Eppendorf polypropylene tube and evaporated under a gentle stream of nitrogen. The residue was dissolved in ethanol (20 $\mu$L), and a portion (2 $\mu$L) of this solution was counted in a scintillation counter, while the remaining material was loaded onto a previously dried preparative TLC plate (silica gel GF, 20 cm × 20 cm, Analtech, Inc.). A standard solution of $TXB_2$, 6-keto-$PGF_{1\alpha}$, $PGD_2$, and $PGE_2$ (10 $\mu$L, 1 mg/mL each) was also loaded on to the same plate as a reference.

In order to effectively separate the major prostanoid metabolites, duplicate plates were eluted separately in two different solvent systems: development with a mixture of $CHCl_3$, MeOH, AcOH, and water (90:8:1:0.8) three times separated $TXB_2$ from $PGE_2$ and 6-keto-$PGF_{1\alpha}$ whereas development twice with the saturated organic layer from a mixture of EtOAc, isooctane, AcOH, and water (11:5:2:10) separated 6-keto-$PGF_{1\alpha}$ from $PGE_2$ and $TXB_2$. The chromatographic bands were located by iodine vapor staining as well as scanning for radioactivity. The bands with measurable radioactivity were removed from the plates and counted in a scintillation counter.

**(d) Ex Vivo Radioimmunoassay (RIA) Determination of Serum $TXB_2$ Levels.** Compounds **7e**, **21**, **23**, **24**, **29**, and dazoxiben **26** were suspended in 0.5% aqueous sodium (carboxymethyl)cellulose as a vehicle and dosed orally in male rats (Sprague–Dawley) by gavage. After 2 or 3 h, depending on the chosen test material, blood was collected into a syringe containing no anticoagulant. The blood was allowed to clot at 37 °C for 1 h. The serum was collected by centrifuging the blood sample at 2000*g* for 30 min. Determinations of the serum $TXB_2$ levels were made using the protocol described in a commercial RIA kit (New England Nuclear, NEK-024A).

**(e) Ex Vivo Platelet Aggregation Experiment with 7e, 23, and Dazoxiben 26.** Male rats (Sprague–Dawley) were anesthetized with pentobarbital (50 mg/kg ip), and blood was collected from the abdominal aorta into a syringe (12 mL) containing 1.2 mL of 1.9% sodium citrate (final concentration 0.19% sodium citrate). The blood was transferred into a plastic conical tube (15 mL) and centrifuged at 200*g* for 15 min at room temperature to obtain platelet-rich plasma (PRP). The red cell fraction was centrifuged further at 1000*g* for 15 min to yield platelet-poor plasma (PPP). PRP was diluted by 2.5-fold with Tyrode's buffer (0.2 g/L KCl; 1.0 g/L $NaHCO_3$; 58 mg/L $NaH_2PO_4$; 8.5 g/L NaCl; 1.0 g/L dextrose; pH 7.4) and used for platelet aggregation studies. PPP was similarly diluted by 2.5-fold with Tyrode's buffer and used as a reference for platelet aggregometry. Aggregation was followed by the turbimetric method of Born[21] using a Payton dual-channel aggregometer with PPP set at 90% transmittance and PRP set at 10% transmittance. An aliquot of diluted PRP (0.49 mL) was incubated in a siliconized cuvette at 37 °C with stirring (500 rpm) in the aggregometer for 2 min. An appropriate amount of PAM was added and incubated for an additional 3 min. Platelet aggregation was induced by the addition of thrombin (10 $\mu$L). The pooled and diluted PRP of the control rats was used to determine the minimum amount of thrombin necessary to induce full platelet aggregation. Using the $TXA_2$ synthase inhibitor dazoxiben **26**, the appropriate amount of PAM for ex vivo experiments was determined as that necessary to give an $IC_{50}$ value of 1–5 $\mu$M in aggregation experiments performed with pooled control rat PRP.

Test materials were prepared as suspensions in a 0.5% aqueous sodium (carboxymethyl)cellulose (CMC) with the aid of a hand homogenizer. Appropriate dosages of the suspended test materials were administered to rats (225–250 g) orally by gavage. A control group of rats ($n$ = 4) was sacrificed at the beginning of the experiment in order to establish the amounts of PAM and thrombin to be used in this study. The study conducted with **7e**, **23**, and dazoxiben **26** employed a total of 24 rats, where three groups ($n$ = 6) of dosed rats were sacrificed at 3-, 5-, and 8-h time points after drug administration. A vehicle control group was comprised of rats ($n$ = 3) sacrificed 3 and 8 h after oral dosing with 0.5% aqueous CMC (1 mL). Ex vivo platelet aggregation experiments were performed as described above.

