vigorously with 10 mL of toluene scintillator, and the radioactivity in the filters was counted with a Packard Tris-Carb scintillation counter (B-2450). IC_{50} values of the test compounds (the concentrations causing 50% inhibition of ^3H-labeled ligand specific binding) were determined by probit analysis.

Acknowledgment. We are grateful to Dr. M. Hashimoto, the Director of the Laboratories. Thanks are also due to the staff of the Physical & Analytical Chemistry Division of our laboratories for elemental analyses and spectral measurements.

Registry No. **1a**, 112885-23-1; **1b**, 112885-33-3; **2**, 112913-96-9; **3**·AcOH, 131322-23-1; **4** (R_2 = 3-$CF_3C_6H_4CH_2$), 112887-04-4; **5** (R_2 = 3-$CF_3C_6H_4CH_2$), 131322-24-2; **6**, 4093-28-1; **7**, 59-06-3; **8**, 4235-43-2; **9**, 112914-09-7; **10a**, 7206-70-4; **10b**, 108282-38-8; **11**, 112914-03-1; **12**, 131322-25-3; **13**, 131322-26-4; **14**, 110859-48-8; **15**, 112887-44-2; **16**, 112914-11-1; **17**, 112885-36-6; **18**, 112914-02-0;)19a, 131322-27-5; **19a**·$2C_4H_4O_4$, 131322-36-6; **19b**, 112886-52-9; **20b**, 131322-28-6; **20b**·HCl, 112886-53-0; **21b**, 112886-55-2; **21b**·HCl, 112886-54-1; **22a**, 112885-14-0; **22a**·$C_4H_4O_4$, 112885-15-1; **23a**, 112885-17-3; **23b**, 112885-41-3; **23b**·1.25HCl, 131322-37-7; **24a**, 112885-18-4; **24a**·$2C_4H_4O_4$, 112885-19-5; **24b**, 112886-56-3; **24b**·$C_4H_4O_4$, 112886-57-4; **25a**, 112885-20-8; **25a**·$0.5C_4H_4O_4$, 112885-21-9; **25b**, 112886-58-5; **25b**·$2C_4H_4O_4$, 112886-59-6; **26a**, 112885-05-9; **26a**·$1.5C_4H_4O_4$, 112885-06-0; **27a**, 112885-07-1; **27a**·$1.5C_4H_4O_4$, 112885-08-2; **27b**, 112886-49-4; **28a**, 112885-02-6; **29a**, 112885-03-7; **20a**·$C_4H_4O_4$, 112885-04-8; **30a**, 112885-11-7; **31a**, 112885-09-3; **31a**·$1.5C_4H_4O_4$, 112885-10-6; **32a**, 112884-98-7; **33a**, 112884-97-6; **33a**·$0.5C_4H_4O_4$, 131322-38-8; **34a**, 131322-29-7; **34a**·HCl, 131322-39-9; **34b**, 131322-35-5; **35b**, 131322-30-0; **35b**, 131322-30-0; **35b**·HCl, 131322-40-2; **36a**, 112885-32-2; **36a**·$C_4H_4O_4$, 112885-35-5; **36b**, 112886-47-2; **37a**, 112884-99-8; **37a**·$C_2H_2O_4$, 112885-00-4; **37b**, 112886-45-0; **37b**·$C_2H_2O_4$, 112886-46-1; **38a**, 112885-01-5; **38b**, 112886-48-3; **39a**, 112885-13-9; **40b**, 112886-44-9; **41b**, 131322-31-1; **42b**, 131322-32-2; **43b**, 131322-33-3; **44a**, 112885-12-8; **44b**, 112886-50-7; **45a**, 112884-94-3; **45a**·$C_4H_4O_4$, 112884-95-4; **45b**, 112886-38-1; **45b**·$C_2H_2O_4$, 112886-39-2; **46a**, 112884-92-1; **46a**·$C_2H_2O_4$, 112884-93-2; **47b**, 112886-37-0; **48a**, 112913-72-1; **49a**, 112884-96-5; **50b**, 112886-42-7; **50b**·$C_2H_2O_4$, 112886-43-8; **51b**, 112886-40-5; **51b**·$C_2H_2O_4$, 112886-41-6; **52b**, 112885-34-4; **53b**, 131322-34-4; **53b**·$1.75C_4H_4O_4$, 131322-41-3; **54b**, 112914-14-4; **55b**, 112914-15-5.

Structure–Activity Relationship of Quinazolinedione Inhibitors of Calcium-Independent Phosphodiesterase

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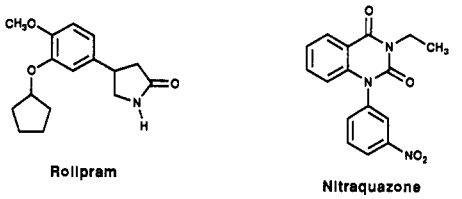
A series of quinazolinediones and azaquinazolinediones is described which possess potent inhibitory activity toward the calcium-independent phosphodiesterase enzyme (CaIPDE). In vivo testing showed that this in vitro activity translates to animal models predictive of chronic diseases such as depression and inflammation. These results support the hypothesis that inhibition of CaIPDE may lead to useful activity in such chronic diseases.

