Preparation and Evaluation of Electrophilic Derivatives of Phenylbutazone as Inhibitors of Prostaglandin-H Synthase

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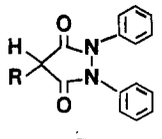
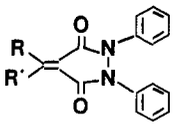
The chemical syntheses and biological evaluation of several potential irreversible inhibitors for prostaglandin (PGH) synthase are described. These inhibitors were modeled after the nonsteroidal antiinflammatory (NSAI) drug phenylbutazone (4-*n*-butyl-1,2-diphenyl-3,5-pyrazolidinedione). Electrophilic functionalities such as an α-bromoacetamide, an α-chloroacetamide, a phenylurethane, a propargyl chloride, and several α,β-unsaturated Michael acceptors were incorporated at the 4-position of the pyrazolidinedione ring structure. None of the derivatives showed evidence of irreversible inhibition of PGH synthase, although several were nearly as potent inhibitors of this enzyme as phenylbutazone. The nitrile obtained from 1,4-conjugate addition of cyanide to one of the unsaturated derivatives was considerably more potent as an inhibitor of PGH synthase than was phenylbutazone.

The specific acetylation of prostaglandin (PGH) synthase by aspirin, as revealed by Roth,[1] has provided a means to obtain significant structural information for this enzyme, as well as suggesting a possible mechanism for inhibition of this enzyme by this commonly employed nonsteroidal antiinflammatory (NSAI) agent. However, since most other effective NSAI agents lack a similar innate electrophilic reactivity, extrapolation of Roth's methods to other inhibitors of this enzyme cannot be directly accomplished. Our goal in this work was to investigate the consequences of the incorporation of electrophilic functionality into the structural framework of another known PGH synthase inhibitor, phenylbutazone (**1a**).



R
1a, n-Bu
b, H
c, CH_2CN
d, $CH_2CH_2NH_2$
e, $CH_2CH_2NH\text{-}CO\text{-}CH_2Br$
f, $CH_2CH_2NH\text{-}CO\text{-}CH_2Cl$
g, $CH_2CH_2NH\text{-}CO\text{-}OPh$
h, $CH_2C{\equiv}CCH_2Cl$
i, $C(Me)_2CN$



R	R′
2a, H	n-Pr
b, H	t-CH=CH-Ph
c, Me	Me
d, Me	Et
e, Me	Ph

The history of development and clinical utility of phenylbutazone has recently been reviewed.[2,3] Phenylbutazone has been claimed to be an irreversible inhibitor of PGH synthase[4,5] and is somewhat unusual in its equal inhibition of both the cyclooxygenase and peroxidase enzymatic functions of this enzyme. Phenylbutazone (150 μM) has been shown to inhibit 93% of the cyclooxygenase activity and 96% of the peroxidase activity of the microsomal PGH synthase preparation.[6] All of the other NSAI drugs tested in these studies were shown to selectively inhibit the cyclooxygenase activity.[6] Furthermore, phenylbutazone underwent oxidization by the peroxidase activity of PGH synthase in an $^{18}O_2$ atmosphere to form 4-[^{18}O]hydroxyphenylbutazone.[7] Reed et al.[8] suggested that phenylbutazone incorporated oxygen to initially form 4-hydroperoxyphenylbutazone, which served to stimulate the oxidative activity of the system yielding undefined species capable of inactivating the enzyme.

The structure–activity relationship (SAR) of phenylbutazone analogues as inhibitors of PGH synthase is difficult to interpret due to the many different kinds of assays used to evaluate inhibitory activity.[9] Thus far, only phenylbutazone and oxyphenbutazone (the mono-4′-hydroxy metabolite) have been tested and shown to be inhibitors of microsomal PGH synthase in vitro. Hafliger[10] noted among an extensive series of phenylbutazone analogues that substitution at the 4-position with carbon chains from three to five carbons showed the best in vivo antiinflammatory activity (rat paw edema), although chains up to 10 carbon atoms also produced compounds with good activity. Also, he reported that substitution in the aromatic rings did not change activity significantly. Incorporation of ketone, vinyl chloride, ester, sulfide, and alkene functionality in the alkyl chain at the 4-position has maintained antiinflammatory activity in the phenylbutazone series.[10-15]

This paper describes the syntheses of several electrophilic phenylbutazone analogues (**1e–h**, **2a–e**) containing different types of potential alkylating groups at the 4-position and the inhibitory activity of these derivatives on microsomal PGH synthase. These derivatives were designed as potential affinity labels for PGH synthase, but none were able to irreversibly inhibit the enzyme.