**(f) Blood Pressure and Heart Rate Measurements in Conscious Spontaneously Hypertensive Rats (SHR).** Male SHR (Charles River or Taconic Farms) weighing 200–250 g and >16 weeks in age were employed in this model. Animals used in the evaluation of test materials listed in Tables VIII and IX had predose basal systolic blood pressures measuring >170 mmHg. Each dosage time-point group was comprised of four rats where the test material was administered as a solution (5 mg/mL) in either *N,N*-dimethylacetamide or PEG-400 by gavage. A control group of eight rats per experiment were gavaged with vehicle alone. Conscious systolic blood pressures were monitored at the described time points by using the tail cuff method. Heart rates were derived electronically from systolic blood pressure measurements.

**(g) Collagen–Epinephrine-Induced Pulmonary Thromboembolism.** The experimental procedure for this model has been described previously.[12,17]

**Chemistry.** All melting points are uncorrected and were obtained on a Thomas-Hoover capillary melting point apparatus. The structures of all compounds were confirmed by their IR and $^1$H NMR spectra. The IR spectra were recorded on a Sargent-Welch 3-200 spectrophotometer and the $^1$H NMR spectra were obtained on a Varian EM390 or a Bruker WM300 spectrometer using tetramethylsilane as an internal standard. Elemental analyses were performed by Syntex Analytical Research Services or Atlantic Micro Lab, Atlanta, GA, and found to be within 0.4% of theoretical values unless otherwise noted.

**6-Acetyl-3,4-dihydroquinolin-2(1*H*)-one (2a).** Acetyl chloride (3.20 mL, 3.53 g, 45.0 mmol) was added to a stirred suspension of **1** (4.40 g, 29.9 mmol) and $AlCl_3$ (27.0 g, 0.20 mol) in $CS_2$ (30 mL) at 0 °C. The reaction mixture was stirred at ambient temperature for 1 h and heated at reflux for 18 h whereupon the mixture was poured on to crushed ice. The precipitate was collected by filtration, washed with water, and air-dried. Recrystallization of the crude product from EtOAc afforded **2a** (4.27 g, 76%) as fine needles, mp 161–163 °C. Anal. ($C_{11}H_{11}NO_2$) C, H, N.

(20) (a) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. An Enzyme Isolated from Arteries Transforms Prostaglandin Endoperoxides to an Unstable Substance that Inhibits Platelet Aggregation. *Nature (London)* **1976**, *263*, 663–665. (b) Neichi, T.; Tomisawa, S.; Kubodera, N.; Uchida, Y. Enhancement of $PGI_2$ Formation by a New Vasodilator, 2-Nicotinamidoethyl Nitrate in the Coupled System of Platelets and Aortic Microsomes. *Prostaglandins* **1980**, *19*, 577–586.

(21) Born, G. V. R. Quantitative Investigations into the Aggregation of Blood Platelets. *J. Physiol.* **1962**, *162*, 67P–68P.

The following ketones were prepared similarly. 6-Benzoyl-3,4-dihydroquinolin-2(1*H*)-one (**2b**): 90% yield; mp 205–207 °C. Anal. ($C_{16}H_{19}NO_2$) C, H, N. 6-Hexanoyl-3,4-dihydroquinolin-2(1*H*)-one (**2c**): 85% yield; mp 145–147 °C (EtOAc). Anal. ($C_{15}H_{19}NO_2$) C, H, N.

**6-[1-(1*H*-Imidazol-1-yl)ethyl]-3,4-dihydroquinolin-2-(1*H*)-one (4a).** Sodium borohydride (840 mg, 22.3 mmol) was added to a stirred suspension of **2a** (4.20 g, 22.3 mmol) in MeOH at 0 °C. The reaction was stirred for 2 h, and the solvent was removed under reduced pressure. Water (25 mL) was added to the solid residue followed by the addition of EtOAc (75 mL). The mixture was stirred and the resulting suspension filtered to afford **3a** (R = $CH_3$) (2.80 g), mp 170.5–172 °C. Anal. ($C_{11}H_{13}NO_2$) C, H, N. Additional material (0.89 g) was isolated from the filtrate.