The phosphodiesterase (PDE) enzymes have been classified into four groups based on selective inhibition by a number of structurally diverse compounds.[1] We have studied the calcium-independent, low K_m, cAMP-selective PDE (CaIPDE) which is selectively inhibited by the antidepressant drug rolipram[2] (Chart I) as a target for novel antidepressant agents,[3] and in this context, we sought new structures to complement our work. Our attention was thus attracted to a report[4] describing the drug nitraquazone, coded TVX 2706, which was characterized as a phosphodiesterase (PDE) inhibitor with antiinflammatory activity in vivo. Despite its marked structural dissimilarity to rolipram, nitraquazone proved to be both a selective CaIPDE inhibitor and a potent binder to the [^3H]rolipram binding site.[5] Our attempts to understand the structural basis for the inhibition of CaIPDE by both nitraquazone and rolipram led us to synthesize an exceptionally potent CaIPDE inhibitor, CP-77,059 (**3e**). The discovery of **3e** and its biological characterization are chronicled in this paper.

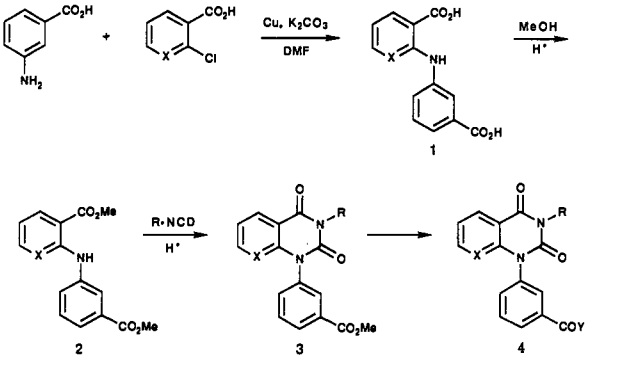
Chart I



Chemistry

The synthetic methodology developed for the analogues of nitraquazone (Table III) is shown in Scheme I; it is based on literature precedent for the preparation of the quinazolinedione ring system by the reaction of an anthranilate derivative with isocyanate under acid catalysis.[6]

Scheme I. Preparation of Quinazolinediones and Pyridopyrimidinediones



(1) Reeves, M. L.; Leigh, B. K.; England, P. J. *Biochem. J.* 1987, *241*, 535.
(2) Eckmann, F.; Fichte, K.; Meya, U.; Sastre-y-Hernandez, M. *Curr. Ther. Res.* 1988, *43*, 291.
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Table I. In Vitro Characterization of PDE Inhibitors 3 and 4

compd	CaIPDE-A[a]	CaIPDE-B[b]	rolipram binding[c]
3a		3.8	
3b	>10	>100	
3c	1.0 ± 0.6 (2)	0.23	180
3d		25.9	
3e	0.040 ± 0.016 (6)	0.13	1.3 ± 0.3 (5)
3f	0.070	1.52	2.5 ± 1.3 (2)
3g	0.10	0.89	3.2 ± 0.1 (2)
4a	≫10		≫1000
4b		21.4	
rolipram	0.49 ± 0.22 (38)	0.59	2.6 ± 0.1 (12)
nitraquazone	1.9 ± 1.0 (4)	11.4	14 ± 1 (5)

[a] Inhibition of calcium-independent phosphodiesterase in rat brain cortex, IC_{50}, μM units. IC_{50} values were determined from dose–response curves of 3 log concentrations of the test compounds; each concentration was run in triplicate. Mean ± standard deviation for (*N*) separate assays was determined for **3c**, **3e**, rolipram, and nitraquazone. [b] Inhibition of calcium-independent phosphodiesterase in guinea pig lung, IC_{50}, μM units. IC_{50} values were determined from dose–response curves of 4 log concentrations of the test compounds; each concentration was run in triplicate. [c] Binding to the [^{3}H]rolipram binding site in rat brain, IC_{50}, nM units. IC_{50} values were determined from dose–response curves of 3 log concentrations of the test compounds; each concentration was run in triplicate. Mean ± standard deviation for (*N*) separate assays was determined for **3e–g**, rolipram, and nitraquazone.

The N-phenylanthranilate derivatives were prepared by coupling of *m*-aminobenzoic acid with 2-chlorobenzoic or 2-chloronicotinic acid catalyzed by cuprous ion,[7] followed by esterification. Ring formation catalyzed by camphorsulfonic acid in refluxing xylene proceeded in modest yield. Those isocyanates which were not commercially available were prepared in situ by reaction of the appropriate carboxylic acid with diphenyl phosphorazidate.[8]