Chemical Results and Discussion

1,2-Diphenyl-3,5-pyrazolidinedione (**1b**) was a key intermediate in the syntheses of the potential affinity labels

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1e–h modeled after phenylbutazone (**1a**). Tsumaki[16] first prepared **1b** in 24% yield by condensing malonyl chloride and 1,2-diphenylhydrazine in ether. The other widely used method for the construction of 3,5-pyrazolidinediones is the condensation between malonic esters and hydrazines catalyzed typically by sodium alkoxides.[11,17-20] After low yields of **1b** were obtained from this method, an alternate condensation procedure was developed. Use of sodium hydride at reflux in chlorobenzene afforded **1b** in 74% yield. This procedure represents a significant improvement over past methods.

The common intermediate for the synthesis of compounds **1e–g** was the aminoethyl-substituted pyrazolidine **1d**. Although this zwitterion had been previously synthesized in a three-step procedure starting from **1b**,[21] our desire to obtain **1d** in a more straightforward manner led to investigation of alternate procedures. The reaction between 2-bromoethylamine hydrobromide and **1b** was unsuccessful under a wide variety of reaction conditions. A one-step synthesis of nitrile **1c**, however, was accomplished in 90% yield by alkylation of **1b**, with iodoacetonitrile with potassium carbonate in ethanol. Compound **1d** could then be readily obtained by lithium aluminum hydride reduction of **1c** in approximately 50% yield.[21] Many attempts were made to improve the yield of this reduction. For example, catalytic hydrogenation of the nitrile using the procedure of Secrist and Logue (H_2/PtO_2/$CHCl_3$)[22] produced some **1d**; however, much of the starting nitrile **1c** remained even after extended reaction times (more than 3 days). Reduction of this nitrile in the presence of ammonia or hydrazine was not explored due to anticipated complications.

Bromoacetamide **1e** was obtained in moderate yield (53%) from the acylation of **1d** with bromoacetyl bromide. An initial attempt to prepare **1e** using bromoacetyl chloride rather than the bromide resulted in a mixture of **1e** and **1f** in a 2:1 ratio (determined by NMR). This mixture presumably arose by displacement of bromide by chloride under the conditions of the reaction. The mixture of **1e** and **1f** was converted to the pure bromoacetamide (**1e**) in quantitative yield by using a modified procedure by Willy et al.[23] (100 equiv of EtBr, 4 equiv of NaBr in *N*-cyclohexyl-2-pyrrolidinone). Chloroacetamide **1f** was prepared directly in low yield (24%) by reaction of chloroacetyl chloride and amine **1d**. This α-chloro derivative (**1f**) was designed to act as a slightly less reactive electrophile than **1e**. The reaction of amine **1d** with phenyl chloroformate in the presence of $KHCO_3$ provided phenylcarbamate **1g** in 48% yield. Synthesis of the propargyl chloride derivative **1h** was accomplished in 33% yield by the monoalkylation of **1b** by 1,4-dichloro-2-butyne.

Synthesis of the α,β-unsaturated phenylbutazone analogue **2a** was accomplished in 35% yield via a condensation between **1b** and *n*-butyraldehyde in the presence of 3-Å molecular sieves. Reaction of **1b** with propionaldehyde was reported to give a low (13%) yield of the propylidene analogue.[24] Due to the excellent inhibitory activity of **2a**, several additional substituted methylene derivatives of phenylbutazone were prepared. Dissolving **1b** in excess acetone afforded **2c** in 83% yield.[16] The facile conversion of **2c** to the nitrile **1i** with potassium cyanide in aqueous ethanol at room temperature demonstrated the high reactivity of **2c** as a Michael acceptor.[25] Reaction of **1b** with *trans*-cinnamaldehyde and 2-butanone in aqueous ethanol, with glycine as a catalyst at room temperature, provided **2b** and **2d** in yields of 82% and 73%, respectively.[26,27] However, the corresponding condensation with acetophenone did not go to completion even with heating to reflux. A neat reaction, however, provided the desired product (**2e**)[16] in 27% yield.