A solution of alcohol **3a** (3.63 g, 19.0 mmol) in THF (25 mL) was added to a cooled (0 °C) solution of thionylbis(imidazole) (prepared previously by reaction of imidazole (10.39 g, 152 mmol) with thionyl chloride (4.52 g, 38.0 mmol) in 75 mL of THF and filtration of the precipitated imidazole hydrochloride). The reaction mixture was stirred for 1 h at 0 °C and an additional 15 h at ambient temperature before the solvent was removed under reduced pressure. The residue was partitioned between EtOAc (50 mL) and water (25 mL), the organic layer was separated, and the aqueous layer was repeatedly extracted with EtOAc (4 × 50 mL). The combined organic extracts were washed with brine (75 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel using MeOH/$CH_2Cl_2$ (6:94) as the eluent to afford **4a** (1.74 g, 38%) as a solid, mp 173–175 °C (EtOAc/MeOH). Anal. ($C_{14}H_{15}N_3O$), C, H, N.

The following compounds were prepared similarly using ketones **2b** and **2c**, respectively: 6-(1*H*-imidazol-1-yl)phenylmethyl-3,4-dihydroquinolin-2(1*H*)-one (**4b**): 74% yield; 6-[1-(1*H*-imidazol-1-yl)hexyl]-3,4-dihydroquinolin-2(1*H*)-one (**4c**): 57% yield.

**6-(1*H*-Imidazol-1-ylacetyl)-3,4-dihydroquinolin-2(1*H*)-one (5).** Chloroacetyl chloride (5.34 mL, 7.57 g, 67.0 mmol) was added over 10 min to a stirred suspension of 1 (6.60 g, 44.9 mmol) and $AlCl_3$ (40.0 g, 0.30 mol) in $CS_2$ (50 mL) at 0 °C. The reaction mixture was stirred at ambient temperature for 1 h and heated under reflux for 3 h before the mixture was poured onto crushed ice. The precipitate was collected by filtration, washed with water, and air-dried to afford **2d** (9.70 g, 97%) which was used without further purification.

A solution of **2d** (9.00 g, 39.4 mmol) and imidazole (13.4 g, 197 mmol) in DMF (40 mL) was heated at 80 °C for 18 h. The reaction was diluted with water (1 L), and the resulting precipitate was collected by filtration. The solid was washed with water and air-dried to afford **5** (8.51 g, 85%) which was pure enough for further use; mp 269–273 °C. Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

**(*E*)-6-[2-(1*H*-Imidazol-1-yl)ethenyl]-3,4-dihydroquinolin-2(1*H*)-one (7e).** Sodium borohydride (760 mg, 20.0 mmol) was added to a stirred suspension of **5** (5.14 g, 20.0 mmol) in EtOH (100 mL) at 0 °C. The reaction was allowed to warm to ambient temperature and stirred for 15 h. The reaction mixture was then evaporated to dryness under reduced pressure, and the residue was dissolved in anhydrous HCOOH (100 mL). The mixture was heated for 72 h, the solvent was removed under reduced pressure, and the residue was neutralized with aqueous $K_2CO_3$. The precipitate was filtered off, washed with water, and air-dried. Purification by chromatography on silica gel eluting with MeOH/$CH_2Cl_2$ (5:95) gave **7e** (2.72 g, 57%).

**(*E*)-6-[1-(1*H*-Imidazol-1-yl)propen-2-yl]-3,4-dihydroquinolin-2(1*H*)-one (7a) and 6-[3-(1*H*-Imidazol-1-yl)-propen-2-yl]-3,4-dihydroquinolin-2(1*H*)-one (8).** A solution of 2.8 M MeMgCl (15.6 mL, 43.6 mmol) in THF was added to a suspension of **5** (2.23 g, 8.74 mmol) in THF (50 mL) at 0 °C. After being stirred for 1 h, the reaction mixture was warmed to ambient temperature, and stirring was continued for an additional 15 h. The mixture was poured on to crushed ice and extracted with $CH_2Cl_2$ (4 × 75 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to afford a yellow foam. Chromatography on silica gel eluting with MeOH/$CH_2Cl_2$ (10:90) gave alcohol **6a** (1.03 g, 43%) as a foam: $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 1.33 (s, 3 H), 2.30–2.58 (m, 2 H, $COCH_2$), 2.70–3.00 (m, 2 H, $ArCH_2$), 3.75 (br s, 1 H, OH); 4.06 (m, 2 H, $CH_2N$), 6.77 (s, 1 H, imidazole H-4), 6.80 (d, $J$ = 9.0 Hz, 1 H, H-8), 6.97 (s, 1 H, H-5), 7.18 (d, $J$ = 9.0 Hz, 1 H, H-7), 7.23 (s, 1 H, imidazole H-5), 7.39 (s, 1 H, imidazole H-2).