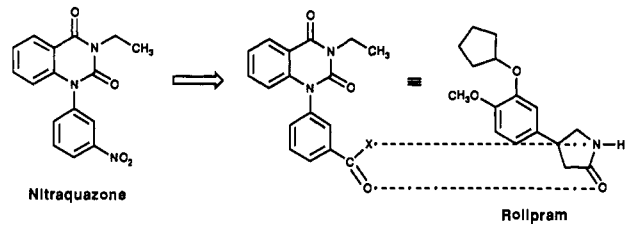
Biology

The procedures for testing compounds for activity against CaIPDE and for binding at the [^{3}H]rolipram binding site are those described previously.[9] Standard animal models of depression and inflammation were used to evaluate in vivo activity. Thus, depression may be at least partially related to dysfunction of noradrenergic neurotransmission; by causing depletion of noradrenaline, reserpine-induced hypothermia may thus serve as a model of depression. Rolipram, and other inhibitors of CaIPDE, are active in this test, presumably by virtue of their elevation of levels of the second messenger, cyclic adenosine monophosphate (cAMP), in noradrenergic neurons. The forced swim test produces behavior in mice similar to depression in that rolipram and other antidepressants reverse the behavioral deficit. The rat foot edema test in rats reliably predicts the antiinflammatory activity of the nonsteroidal antiinflammatory drugs (NSAIDs) developed thus far and allows an estimate of the effective clinical dose in man.

Results and Discussion

Our finding, reported previously,[5] that nitraquazone binds with high affinity to the [^{3}H]rolipram binding site is surprising because of the structural dissimilarity of the two compounds. In searching for ways to overlap their basic structural elements, we developed the hypothesis that the nitro group of nitraquazone and the lactam group of rolipram might occupy the same site on the [^{3}H]rolipram binding site. In contrast to the carbonyl groups of nitraquazone which are structurally rigid and inflexible, the nitro group of nitraquazone provided better overlap with the lactam carbonyl moiety of rolipram because both groups can rotate freely. As shown in Scheme II, we examined replacement of the nitro group by carbonyl functions that would mimic its charge distribution in order to test our hypothesis.

Scheme II



The results of in vitro testing of the compounds resulting from this effort are summarized in Table I, where it can be seen from compound **3a** that a carboxylate ester does indeed fulfill much of the function of the nitro group, both in binding at the [^{3}H]rolipram binding site and in inhibition of CaIPDE. Variation of the side chain group at N-3, compounds **3b** and **3c**, showed that increased size and lipophilicity improve CaIPDE activity. Transformation of the carboxylate ester of **3c** to an acid or amide function, compounds **4a** and **4b**, respectively afforded substantial or complete loss of activity. Although the acid group in **4a** would seem a suitable bioisosteric replacement for a nitro group, its negative charge may well exclude it from binding to CaIPDE or the [^{3}H]rolipram binding site. The aromatic amide group in **4b** is apparently not an effective bioisostere for the nitro group in this system due to its weaker electron-withdrawing properties. Finally, the pyridopyrimidinedione nucleus also afforded potent CaIPDE inhibitory activity, provided the SAR established for the parent series was observed. Thus, **3d** was less potent than the more lipophilic analogues **3e–g**. Even though the activity of compounds as CaIPDE inhibitors showed limited correlation with their binding at the [^{3}H]rolipram binding site, potent [^{3}H]rolipram binding compounds were also good CaIPDE inhibitors. In addition, the selectivity of these compounds for CaIPDE was determined by assaying them with a calcium-dependent PDE preparation.[9] In all cases, IC_{50} values for inhibition of calcium-dependent PDE were much greater than 10 μM.

Characterization of selected members of this series in animal models of chronic disease afforded the results shown in Table II. Potent in vitro activity translated well

(6) Durant, G. J. *Chem. Ind.* **1965**, 1428.
(7) In analogy with the preparation of the corresponding initial intermediate in the synthesis of nitraquazone, see: Lehmstedt, K.; Schrader, K. *Chem. Ber.* **1937**, *70*, 838. Ullman, F. *Liebigs Ann. Chem.* **1907**, *355*, 330.
(8) Commercially available from Aldrich Chemical Co., see: Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.
(9) Davis, C. *Biochim. Biophys. Acta* **1984**, *797*, 354. The CaIPDE preparation described herein which was used in these assays is believed to be a mixture of several isozymes with similar high affinity for cAMP (Davis, C., personal communication).

Table II. In Vivo Characterization of PDE Inhibitors 3 and 4

no.	mouse reserpine-induced hypothermia[a]	mouse swim test[b]	rat foot edema[c]
3a			19
3b	<0.32		5
3c	0.1	>32	25
3e	0.01	0.01	50*
3f	0.003	1.0	
3g	0.001	1.0	
rolipram	0.00003	1.78	41
nitraquazone	0.001	3.2	43*

[a] Minimal effective dose for inhibition of reserpine-induced hypothermia in mice, in mg/kg po. [b] Minimal effective dose for inhibition of immobility in the mouse forced swim test, in mg/kg po. [c] Percent inhibition of carrageenan-induced rat foot edema at 32 mg/kg po, except where indicated* at 3.2 mg/kg po.