Table I. Electrophilic Phenylbutazone (PB) Analogues as Inhibitors of PGH Synthase

no.	% control[a] 25 μM	% control[a] 100 μM
1a (PB)	25 ± 1	11 ± 1
1e		79 ± 4
1f		90 ± 2[b]
1g	78 ± 2	42 ± 2
1h	35 ± 1	10 ± 1
1i	23 ± 1	5 ± 0.3
2a	29 ± 1	18 ± 1
2b		96 ± 4[b]
2c	89 ± 1	63 ± 5[b]
2d	49 ± 1	21 ± 1
2e	73 ± 2	31 ± 1

[a] Reported values are the calculated mean ± standard error (SE) for triplicate determinations. All values, except those indicated by footnote *b*, represent statistically significant inhibition of PGH synthase above the 95% confidence level as determined by the Student's *t* test. [b] These values do not represent statistically significant inhibition of PGH synthase as determined by the Student's *t* test.

PGH Synthase Enzyme Inhibition Studies

Inhibition of enzyme activity for PGH synthase was determined by measuring oxygen consumption with a Clark oxygen electrode as described in detail in the Experimental Section. The inhibition, or lack thereof, of each analogue at 25 and 100 μM was calculated as a percent of the control value (mean ± SE, $N = 3$). Experiments to determine time dependence in the inhibitory process were performed in duplicate. If any analogue did not produce more than a 35% inhibition (65% of control) at 100 μM, it was not tested at 25 μM. The statistical significance of observed inhibition was determined by a Student's *t* test evaluation.

The phenylbutazone analogues having electrophilic groups, which were designed as potential affinity labels, exhibited a wide range of inhibitory potencies as illustrated in Table I. The propargyl chloride **1h** and the alkylidene derivatives **2a** and **2d** were the most active inhibitors with potencies comparable to that of the prototype phenylbutazone. The two most potent derivatives in this series, **1h** and **2a**, along with phenylbutazone **1a** and nitrile **1i**, were tested for time-dependent inhibition, which would be expected for irreversible inhibitors, but none showed any increase in inhibition with time (up to 40 min). This result is especially interesting, as previous reports had

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claimed that phenylbutazone (**1a**) itself was an irreversible inhibitor of the enzyme.[3,4]

The haloacetamides **1e** and **1f**, which are more polar than phenylbutazone, were much less active, but phenylurethane **1g** showed a better inhibitory effect than these analogues. The acetone condensation product **2c**, which may lack a large enough substituent at the 4-position for sufficient lipophilicity, was less active. Analogue **2d**, the homologue of **2c**, was considerably more potent. The acetophenone condensation produce **2e** also showed some inhibitory activity, but the highly conjugated **2b** was essentially inactive. The observed inhibitory activity of these α,β-unsaturated derivatives demonstrates an apparent exception to the previously reported requirement of an acidic enolic proton at the 4-position for activity in the phenylbutazone series.[28] Nitrile derivative **1i** was the most potent inhibitor of all and was superior to phenylbutazone itself. In fact, even at 5 μM, **1i** inhibited the enzyme to 26 ± 1% of control, while phenylbutazone at 5 μM gave 71 ± 2% of control. Further studies are necessary to evaluate whether the release of cyanide (which has been observed to inhibit this enzyme[29]) was a factor in this observed inhibition. Since nitrile **1i** is much more potent than NaCN as an inhibitor, its hydrophobicity and affinity for the inhibitory site must contribute to this effect.

In conclusion, neither the more potent electrophilic phenylbutazone analogues tested (**1a**, **2a**) nor phenylbutazone showed any time-dependent increase in inhibition, one of the first criteria for evaluation of a potential affinity label. Nevertheless, several phenylbutazone derivatives (**1a**, **2a**, **2d**) were synthesized that proved to be nearly as potent as the prototype in inhibition of PGH synthase. One inhibitor (**1i**) was superior to phenylbutazone in the inhibition of PGH synthase. It is questionable whether the acidic proton at the 4-position is necessary for activity since several α,β-unsaturated Knoevenagel condensation products (**2a**,**d**,**e**) were quite active despite the absence of an acidic proton at this position. However, as pointed out by a reviewer, each of these analogues would be susceptible to enolization via removal of a γ-proton under appropriate conditions. Experiments to determine the relative acidity of these γ-protons have not been performed. In general, the inhibitory activity observed in this series of compounds seems consistent with previous observations that emphasize the importance of hydrophobic substitution in the 4-position of the pyrazolidinedione ring.[10,11]