A solution of **6a** (1.00 g, 3.64 mmol) in anhydrous HCOOH (25 mL) was heated at 90 °C for 24 h. The reaction was cooled and poured into aqueous $K_2CO_3$ (200 mL, 1 M). The resulting white precipitate was collected by filtration, washed with water, and air-dried. Chromatography on silica gel eluting with MeOH/$CH_2Cl_2$ (5:95) gave **7a** (370 mg, 40%). Further elution afforded **8** (120 mg, 13%) in addition to a mixture of **7a** and **8** (260 mg, 28%).

**6-[1-(1*H*-Imidazol-1-yl)prop-2-yl]-3,4-dihydroquinolin-2-(1*H*)-one (9a).** Hydrogenation of a mixture of **7a** and **8a** (260 mg, 1.03 mmol) over 10% Pd/C (25 mg) in EtOH (10 mL) at 50 psi gave **9a** (250 mg, 96%).

**6-[1-Phenyl-2-(1*H*-imidazol-1-yl)ethyl]-3,4-dihydroquinolin-2(1*H*)-one (9b).** Use of PhMgBr as the Grignard reagent in the procedure described for the preparation of **7a** afforded an inseparable *E* and *Z* mixture (4:1) of 6-[1-phenyl-2-(1*H*-imidazol-1-yl)ethenyl]-3,4-dihydroquinolin-2(1*H*)-one (**7b**) in 85% yield as a foam. Atmospheric hydrogenation of **7b** (390 mg, 1.24 mmol) over 10% Pd/C (200 mg) in EtOH (10 mL) gave **9b** (300 mg, 77%).

**6-[1-(1*H*-Imidazol-1-ylmethyl)hexyl]-3,4-dihydroquinolin-2(1*H*)-one (9c).** Use of $n$-$C_5H_{11}$MgBr as the Grignard reagent in the procedure described for the preparation of **7a** afforded an inseparable *E* and *Z* mixture (2:1) of 6-[2-(1*H*-imidazol-1-yl)-1-pentylethenyl]-3,4-dihydroquinolin-2(1*H*)-one (**7c**) in 48% yield as a gum. Atmospheric hydrogenation of **7c** (400 mg, 1.29 mmol) over 10% Pd/C (200 mg) in EtOH (10 mL) gave **9c** (390 mg, 95%).

**6-(3-Pyridinylcarbonyl)-3,4-dihydroquinolin-2(1*H*)-one (10a).** Nicotinyl chloride hydrochloride (16.0 g, 89.9 mmol) was added to a stirred suspension of 1 (8.80 g, 59.9 mmol) and $AlCl_3$ (54.0 g, 405 mmol) in $CS_2$ (100 mL). The mixture was heated at reflux for 72 h, cooled to ambient temperature, and poured on to crushed ice. The resulting mixture was poured into a solution of NaOH (125 g) in water (300 mL) and extracted with MeOH/$CH_2Cl_2$ (10:90; 3 × 250 mL). The organic extracts were washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure to afford **10a** (12.1 g, 80%) sufficiently pure for further use, mp 195–202 °C. Anal. ($C_{15}H_{12}N_2O_2{\cdot}0.25H_2O$) C, H; N.