Table III. Physical Data

no.	formula	method	mp, °C	% yield	analysis
1a	$C_{14}H_{11}NO_4$	A	277–279	69	C, H, N
1b	$C_{13}H_{10}N_2O_4 \cdot {}^1/_4H_2O$	A	275–280	72	C, H, N
2a	$C_{16}H_{15}NO_4$	B	oil	61	C, H, N
2b	$C_{15}H_{14}N_2O_4$	B	105–106	13	C, H, N
3a	$C_{18}H_{16}N_2O_4$	C	135–136	22	C, H, N
3b	$C_{16}H_{12}N_2O_4$	C	274–276	8	C, H, N
3c	$C_{23}H_{18}N_2O_4$	C	151–153	45	C, H, N
3d	$C_{17}H_{15}N_3O_4 \cdot {}^1/_4H_2O$	C	186–187	4	C, H, N
3e	$C_{22}H_{17}N_3O_4$	C	157–160	31	C, H, N
3f	$C_{21}H_{21}N_3O_4$	D	149–151	5	C, H, N
3g	$C_{23}H_{23}N_3O_4$	D	157–159	20	C, H, N
4a	$C_{22}H_{16}N_2O_4 \cdot {}^1/_2H_2O$		260–262	95	C, H, N
4b	$C_{23}H_{19}N_3O_3 \cdot {}^1/_2H_2O$		250–251	87	C, H, N

to animal models for depression, and **3e**, which in addition to potent inhibitory activity against both central and peripheral CaIPDE enzymes has potent [^{3}H]rolipram binding activity, also showed antiinflammatory activity comparable to that of nitraquazone. This result is remarkable in light of the rapid metabolic hydrolysis of **3e** to the corresponding acid by rat plasma;[10] **3e** is thus active in vivo presumably by virtue of its very potent in vitro activity. Thus, although proving a liability in terms of bioavailability, the use of an ester as a bioisostere for the nitro or lactam group has provided a valuable hypothesis for the development of new CaIPDE inhibitors and may find applications in other systems as well.

Conclusion

Recent work on CaIPDE has suggested that it is involved in controlling basal levels of cAMP in various cell types; it thus may play a role in the long-term pathology of chronic disease.[11] This hypothesis is supported by the finding that rolipram is effective in the treatment of depression.[12] Our finding that a completely different structural class of CaIPDE inhibitors, exemplified by compound **3e**, is effective in animal models of depression and inflammation adds fresh support to this hypothesis. Future reports from these laboratories will present an account of our efforts to evaluate the potential for such applications of CaIPDE inhibitors.

Experimental Section

Melting points were obtained on a Hoover melting point apparatus and are uncorrected. NMR spectra were obtained on a Varian XL-300 or a Bruker AM-300 spectrometer. IR spectra were obtained on Perkin-Elmer 283B or 1420 spectrometers. Mass spectra were obtained on a Finnegan 4510 mass spectrometer, and high-resolution mass spectra were obtained on an AE-9 instrument. TLC analyses were carried out on EM Kieselgel 60 F_{254} 5 × 20 cm plates. Elemental analyses were carried out by the Analytical Laboratory of Pfizer Central Research and are within ±0.4% of theory unless otherwise noted.

Method A. 2-[(3-Carboxyphenyl)amino]benzoic Acid (1a). To a 500-mL round-bottomed flask equipped with condenser and N_2 inlet were added 15.7 g (0.10 mol) of 2-chlorobenzoic acid, 23.3 g (0.17 mol) of 3-aminobenzoic acid, 23.5 g (0.17 mol) of potassium carbonate, 50 mg of copper powder, and 40 mL dimethylformamide. The mixture was heated to reflux, and three 50-mg portions of methanol-washed copper(I) bromide were added. The reaction was refluxed for 3.5 hr, cooled, and poured into 1 N HCl. The mixture was stirred for 10 min and filtered, and the filtered solid washed with water, methanol, and ether, and dried to afford 19 g (74%) of a gray solid: mp 277–279 °C; ^{1}H NMR (DMSO-d_6) δ 6.86 (t, 1 H), 7.3–8.0 (m, 7 H); IR (KBr) 1684 (C = O) cm^{-1}; MS m/z 257 (100, parent), 239 (44), 222 (28), 221 (76), 195 (69), 167 (55), 166 (44), 165 (42), 139 (27). Anal. ($C_{14}H_{11}NO_4$) C, H, N.

2-[(3-Carboxyphenyl)amino]nicotinic acid (1b) was prepared according to method A in 81% yield: mp 275–280 °C; ^{1}H NMR (DMSO-d_6) δ 6.90 (m, 1 H), 7.43 (t, J = 8, 1 H), 7.58 (d, J = 8, 1 H), 7.94 (m, 1 H), 8.29 (m, 2 H), 8.42 (m, 1 H), 10.56 (bs, 1 H, N H), 12.5 (bs, 2 H, CO_2H); IR (KBr) 1720, 1652 (C = O) cm^{-1}; MS m/z 258 (96, parent), 257 (100), 239 (82), 168 (65), 65 (66). Anal. ($C_{13}H_{10}N_2O_4 \cdot {}^1/_4H_2O$) C, H, N.