Experimental Section

A. General Procedures. Melting points were determined with a Thomas-Hoover or Mel-Temp capillary apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 281 spectrophotometer. Nuclear magnetic resonance spectra were obtained with a JEOL FX90Q or Nicolet NT 300 spectrometer. Mass spectra were obtained on an AEI MS-30 mass spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoeniz, AZ. Thin-layer chromatography was performed on precoated silica gel plates (HLF, Analtech, Newark, DE). Silica gel (230–400 mesh, E. Merck) was used for all flash column chromatography.[33] All chemicals and solvents were reagent grade and were distilled or recrystallized according to literature procedures when necessary. Ultracentrifugation was performed on a Beckman L2-65B ultracentrifuge.

B. Chemistry. 1,2-Diphenyl-3,5-pyrazolidinedione (1b). To a mixture of NaH (0.12 mol, 2.9 g) as a 50% mineral oil dispersion and 1,2-diphenylhydrazine (0.10 mol, 18.4 g) in chlorobenzene (80 mL) was added diethyl malonate (0.11 mol, 17.6 g) dropwise at 0 °C. Following the addition of diethyl malonate, the solution was refluxed for 5 h. Refluxing was continued for another 4 h during which time a short-path distillation head was installed to remove the EtOH formed from the condensation reaction. CH_2Cl_2 (150 mL) and H_2O (150 mL) were added to the cooled reaction mixture, and the layers were separated. The aqueous layer was washed with additional CH_2Cl_2 (50 mL) prior to acidification with ice-cold 2 N HCl to yield **1b** (18.6 g, 74%) as a pale yellow solid. Pyrazolidinedione **1b** was crystallized from EtOH to yield off-white plates: mp 178–179 °C (lit.[16,34] mp 178 °C); IR (Nujol) 3120, 3080, 1755, 1725, 1600, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.57 (s, 2 H), 7.01–7.33 (m, 10 H).

4-(Cyanomethyl)-1,2-diphenyl-3,5-pyrazolidinedione (1c). Freshly distilled iodoacetonitrile (23.0 mmol, 3.8 g) was added to a suspension of **1b** (20.0 mmol, 5.0 g) and K_2CO_3 (80.0 mmol, 11.0 g) in EtOH (80 mL), and this reaction mixture was refluxed for 5 h. The EtOH was removed in vacuo, and the resulting solid was dissolved in water (50 mL) and 10% Na_2SO_3 (10 mL) before washing with Et_2O (20 mL). The aqueous layer was acidified with ice-cold 2 N HCl to afford **1c** (5.2 g, 90%) as a tan powder. Nitrile **1c** was crystallized from EtOH to yield tan cubes: mp 137–139 °C (lit.[21] mp 140 °C); IR (Nujol) 3060, 2260, 1745, 1600, 1570, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.10 (d, J = 5 Hz, 2 H), 3.65 (t, J = 5 Hz, 1 H), 7.19–7.38 (m, 10 H).

4-(2-Aminoethyl)-1,2-diphenyl-3,5-pyrazolidinedione (1d). Following the procedure of Logemann et al.[21] nitrile **1c** (15.0 mmol, 4.36 g) in THF (20 mL) was added dropwise over a period of 20 min to a suspension of LAH (30.0 mmol, 1.14 g) in Et_2O (40 mL) at room temperature. The reaction mixture was stirred for an additional 1.25 h before adding 5-mL portions of 10% NH_4Cl and 2 N KOH to quench the reaction. Filtration through Celite and solvent removal in vacuo produced a light brown solid to which Et_2O (15 mL), EtOAc (15 mL), and saturated sodium citrate (60 mL) were added. The aqueous layer was acidified to pH 6.7 with small aliquots of ice-cold 2 N HCl at 0 °C to yield **1d** (2.25 g, 51%) as shiny tan plates: mp 190–191 °C (lit.[21] mp 195 °C); IR (Nujol) 3070, 1665, 1600, 1555, 1515 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.32 (t, J = 7 Hz, 2 H), 2.91 (t, J = 7 Hz, 2 H), 6.88–7.36 (m, 10 H).