Replacement of the nicotinyl chloride employed in the above procedure by 3-pyridinylacetyl chloride hydrochloride gave 6-(3-pyridinylacetyl)-3,4-dihydroquinolin-2(1*H*)-one (**10b**) in a 73% yield; mp 207–209 °C ($CHCl_3$/EtOH). Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

**6-[(3-Pyridinyl)phenylhydroxymethyl]-3,4-dihydroquinolin-2(1*H*)-one (11b).** A solution of PhMgBr (4.0 mL, 3.0 M in ether, 12.0 mmol) was added dropwise over 10 min to a stirred suspension of ketone **10a** (1.0 g, 4.0 mmol) in THF (20 mL) at 0 °C. The reaction mixture was stirred for 1 h and then allowed to warm to ambient temperature. After being stirred for 15 h, the reaction mixture was quenched with saturated aqueous $NH_4Cl$ (25 mL), extracted with EtOAc (2 × 75 mL), and dried ($MgSO_4$). The extract was evaporated under reduced pressure and purified by column chromatography initially eluting with MeOH/$CH_2Cl_2$ (2:98) to (5:95) to afford alcohol **11b** (0.49 g, 37%) as a yellow foam: $^1$H NMR (90 MHz, $CDCl_3$) $\delta$ 2.45–3.00 (m, 4 H, $CH_2CH_2$), 3.5 (br s, 1 H, OH), 6.82 (d, $J$ = 8.9 Hz, 1 H, dihydroquinolinone H-8), 6.90–7.40 (m, 3 H, dihydroquinolinone H-5,6 and pyridine H-4), 7.28 (s, 5 H, $C_6H_5$), 7.45 (m, 1 H, pyridine H-3), 8.45 (m, 2 H, pyridine H-2,6); mass spectrum exact mass 330.136522 (−0.9 ppm error).

**6-[(3-Pyridinyl)phenylmethyl]-3,4-dihydroquinolin-2-(1*H*)-one (12).** Trifluoroacetic acid (5 mL) was added portionwise under a stream of nitrogen to a mixture of alcohol **11b** (0.24 g, 0.73 mmol) and $NaBH_4$ (0.28 g, 7.26 mmol) with vigorous stirring at 0 °C. After the considerable foaming had ceased, the yellow solution was stirred at ambient temperature for 30 min. The $CF_3COOH$ was removed under reduced pressure, and the residue was basified with dilute aqueous $K_2CO_3$. The yellow gum which separated was stirred with EtOAc, separated from the aqueous phase, and dried ($MgSO_4$). The organic extract was evaporated, and the residue was purified by column chromatography using MeOH/$CH_2Cl_2$ (3:97) as the eluent to afford **12** (0.15 g, 66%) as a white solid.

**(*E*)-6-[1-(3-Pyridinyl)propen-2-yl]-3,4-dihydroquinolin-2(1*H*)-one (13a).** Using **10b** as the ketone employed in the procedure described for the preparation of **7a**, **13a** was prepared in 50% yield.

**(*E*)-6-[1-Phenyl-2-(3-pyridinyl)ethenyl]-3,4-dihydroquinolin-2(1*H*)-one (13b).** Use of PhMgBr as the Grignard reagent in the procedure described for the preparation of **13a** afforded **13b** in 20% yield.

**(*E*)-6-[2-(3-Pyridinyl)-1-pentylethenyl]-3,4-dihydroquinolin-2(1*H*)-one (13c).** Use of *n*-$C_5H_{11}MgBr$ as the Gringnard reagent in the procedure described for the preparation of **13a** afforded **13c** in 25% yield, isolated as its hydrochloride salt.

**(*E*)-6-[2-(3-Pyridinyl)ethenyl]-3,4-dihydroquinolin-2-(1*H*)-one (13d).** Use of ketone **10b** in the procedure described for the preparation of **7e** afforded **13d** in 62% yield.

**6-[1-(3-Pyridinyl)prop-2-yl]-3,4-dihydroquinolin-2(1*H*)-one (14a).** Hydrogenation of **13a** (0.16 g, 0.61 mmol) over 10% Pd/C (20 mg) in EtOH (10 mL) at 50 psi gave **14a** (0.16 g, 100%) as a colorless gum.

**6-[1-(3-Pyridinylmethyl)hexyl]-3,4-dihydroquinolin-2-(1*H*)-one (14c).** Hydrogenation of **13c** (0.15 g, 0.47 mmol) over 10% Pd/C (25 mg) in EtOH (10 mL) at 50 psi gave **14c** (0.13 g, 86%) as a colorless gum.