Method B. Methyl 2-[(3-Carbomethoxyphenyl)amino]benzoate (2a). The acid from the previous step was taken up in 250 mL of methanol and the solution saturated with HCl, refluxed 40 h, cooled, and evaporated. The residue was chromatographed on silica gel using methylene chloride as eluent to afford 17.46 g (61% overall) of a yellow oil: ^{1}H NMR ($CHCl_3$) δ 3.898 (s, 3 H), 3.900 (s, 3 H), 6.77 (m, 1 H), 7.2–7.4 (m, 4 H), 7.73 (m, 1 H), 7.95 (m, 1 H), 7.98 (m, 1 H), 9.55 (bs, 1 H, NH); IR (KBr) (C = O) 1730 cm^{-1}. Anal. ($C_{16}H_{15}NO_4$) C, H, N.

Methyl 2-[(3-carbomethoxyphenyl)amino]nicotinate (2b) was prepared according to method B in 13% yield: mp 105–106 °C; NMR ($CDCl_3$) δ 3.91 (s, 3 H), 3.93 (s, 3 H), 6.74 (m, NH, 1 H), 7.38 (m, 1 H), 7.70 (m, 1 H), 8.00 (m, 1 H), 8.3 (m, 3 H), 10.27 (bs, 1 H); IR (KBr) 1713 (CO), 1682 (CO), 1602, 1585 cm^{-1}; MS (M^+) m/z 286 (92, parent), 285 (100). Anal. ($C_{15}H_{14}N_2O_4$) C, H, N.

Method C. 1-(3-Carbomethoxyphenyl)-3-ethylquinazoline-2,4-dione (3a). To a 25-mL round-bottomed flask equipped with condenser and N_2 inlet were added 3.0 g (10.5 mmol) of methyl 2-[(3-carbomethoxyphenyl)amino]benzoate, 0.83 mL (10.5 mmol) of ethyl isocyanate, 1 mg of camphorsulfonic acid, and 3 mL of xylene. The solution was refluxed for 6 days, cooled, and evaporated. The residue was taken up in ethyl acetate and the product was crystallized by adding isopropyl ether at boiling until most of the ethyl acetate had been replaced and then cooling the solution to 0 °C, affording 0.76 g (22%) of a crystalline solid: mp 135–136 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 6, 3 H), 3.88 (s, 3 H), 4.00 (q, J = 6, 2 H), 6.41 (d, 1 H), 7.1–8.0 (m, 7 H); IR (KBr) 1722, 1663 (C = O) cm^{-1}; MS m/z 324 (87, parent), 325 (27), 296 (29), 222 (38), 221 (100), 194 (63), 166 (48), 165 (30), 139 (20), 111 (67), 83 (45), 70 (22), 59 (25). Anal. ($C_{18}H_{16}N_2O_4$) C, H, N.

1-(3-Carbomethoxyphenyl)quinazoline-2,4-dione (3b) was prepared according to method C in 8% yield: mp 274–276 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (2, 3 H), 6.45 (d, 1 H), 7.2–8.2 (m, 7 H), 11.77 (bs, 1 H); IR (KBr) 1685 (C = O) cm^{-1}; MS m/z 296 (100, parent), 297 (25), 221 (71), 194 (25), 82 (24), 69 (22). Anal. ($C_{16}H_{12}N_2O_4$) C, H, N.

1-(3-Carbomethoxyphenyl)-3-benzylquinazoline-2,4-dione (3c) was prepared according to method C in 45% yield: mp 151–153 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3 H), 5.19 (s, 2 H), 6.48 (d, 1 H), 7.2–8.2 (m, 12 H); IR (KBr) 1728, 1706, 1660 (C = O) cm^{-1}; MS 387 (11), 386 (51, parent), 222 (22), 221 (74), 194 (30), 166 (28), 91 (100), 59 (23). Anal. ($C_{23}H_{18}N_2O_4$) C, H, N.

1-(3-Carbomethoxyphenyl)-3-ethylpyrido[2,3-d]pyrimidine-2,4-dione (3d) was prepared according to method C in 6% yield: mp 186–187 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 6, 3 H), 3.83 (s, 3 H), 3.97 (q, J = 6, 2 H), 7.31 (m, 1 H), 7.66 (m, 2 H), 8.03 (m, 2 H), 8.4–8.5 (m, 2 H); IR (KBr) 1719, 1671 (C = O)

(10) We thank Dr. L. Tremaine for this unpublished data.
(11) Donaldson, J.; Brown, A. M.; Hill, S. J. *Biochem. Pharmacol.* 1988, *37*, 715.
(12) Hebenstreit, G. F.; Fellerer, K.; Fichte, K.; Geyer, N. *Pharmacopsychiatry* 1989, *22*, 156.

cm^{-1}; MS m/z 325 (51, parent), 324 (100), 253 (23), 168 (22). Anal. ($C_{17}H_{15}N_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

1-(3-Carbomethoxyphenyl)-3-benzylpyrido[2,3-*d*]pyrimidine-2,4-dione (3e) was prepared according to method C in 31% yield: mp 157–160 °C; ^1H NMR (DMSO-d_6) δ 3.84 (s, 3 H), 5.12 (s, 2 H), 7.2–8.1 (m, 10 H), 8.45 (m, 2 H); IR (KBr) 1723 (C=O), 1677 (C=O), 1594 cm^{-1}; MS m/z 387 (100, parent), 283 (31), 253 (11), 226 (16), 195 (14), 181 (11.5), 168 (18), 91 (28). Anal. ($C_{22}H_{17}N_3O_4$) C, H, N.