4-(5-Bromo-4-oxo-3-azapentyl)-1,2-diphenyl-3,5-pyrazolidinedione (1e). Bromoacetyl bromide (1.2 mmol, 0.25 g) in THF (2 mL) was added dropwise to a mixture of amine **1d** (1.0 mmol, 0.30 g) and $KHCO_3$ (1.2 mmol, 0.12 g) in THF (10 mL) at 0 °C, and the mixture was stirred for 6.5 h. After the THF was removed in vacuo, EtOAc (10 mL), Et_2O (10 mL), and 2 N K_2CO_3 were added to the residue. The organic layer was extracted with 2 N K_2CO_3 (10 mL); the aqueous layer was extracted with Et_2O (5 mL), and the aqueous phases were combined. Acidification with ice-cold 2 N HBr produced a white precipitate, which was purified by flash chromatography (silica, CH_2Cl_2/MeOH/HOAc, 500:5:1) to afford **1e** (0.22 g, 53%) as a white solid. Amide **1e** was recrystallized from EtOH to yield shiny white needles: mp 139–141 °C; IR (KBr) 3380, 3060, 2980, 2940, 2900, 1750, 1715, 1655, 1600, 1540 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.22–2.44 (m, 2 H), 3.49 (t, J = 6 Hz, 1 H), 3.61 (t, J = 6 Hz, 2 H), 6.98–7.37 (m, 10 H); MS, m/e (relative intensity) 415/417 (M^+, 1.7/1.6), 335 (60), 278 (79), 264 (39), 184 (23), 119 (19), 93 (90), 82 (100), 80 (91), 77 (25). Anal. ($C_{19}H_{18}BrN_3O_3$) C, H, N.

In addition, the procedure of Willy et al.[23] was used to convert a mixture of **1e** and **1f** obtained from the use of bromoacetyl chloride rather than bromoacetyl bromide in the preparation of **1d**. The mixture (0.078 g) was added to *N*-cyclohexylpyrrolidinone (2 mL) containing NaBr (0.8 mmol, 0.082 g) and EtBr (20.0 mmol, 2.18 g), and the mixture was stirred for 4 days at room temperature to afford pure **1e** after a similar workup procedure.

4-(5-Chloro-4-oxo-3-azapentyl)-1,2-diphenyl-3,5-pyrazolidinedione (1f). Chloroacetyl chloride (1.1 mmol, 0.12

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g) in THF (1 mL) was slowly dripped into a solution of amine **1d** (1.0 mmol, 0.30 g) in dry pyridine (10 mL) at 0 °C, and the mixture was stirred for 2 h. The reaction mixture was then slowly added to a stirred mixture of ice and 2 N HCl (125 mL) to provide a tan solid, which was further purified by flash chromatography (silica, CH_2Cl_2/MeOH/HOAc, 500:5:1). Amide **1f** (0.09 g, 24%) was obtained as a white solid after azeotropic removal of the residual HOAc by *n*-heptane and was crystallized from EtOH to yield shiny white needles: mp 127–128 °C; IR (KBr) 3370, 3060, 2970, 2940, 2900, 1750, 1720, 1660, 1600, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24–2.46 (m, 2 H)8 3.50 (t, J = 6 Hz, 1 H), 3.71 (t, J = 6 Hz, 2 H), 3.97 (s, 2 H), 7.00–7.38 (m, 10 H); MS, m/e (relative intensity) 371/373 (M^+, 4.0/1.6), 335 (22), 278 (30), 265 (23), 183 (17), 159 (12), 93 (100), 77 (99). Anal. ($C_{19}H_{18}ClN_3O_3$) C, H, N.

4-(4-Phenoxy-4-oxo-3-azabutyl)-1,2-diphenyl-3,5-pyrazolidinedione (1g). Phenyl chloroformate (1.2 mmol, 0.19 g) was added in one portion to a stirred solution of amine **1d** (1.0 mmol, 0.30 g) and $KHCO_3$ (1.0 mmol, 0.10 g) in CH_2Cl_2 (10 mL) at room temperature. This reaction mixture was refluxed for 9 h. The reaction mixture was filtered, and the CH_2Cl_2 was removed in vacuo to afford a solid. Purification by flash chromatography (silica, EtOAc/petroleum ether, 1:1) afforded urethane **1g** (0.20 g, 48%) as a clear oil, which crystallized from EtOH: mp 132–133 °C; IR (Nujol) 3310, 3060, 1710, 1675, 1615, 1600, 1540 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.22–2.48 (m, 2 H), 3.44–3.76 (m, 3 H), 5.70 (br s, 1 H), 6.98–7.48 (m, 15 H); MS, m/e (relative intensity) 415 (M^+, 1.2), 321 (28), 265 (13), 94 (100), 66 (22). Anal. ($C_{24}H_{21}N_3O_4$) C, H, N.