**6-Bromo-3,4-dihydroquinolin-2(1*H*)-one (15).**[22] *N*-Bromosuccinimide (8.90 g, 50.0 mmol) was added in portions to a stirred solution of 3,4-dihydroquinolin-2-one (7.35 g, 50.0 mmol) in DMF. After the exotherm had subsided (ca. 10–15 min), the reaction mixture was heated in an oil bath at 100 °C for an additional 15 min. The mixture was cooled to ambient temperature and diluted with water (100 mL), and the precipitate was collected by filtration. Recrystallization from EtOH afforded colorless needles (7.80 g, 69%), mp 168–169 °C. Anal. ($C_9H_8BrNO$) C, H, N.

**6-[2-(Trimethylsilyl)ethynyl]-3,4-dihydroquinolin-2-(1*H*)-one (16).** A solution of **15** (8.00 g, 35.4 mmol) in $Et_3N$ (53 mL) and pyridine (11 mL) was deoxygenated by passing a stream of $N_2$ through the mixture for 15 min. (Trimethylsilyl)acetylene (10.0 mL, 6.95 g, 70.8 mmol), $(Ph_3P)_2PdCl_2$ (460 mg, 0.64 mmol), and CuI (62 mg, 0.32 mmol) were added, and the reaction mixture was heated at 90 °C for 72 h. The solvent was removed under reduced pressure, and the residue was partitioned between $CH_2Cl_2$ (200 mL) and water (100 mL). The organic extract was dried ($Na_2SO_4$) and evaporated under reduced pressure, and the residue was purified by chromatography on silica gel using $MeOH/CH_2Cl_2$ (2.5:97.5) as the eluent to afford **16** (6.40 g, 75%), mp 176–177 °C. Anal. ($C_{14}H_{17}NOSi$) C, H, N.

**6-Ethynyl-3,4-dihydroquinolin-2(1*H*)-one (17).** A mixture of **16** (6.00 g, 24.7 mmol) and finely powdered $K_2CO_3$ (250 mg, 1.81 mmol) in MeOH (40 mL) was stirred for 18 h. The solvent was removed under reduced pressure, and the residue was partitioned between $MeOH/CH_2Cl_2$ (10:90, 150 mL) and water (75 mL). The organic extract was washed with water (50 mL) and brine (50 mL) and dried ($Na_2SO_4$). The extract was evaporated under reduced pressure, and the crude product was purified by chromatography on silica gel using $MeOH/CH_2Cl_2$ (2.5:97.5) as the eluent to afford **17** (4.17 g, 99%), mp 182–184 °C. Anal. ($C_{11}H_9NO$) C, H, N.

**6-[2-(3-Pyridinyl)ethynyl]-3,4-dihydroquinolin-2(1*H*)-one (18).** Use of **17** as the acetylene and 3-bromopyridine as the bromide in the procedure described for the preparation of **16** gave **18**.

(22) Loev, B.; Kormendy, M. F. Sulfostyril (2,1-Benzothiazine 2,2-Dioxide). I. Preparation and Reactions of 3,4-Dihydrosulfoxtyril. *J. Org. Chem.* **1965**, *30*, 3163–3166.

**6-[2-(3-Pyridinyl)ethyl]-3,4-dihydroquinolin-2(1*H*)-one (14d).** Atmospheric hydrogenation of **18** (1.00 g, 4.03 mmol) over 10% Pd/C (200 mg) in EtOH (25 mL) gave **14d** (965 mg).

**6-[3-(1*H*-Imidazol-1-yl)propynyl]-3,4-dihydroquinolin-2-(1*H*)-one (20).** Use of *N*-propargylimidazole **19** as the acetylene and **15** as the bromide in the procedure described for the preparation of **16** gave **20**.

**(*Z*)-6-[3-(1*H*-Imidazol-1-yl)propen-1-yl]-3,4-dihydroquinolin-2(1*H*)-one (21).** A suspension of 5% $Pd/BaSO_4$ (75 mg) in pyridine (8 mL) was hydrogenated for 15 min before introduction of a solution of **20** (502 mg, 2.00 mmol) in pyridine (3 mL). Hydrogenation was continued until the theoretical uptake was achieved; the mixture was diluted with EtOH (50 mL) and filtered through a pad of Celite. The solvent was evaporated under reduced pressure, and the resulting solid was recrystallized from EtOAc/MeOH to afford **21** (430 mg).