Method D. 1-(3-Carbomethoxyphenyl)-3-(cyclopentylmethyl)pyrido[2,3-*d*]pyrimidine-2,4-dione (3f). To a 35-mL round-bottomed flask equipped with a condenser and N_2 inlet were added 1.0 g (3.496 mmol) of methyl 2-[(3-carbomethylphenyl)amino]nicotinate, 0.44 g (3.496 mmol) of cyclopentylacetic acid, 4 mL of dry xylene, 0.83 mL (3.846 mmol) of diphenyl phosphorazidate, and 0.54 mL (3.846 mmol) of triethylamine. The reaction was heated to 70 °C, whereupon a gas was evolved, and 5 mg of camphorsulfonic acid was added once gas evolution had ceased. The reaction was heated at reflux for 2.5 days, cooled, and chromatographed on silica gel using ethyl acetate in methylene chloride as eluent to give 67 mg (5.1% yield) of a white solid: mp 149–151 °C; ^1H NMR (DMSO-d_6) 1.2–1.7 (m, 8 H), 2.3–2.4 (m, 1 H), 3.87 (s, 3 H), 3.91 (d, 2 H), 7.3–8.5 (m, 7 H); IR (KBr) 1721 (C=O), 1677 (C=O), 1593 cm^{-1}; MS m/z 379 (19, parent) 299 (20), 298 (100), 296 (101), 267 (10), 266 (50), 253 (10), 195 (16), 168 (12), 167 (17). Anal. ($C_{21}H_{21}N_3O_4$) C, H, N.

1-(3-Carbomethoxyphenyl)-3-(norbornylmethyl)pyrido[2,3-*d*]pyrimidine-2,4-dione (3g) was prepared according to method D in 20% yield: mp 157–159 °C; ^1H NMR ($CDCl_3$) δ 1.0–1.2 (m, 6 H), 1.3–1.5 (m, 4 H), 2.0–2.1 (m, 1 H), 3.7–4.0 (m, 2 H), 3.81 (s, 3 H), 7.1–8.5 (m, 7 H); IR ($CHCl_3$) 1700 (C=O), 1650 (C=O), 1580 cm^{-1}; MS m/z 405 (5, parent), 299 (20), 298 (100), 266 (33), 195 (13), 167 (14), 95 (17), 85 (11), 83 (19), 67 (15). Anal. ($C_{23}H_{23}N_3O_4$) C, H, N.

1-(3-Carboxyphenyl)-3-benzylquinazoline-2,4-dione (4a). To a 500-mL round-bottomed flask equipped with condenser and N_2 inlet were added 3.86 g (10 mmol) of 1-(3-carbomethoxyphenyl)-3-benzylquinazoline-2,4-dione, 24.1 g (180 mmol) of lithium iodide, and 250 mL of dimethylformamide. The reaction was refluxed for 36 h, cooled, and added to 1 N HCl. The mixture was stirred for 20 min and filtered, and the solid washed with water and dried to afford 3.55 g (95%): mp 260–262 °C; ^1H NMR (DMSO-d_6) δ 5.18 (s, 2 H), 6.49 (d, 1 H), 7.2–8.2 (m, 12 H); IR (KBr) 1667 (C=O) cm^{-1}; MS m/z 373 (26), 372 (100, parent), 239 (18), 221 (27), 195 (17), 91 (18). Anal. ($C_{22}H_{16}N_2O_4 \cdot {}^1/_2H_2O$) C, H, N.

1-[3-(*N*-Methylcarbamoyl)phenyl]-3-benzylquinazoline-2,4-dione (4b). To a 100-mL three-necked round-bottomed flask equipped with septum and N_2 inlet were added 0.50 g (1.34 mmol) of 1-(3-carboxyphenyl)-3-benzylquinazoline-2,4-dione, 0.15 mL (1.34 mmol) of *N*-methylmorpholine, 10 mL of methylene chloride, and 1.5 mL of dimethylformamide. The solution was cooled to −10 °C and 0.17 mL (1.34 mmol) of isobutyl chloroformate added. The reaction was stirred at −10 °C for 10 min, then methylamine gas was bubbled through the solution for 10 min. The reaction was allowed to warm to room temperature and stirred for 3 days. It was taken up in methylene chloride, washed with 1 N HCl and brine, dried, and evaporated. The residue was chromatographed on silica gel using methylene chloride/ethyl acetate as eluent to afford 0.45 g (87%) of a white, crystalline solid after trituration with isopropyl ether: mp 250–251 °C; ^1H NMR (DMSO-d_6) δ 2.79 (m, 3 H), 5.16 (s, 2 H), 6.43 (m, 1 H), 7.2–8.1 (m, 12 H), 8.53 (m, 1 H); IR (KBr) 1702, 1657 (C=O) cm^{-1}; MS m/z 385 (12, parent), 222 (32), 221 (100), 166 (38), 165 (40), 91 (99), 85 (21), 83 (53). Anal. ($C_{23}H_{19}N_3O_3 \cdot {}^1/_2H_2O$) C, H, N.