4-(4-Chloro-2-butynyl)-1,2-diphenyl-3,5-pyrazolidinedione (1h). A mixture of **1b** (2.0 mmol, 0.50 g) and K_2CO_3 (4.0 mmol, 0.55 g) in DMF (10 mL) was stirred for 15 min at room temperature before addition of 1,4-dichloro-2-butyne (2.5 mmol, 0.31 g) in one portion. Additional 1,4-dichloro-2-butyne (2.5 mmol, 0.31 g) was added at 19 h, and the reaction was allowed to stir for a total time of 63 h. The reaction mixture was poured into an ice-cold combination of Et_2O (25 mL) and 2 N K_2CO_3 (75 mL). After the aqueous layer was washed with additional Et_2O, acidification with ice-cold 2 N HCl produced a light-orange solid. Purification by flash chromatography (silica, EtOAc/petroleum ether, 3:7) afforded **1h** (0.22 g, 33%) as a white crystalline solid. Propargyl chloride **1h** was recrystallized from EtOH to yield shiny white needles: mp 154–155 °C; IR (Nujol) 3070, 3040, 3010, 2230, 1760, 1730, 1600, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.07 (m, 2 H), 3.49 (t, J = 4.8 Hz, 1 H, 3.98 (t, J = 2.3 Hz, 2 H), 7.17–7.38 (m, 10 H); MS, m/e (relative intensity) 338/340 (M^+, 31/10), 183 (56), 105 (22), 77 (100), 28 (19). Anal. ($C_{19}H_{15}ClN_2O_2$) C, H, N.

4-(1-Methyl-1-cyanoethyl)-1,2-diphenyl-3,5-pyrazolidinedione (1i). Following a modified procedure of Mustapha et al.,[25] a 2.0 M aqueous solution of KCN (25 mL) was added to α,β-unsaturated pyrazolidinedione **2c** (5.0 mmol, 1.45 g) dissolved in EtOH (25 mL) at room temperature. The yellow solution turned clear immediately and was heated to reflux for 1 h. Acidification of the cooled reaction mixture with ice-cold 2 N HCl (500 mL) produced **1i** (1.56, 98%) as a white powder. Nitrile **1i** was crystallized from EtOH to yield white needles: mp 174–176 °C; IR (Nujol) 3070, 2240, 1750, 1710, 1600, 1490, cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.74 (s, 6 H), 3.30 (s, 1 H), 7.17–7.36 (m, 10 H); MS, m/e (relative intensity) 319 (M^+, 24), 292 (36), 200 (32), 183 (54), 77 (100). Anal. ($C_{19}H_{17}N_3O_2$) C, H, N.

4-Butylidene-1,2-diphenyl-3,5-pyrazolidinedione (2a). Butyraldehyde (11.0 mmol, 0.79 g) was added at once with stirring to a solution of **1b** (10.0 mmol, 2.52 g) in THF (30 mL) containing 3-Å molecular sieves (10.0 g). The reaction mixture was refluxed for 2.25 h. THF was then evaporated, and the yellow residue was purified by flash chromatography (silica, EtOAc/petroleum ether, 3:7) to yield α,β-unsaturated pyrazolidinedione **2a** (1.08 g, 35%) as bright-yellow crystals collected in two crops: mp 157–159 °C; IR (Nujol 3080, 1740, 1720, 1660, 1600, 1500 cm^{-1}; ^1H NMR (Me_2CO-d_6) δ 0.64–2.04 (m, 8 H), 7.16–7.52 (m, 10 H); MS, m/e (relative intensity) 306 (M^+, 100), 214 (35), 198 (72), 186 (16), 93 (37), 77 (24). Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