**6-[3-(1*H*-Imidazol-1-yl)prop-1-yl]-3,4-dihydroquinolin-2-(1*H*)-one (22).** Atmospheric hydrogenation of **21** (220 mg, 0.87 mmol) over 10% Pd/C (200 mg) in EtOH (8 mL) gave crude **22**. Purification by chromatography on silica gel eluting with $MeOH/CH_2Cl_2$ (2.5:97.5) afforded **22** (150 mg).

**6-(3-Pyridinyl)-3,4-dihydroquinolin-2(1*H*)-one (23).** A mixture of bromide **15** (1.00 g, 4.42 mmol), *n*-$Bu_4NBr$ (114 mg, 0.35 mmol), powdered KOH (594 mg, 9.00 mmol), diethyl(3-pyridyl)borane[14] (735 mg, 5.00 mmol), and $(Ph_3P)_4Pd$ (205 mg, 0.18 mmol) in THF (18 mL) was heated at reflux under $N_2$ for 48 h. The solvent was removed under reduced pressure, and the residue was partitioned between 10% $MeOH/CH_2Cl_2$ (125 mL) and water (75 mL). The organic extract was dried ($Na_2SO_4$) and evaporated under reduced pressure, and the residue was purified by column chromatography using $MeOH/CH_2Cl_2$ (5:95) as the eluent to give **23** (700 mg, 71%).

**6-(1*H*-Imidazol-1-yl)-3,4-dihydroquinolin-2(1*H*)-one (24).** A mixture of bromide **15** (1.00 g, 4.42 mmol), imidazole (301 mg, 4.42 mmol), $K_2CO_3$ (611 mg, 4.42 mmol), and CuI (42 mg, 0.22 mmol) in DMF (4 mL) was heated at 90 °C under $N_2$ fo 48 h. The reaction was cooled and diluted with water (50 mL). The mixture was extracted with 10% $MeOH/CH_2Cl_2$ (3 × 25 mL), and the combined organic extracts were washed with water (3 × 25 mL), dried ($Na_2SO_4$), and evaporated under reduced pressure to afford a solid. Purification by column chromatography using $MeOH/CH_2Cl_2$ (2.5:97.5) as the eluent afforded **24** (110 mg, 12%).

**6-[2-(1*H*-Imidazol-1-yl)ethenyl]quinolin-2(1*H*)-one (25).** A solution of **7e** (1.50 g, 6.29 mmol) was stirred in the presence of nickel peroxide (0.63 g, 6.92 mmol) in DMF (15 mL) for 18 h. The reaction was filtered through Celite, and the filter pad was washed with $MeOH/CH_2Cl_2$ (5:95). The filtrate was evaporated under reduced pressure, and the remaining solid was purified by column chromatography using $MeOH/CH_2Cl_2$ (5:95) as the eluent. Carbostyril **25** was isolated (40 mg, 3%) along with recovered **7e** (1.20 g, 80%).

**Registry No.** 1, 553-03-7; **2a**, 62245-12-9; **2b**, 120067-47-2; **2c**, 64483-62-1; **2d**, 61122-82-5; **3a**, 130343-76-9; **4a**, 120067-51-8; **4b**, 120067-40-5; **4c**, 120067-52-9; **5**, 120067-53-0; **7a**, 138260-80-7; (*E*)-**7b**, 138260-81-8; (*Z*)-**7b**, 138260-82-9; (*E*)-**7c**, 138260-83-0; (*Z*)-**7c**, 138260-84-1; **7e**, 138260-79-4; **8**, 120067-76-7; **9a**, 120067-79-0; **9b**, 120067-77-8; **9c**, 120067-80-3; **10a**, 120067-54-1; **11b**, 120067-67-6; **12**, 120067-68-7; **13a**, 138260-85-2; **13b**, 138260-86-3; **13c**, 138260-87-4; **13d**, 138260-88-5; **14a**, 138260-89-6; **14c**, 138260-90-9; **14d**, 138260-92-1; **15**, 3279-90-1; **16**, 138260-91-0; **17**, 120067-46-1; **18**, 120067-82-5; **19**, 18994-77-9; **20**, 120115-82-4; **21**, 138260-93-2; **22**, 120067-81-4; **23**, 99471-41-7; **24**, 119924-94-6; **25**, 138260-94-3; nicotinyl chloride, 10400-19-8; 3-bromopyridine, 626-55-1.