Biological Methods. Measurement of [^{3}H]Rolipram Binding. Fresh rat brain was homogenized in 20 volumes of ice-cold 50 mM Tris/1.2 mM $MgCl_2$ (pH 8.0) for 20 s (position 6.5 on a Polytron). The resulting homogenate was centrifuged at 15000 rpm for 20 min. The pellet was resuspended in 20 volumes of the Tris buffer and recentrifuged as before. The final homogenate was resuspended in Tris buffer to give a protein concentration of 0.5 mg/mL. The binding assay was run with 1.0 mL of the tissue suspension that was incubated with 0.1 mL of [^{3}H]rolipram and 0.2 mL of test compound at various concentrations.

Measurement of Phosphodiesterase Activity. CaIPDE-A. Phosphodiesterase activity was determined with 0.1 mL of a reaction medium containing 50 mM Tris-HCl/5 mM $MgCl_2$ (pH 7.5) buffer and [^{3}H]cAMP (NEN NET-275). The final concentration of cAMP was 1.0 μM containing 400000 dpm of [^{3}H]cAMP. Vehicle or test compound (10 μL) and 10 μL of fresh calcium-independent phosphodiesterase (courtesy of Dr. Craig Davis, University of South Carolina[13]) or boiled enzyme were added to 80 μL of substrate in the Tris/$MgCl_2$ buffer. The incubation was carried out at 37 °C for 8 min and stopped by placing in a boiling water bath. Carrier 5′-AMP (0.5 mL of 5 mM 5′-AMP in 0.1 M Hepes/0.1 M NaCl buffer (pH 8.0) was added and the reaction mixture was chromatographed on a polyacrylamide–boronate affinity gel (Bio-Rad Affi-Gel 601 boronate gel). The unhydrolyzed [^{3}H]cAMP was eluted from the gel with 7.5 mL of the Hepes–NaCl buffer. The [^{3}H]5′-AMP product was eluted with 7 mL of 50 mM NaOAc buffer (pH 4.8). Aliquots (1.0 mL) of the latter eluates were counted in a liquid scintillation counter to determine their relative content of radioactive 5′-AMP.

CaIPDE-B. Lung tissue from guinea pigs was placed in a homogenization buffer (20 mM Bistris, 5 mM 2-mercaptoethanol, 2 mM benzamidine, 2 nM EDTA, 50 mM sodium acetate, pH 6.5) at a concentration of 10 mL/g of tissue. The tissue was homogenized with a Tekmar Tissumizer at full speed for 10 s. Phenylmethanesulfonyl fluoride (PMSF, 50 mM in 2-propanol) was added to the buffer immediately prior to homogenization to give a final PMSF concentration of 50 μM. The homogenate was centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was filtered through gauze and glass wool and then applied to a 17 × 1.5 cm column of DEAE-Sepharose CL-6B, preequilibrated with homogenization buffer, at 4 °C.[1] After the supernatant had passed through the column, the column was washed with homogenization buffer, and PDE eluted with a linear gradient of 0.05–1.0 M sodium acetate. Fractions containing CaIPDE activity, as determined by inhibition by rolipram, were pooled (2 mL of ethylene glycol was added to each fraction) and stored at −20 °C. The assay was then carried out by incubating 25 μL of CaIPDE solution, 25 μL of test compound solution in 4% DMSO in water, 25 μL of test buffer (50 mM Tris, 10 mM $MgCl_2$, pH 7.5), and 25 μL of [^{3}H]cAMP solution (final concentration of [^{3}H]cAMP in assay mixture was 1 μM). The tubes were incubated at 37 °C for 10 min, and the reaction was stopped by placing the tubes in a boiling water bath for 2 min. Wash buffer (0.5 mL, 0.1 M Hepes/0.1 M NaCl, pH 8.5) was added to each tube in an ice bath. The contents of each tube were applied to an Affi-Gel column (boronate affinity gel, 1.2-mL bed volume) previously equilibrated with wash buffer. [^{3}H]cAMP was eluted with 2 × 6 mL wash buffer, and [^{3}H]-5′-AMP eluted with 6 mL of 0.25 M acetic acid. After vortexing, 1 mL of the eluate was added to 3 mL of Atomlight scintillation fluid, vortexed, and counted for tritium.

Reserpine Hypothermia Test. In accordance with the antidepressant screening method of Askew,[14] as modified by Koe and co-workers,[15] mice were individually housed at 18–20 °C in plastic chambers with cardboard bottoms. The animals were injected with reserpine (2 mg/kg sc), and retained at 18–20 °C for 18 h. Rectal temperatures were then measured, after which the animals were treated with either saline or test drug solution. Rectal temperatures were again determined, typically at 0.5, 1.0, 2.0, and 14 h after the second injection. Reserpine-pretreated mice given vehicle exhibit rectal temperatures of 20–21 °C 3 h after injection with vehicle, while antidepressant-treated animals display a temperature increase of several degrees centigrade.