4-(3-Phenyl-2-propenylidene)-1,2-diphenyl-3,5-pyrazolidinedione (2b). To a stirred mixture of **1b** (45.0 mmol, 1.25 g) and glycine (5.0 mmol, 0.38 g) in 70% aqueous EtOH (15 mL) at room temperature was added *trans*-cinnamaldehyde all at once. After the mixture was stirred for 1.3 h, the condensation product **2b** was collected by filtration and washed with H_2O, EtOH, and petroleum ether and then dried to afford a red-orange solid (82% yield). Olefin **2b** was crystallized from EtOH to yield long red-orange needles: mp 195–196 °C (lit.[16] mp 190–192 °C dec); IR (Nujol) 3070, 1725, 1695, 1615, 1590, 1495 cm^{-1}; 300-MHz ^1H NMR ($CDCl_3$) δ 7.15–7.50 (m, 13 H), 7.65–7.68 (m, 2 H), 7.88 (d, J = 12.2 Hz, 1 H), 8.42 (d, J = 12.2 Hz, 1 H), 8.47 (d, J = 11.9 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 120.6, 120.7, 121.7, 123.0, 124.6, 123.0, 124.6, 127.3, 127.4, 129.9, 131.9, 135.3, 149.9, 151.1, 161.3, 162.0.

4-(1-Methylethylidene)-1,2-diphenyl-3,5-pyrazolidinedione (2c). Reagent grade acetone (5 mL) was added to **1b** (1.0 mmol, 0.25 g) and 3-Å molecular sieves (3.0 g). The reaction mixture was refluxed for 2 h before filtration and removal of the acetone in vacuo. Ether (75 mL) was added to dissolve the yellow residue, and this solution was washed successively with 25-mL portions of 2 N HCl, 2 N K_2CO_3, and saturated NaCl before drying over anhydrous Na_2SO_4. After removal of the Et_2O in vacuo, the yellow residue was purified by flash chromatography (silica, EtOAc/petroleum ether, 1:4) to yield (0.24 g, 83%) of α,β-unsaturated pyrazolidinedione **2c** as bright-yellow plates: mp 112–114 °C (lit.[16] mp 113 °C); IR (Nujol) 3070, 1730, 1705, 1635, 1600, 1495 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.67 (s, 6 H), 7.07–7.24 (m, 10 H).

4-(1-Methylpropylidene)-1,2-diphenyl-3,5-pyrazolidinedione (2d). The reaction conditions developed by Bastus[26] and Dakin[27] were used. 1,2-Diphenyl-3,5-pyrazolidinedione (**1b**; 5.0 mmol, 1.26 g), 2-butanone (50.0 mmol, 3.60 g), and glycine (5.0 mmol, 0.38 g) were added to 70% aqueous EtOH (15 mL), and the mixture was stirred for 22 h at room temperature. Following removal of most of the EtOH and H_2O in vacuo, EtOAc (30 mL) and Et_2O (30 mL) were added to the reaction mixture. The organic phase was washed with 2 N K_2CO_3 (2 × 10 mL), dried over anhydrous Na_2SO_4, and then evaporated to yield a yellow solid. Purification by flash chromatography (silica, ethyl acetate/petroleum ether, 1:4) afforded α,β-unsaturated pyrazolidinedione **2d** (1.12 g, 73%) as a bright-yellow oil, which crystallized upon addition of EtOH: mp 116–117 °C; IR (Nujol) 3100, 3065, 3040, 3010, 1730, 1705, 1630, 1600, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, J = 8 Hz, 3 H), 2.68 (s, 3 H), 3.11 (q, J = 8 Hz, 2 H), 7.06–7.48 (m, 10 H). Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

4-(1-Phenylethylidene)-1,2-diphenyl-3,5-pyrazolidinedione (2e). A mixture of pyrazolidinedione **1b** (5.0 mmol, 1.26 g) and acetophenone (10.0 mmol, 1.20 g) was heated with stirring at 85–95 °C for 2.5 h. The cooled reaction mixture was then dissolved in Et_2O (75 mL), extracted sequentially with 2 N KOH (2 × 25 mL) and saturated NaCl (25 mL), and dried over anhydrous Na_2SO_4. Removal of the solvent provided α,β-unsaturated pyrazolidinedione **2e** (0.47 g, 27%) as a yellow solid. Recrystallization from EtOH gave fine silky bright-yellow needles: mp 158–159 °C (lit.[16] mp 148–149 °C); IR (Nujol) 1735, 1705, 1615, 1600, 1495 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.94 (s, 3 H), 7.13–7.53 (m, 15 H).

C. Enzyme Preparation. 1. PGH Synthase Microsomal Preparation. The microsomal enzyme was isolated from sheep seminal vesicles (SSV), obtained from Pel-Freez Biologicals (Rogers, Arkansas), by a modified procedure of Takeguchi et al.[35] Approximately 120 g of SSV was allowed to thaw before removal of the associated fat, muscle, and connective tissues. These were then minced and added to 120 mL of potassium phosphate buffer (100 mM, pH 8.0), containing 5 mM diethyl dithiocarbamate, and 5 mM Na_2EDTA. This mixture was homogenized in a Waring blender for 2 min and centrifuged for 10 min at 12000*g*. The supernatant was filtered through several layers of cheesecloth to remove the fat and recentrifuged for 1.25 h at 100000*g*. The resulting supernatant was decanted, and the pelleted microsomes were cooled to −80 °C and lyophilized. The microsomes were obtained in yields of between 1.0 and 1.5 g from 120 g of crude SSV. For inhibition studies the microsomes were dissolved at a concentration of 1.0 mg/mL in EDTA buffer[36] (100 mM, pH 8.0) containing 1.0 mM phenol, 2.0 mM glutathione, and 1.5% v/v Tween-20. The specific activity of these SSV microsomes

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was 52 ± 2 nmol of O_2 min^{-1} mg^{-1}.

2. Enzyme Assays. Enzyme activity was determined by measuring oxygen consumption in solution with a Yellow Springs polarographic electrode (Clark oxygen electrode, YSI-5331) in conjunction with a Gilson oxygraph (Model K-1C) as previously described.[37] The YSI reaction chamber was modified by uniformly rounding the bottom to permit smaller sample volumes. The temperature of the reaction chamber was maintained at 37 ± 1 °C with a Haake FG water circulator. The enzyme reaction was initiated by the addition of sodium arachidonate solution (5 mg/mL; Nu-Check Preps, Elysian, MN) to provide a 100 μM final concentration (K_m = 5.9 μM)[30] in the 2-mL reaction chamber. All inhibitors were added as 10 or 100 mM solutions in Me_2SO for enzymes assays. The levels of Me_2SO used in the enzyme-inhibition experiments had no effect on enzyme activity. Initial enzyme velocities (dO_2/dt) were obtained by measuring the slopes of the resulting oxygen concentration vs. time curves and are reported as a percent of uninhibited control.

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Functionalized DL-Amino Acid Derivatives. Potent New Agents for the Treatment of Epilepsy

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Structural analogues of the potent known anticonvulsant agent *N*-acetyl-DL-alanine *N*-benzylamide (**1a**) have been prepared (16 examples). The pharmacological activities of these products were evaluated in the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole seizure threshold (sc Met), and the rotorod (Tox) tests. The median effective doses (ED50) and the median toxic doses (TD50) for the most active compounds by both intraperitoneal and oral administration are reported. The most active compounds were *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) and *N*-acetyl-DL-alanine *N*-*m*-fluorobenzylamide (**1m**) along with the parent compound **1a**. The ED50 values in the MES test for these three compounds compared well with phenobarbital, while their high TD50 values contributed to their large protective indexes, which approached that of phenytoin. When tested against four convulsant agents, compounds **1a** and **1d** displayed activity profiles significantly different from those reported for conventionally used antiepileptic drugs.

Amino acids and their derivatives have not had a significant impact in the development of new agents for the treatment of epilepsy. The lack of interest in amino acid type compounds stems from the inability of many of these polar compounds to readily penetrate the blood–brain barrier.[2] Despite this concept, several types of amino acids and their derivatives have demonstrated the ability to prevent chemically, audiogenically, and photically induced seizures. These include derivatives of alicyclic and aromatic amino acids,[3] phosphono derivatives of aliphatic amino acids,[4] *N*-benzoyl- and *N*-phenylacetylglycine amides,[5] and structural analogues of the inhibitory neurotransmitter γ-aminobutyric acid (GABA).[6] The endogenous neuropeptides Met- and Leu-enkephalin have also exhibited anticonvulsant activity in a variety of test animals and may play an important role in the prevention of a static convulsive state or in the maintenance of normal brain function.[7]

Inspection of chemotherapeutic agents possessing central nervous system (CNS) depressant and anticonvulsant activity reveals a common structural pattern (Figure 1). Three functionalities are prevalent in many of these compounds: (1) a vicinal diamine linkage, (2) an oxygen atom on the ethylene chain bridging the two amino groups, and (3) an aromatic ring one carbon removed from an amino residue.[8] Representatives of this structural design are

(1) Abstracted from the Ph.D. dissertation of this author. Additional structure proof, discussion, and experimental and spectral data may be found in this reference.

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