Mouse Swim Test. In accordance with the method of Porsolt and co-workers,[16] as modified by Wallach and Hedley,[17] mice were pretreated with test compounds dissolved in saline and administered po in a volume of 1 mL/100 g body weight. One hour after dosing, the animals were placed in 1-L beakers containing water at 22–24 °C, such that their tails could not touch the beaker bottom. After a 2-min habituation time, the mice were observed

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10 times (once every 30 s) to determine whether or not they were mobile. Antidepressant drugs reduce the score in this test in a dose-dependent fashion.

Rat Foot Edema Test. The procedure of Winter[18] was modified as follows: Adult male, fasted, unanesthetized, Charles River CD Sprague-Dawley rats of 170-205 g body weight were numbered and weighed, and an ink mark was placed on the right lateral malleolus. Each paw was immersed in mercury exactly to the ink mark. The mercury was contained in a plastic cylinder connected to an Omega pressure transducer. The output from the transducer was fed through a Buxco organ volume monitor. The volume of mercury displaced by the immersed paw was read, then printed on a Texas Instruments Silent 700. Drugs were given by gavage in a 25 mL/kg volume. One hour after drug administration, edema was induced by injection of 0.05 mL of a 2-week-old 1% solution of carrageenan in distilled water into the plantar tissue of the marked paws. Immediately thereafter, the volume of the injected paw was measured. The increase in foot volume 3 h after the injection of carrageenan constitutes the individual response. Compounds were considered active if they inhibited the edema response by 30% or more in a group of six rats.

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Registry No. **1a**, 27693-67-0; **1b**, 114918-39-7; **2a**, 114918-37-5; **2b**, 114918-35-3; **3a**, 130985-15-8; **3b**, 130985-16-9; **3c**, 114934-47-3; **3d**, 130985-17-0; **3e**, 114918-24-0; **3f**, 114918-31-9; **3g**, 114918-32-0; **4a**, 114934-48-4; **4b**, 114934-49-5; CalPDE, 9036-21-9; $PhCH_2NCO$, 3173-56-6; 2-chlorobenzoic acid, 118-91-2; 2-chloronicotinic acid, 2942-59-8; ethyl isocyanate, 109-90-0; 2-norbornylacetic acid, 1007-01-8; cyclopentylacetic acid, 1123-00-8; 3-aminobenzoic acid, 99-05-8.

Synthesis and Anticonvulsant Activity of 1-Acyl-2-pyrrolidinone Derivatives

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Several 1-acyl-2-pyrrolidinone derivatives were synthesized as derivatives of γ-aminobutyric acid (GABA), and their pharmacological activities and stabilities were investigated. The derivatives showed anticonvulsant effect on picrotoxin-induced seizure at a dose of 200 mg/kg. In particular, 1-decanoyl-2-pyrrolidinone (**7**) and 1-dodecanoyl-2-pyrrolidinone (**8**) had a high activity. The anticonvulsant activity showed a dose dependency. Some of 1-acyl-2-pyrrolidinone derivatives prolonged sleeping time which was induced by sodium pentobarbital and showed a recovery from disruption of the memory of passive avoidance response, which was induced by an electroconvulsive shock. As shown by the results of the stability study of 1-acetyl-2-pyrrolidinone (**1**), it was degraded in an acidic buffer and an alkaline buffer although 2-pyrrolidinone was stable. 1-Acyl-2-pyrrolidinone derivatives were degraded in liver and brain homogenates of mouse and rat. They showed a degradation rate in rat plasma. Conversion of **8** to GABA in mouse liver homogenate was demonstrated. These results suggested that the pharmacological activity of 1-acyl-2-pyrrolidinone is probably due to the release of GABA by hydrolysis of derivatives although further work is necessary.

Several neurological and neuropsychiatric disorders such as epilepsy and Huntington's disease have been reported to be associated with a decrease in γ-aminobutyric acid (GABA) levels in the central nervous system (CNS).[1-3] These observations suggest that increased levels of GABA in the CNS may be useful in the treatment of such neuropsychiatric disorders. However, attempts to use GABA in clinical trials failed due to the extremely high doses required to force GABA across the blood-brain barrier.[4,5]

Numerous derivatives of GABA, including alkyl ester of GABA,[6,7] γ-acetylenic GABA,[8] aliphatic and steroid esters of GABA,[9-11] Schiff bases of GABA,[12] soft drug of GABA,[13] and isonicotinoyl GABA,[14] have been developed in the hope of facilitating the uptake of GABA into the brain.

Callery et al.[15,16] suggested another possibility, use of 2-pyrrolidinone as a GABA prodrug. 2-Pyrrolidinone, the lactam of GABA, is more lipophilic than GABA and penetrates readily into the CNS. However, attempts to increase whole-brain GABA concentrations with single large doses of 2-pyrrolidinone have been unsuccessful.[4] The failure may be explained by a slow hydrolysis of 2-pyrrolidinone to GABA.[16]

The tight amide bond of 2-pyrrolidinone may be weakened by introduction of an acyl function to the 1-position. Also the lipophilicity of drug may be controlled by the chain length of the acyl function to be introduced. Therefore, several 1-acyl-2-pyrrolidinone derivatives having various alkyl chains were synthesized and their GABA-like pharmacological activities such as anticonvulsant activity and sedative activity were evaluated. Antiamnesic activity

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