The rats were placed in a test chamber and allowed to locate a drinking spout which gave off mild electric shocks (1.0 mA). Only rats making between 3 and 10 licks during a total of 30 s in this presession were used for the animal test. The rats were removed from the chamber, administered compounds and were returned to the home cage for 60 min. The rats were then returned to the test chamber for the 5-min test session. During the 5-min test session, the rats were delivered a shock (2.5 mA) for every drop they drank. The number of shocks received during the 5-min session was recorded automatically.

Passive Avoidance Test.[3,4] Male ddy mice (20–25 g) were kept in a plastic cage in groups of 10 and allowed free access to dry food pellets and water. The mice were trained in a one-trial step-through passive avoidance task. The apparatus consisted of a small white box (15 cm × 10 cm × 9 cm) and a black shock box (15 cm × 15 cm × 14 cm) with a hall at the bottom and a grillotine door. During the acquisition test, they were placed in a small, lighted compartment. Five seconds later, the door was opened. The mice received a 1 mA foot shock after entering the dark compartment and they were then returned to their home cage. Twenty-four hours later, after the acquisition test, mice were placed in the small, lighted box again. Time latency to enter the dark compartment was recorded (maximum = 300 s). Compounds were administered 60 min prior to the acquisition. For evaluation of the effect on scopolamine-induced amnesia, scopolamine 3 mg/kg was administered intraperitoneally 15 min prior to the acquisition test. Compounds were administered 45 min prior to the administration of scopolamine.

BZP Receptor Binding Assays.[23] Wistar rats were decapitated, and the cerebral cortex was dissected. The cortex was homogenized in 20 volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) with a Physcotron (15 s, setting 60, NITI-ON Medical Instruments) and centrifuged (4 °C) for 20 min at 50000*g*. The tissue was resuspended in an equal volume of buffer and recentrifuged. This procedure was repeated two more times. The final pellet was resuspended, frozen in a liquid nitrogen bath, and stored at −80 °C. For the binding assay, the frozen membrane preparations were thawed and centrifuged. The pellet was resuspended in 100 volumes (protein concentrations, 0.5–0.6 mg/mL) of ice-cold 50 mM Tris-HCl buffer containing 100 mM NaCl and 5 mM KCl, and 0.5-mL aliquots of this homogenate were added to 0.1 mL of a solution of [^{3}H]flunitrazepam (final concentration, 56.6 pM) and varying amounts of the test compounds. The mixture was incubated at 0 °C for 20 min, and the incubations were terminated by addition of 2.5 mL of ice-cold incubation buffer, followed by rapid filtration through Whatman GF/C filters. The filters were washed three times with 2.5 mL of the buffer and placed in minivials containing 5 mL of Clear-sol I. After 12 h, the radioactivity was counted with an Aloka LSC-673 liquid-scintillatin counter. Nonspecific binding was determined by parallel experiments with nonradioactive diazepam (final concentration, 100 μM) and accounted for less than 10% of total binding. Each value was determined in duplicate, and IC_{50} values were calculated from semilogarithmic plots. The data are means of at least three individual determinations.

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Additions and Corrections

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James L. Kelley,* Ed W. McLean, Naomi K. Cohn, Mark P. Edelstein, David S. Duch, Gary K. Smith, Mary H. Hanlon, and Robert Ferone*: Synthesis and Biological Activity of an Acyclic Analogue of 5,6,7,8-Tetrahydrofolic Acid, *N*-[4-[[3-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)propyl]amino]benzoyl]-L-glutamic Acid.

Page 561. The following should be inserted after the introduction: E. C. Taylor and coworkers recently reported the synthesis of *N*-[4-[4-(2,4-diamino-6(1*H*)-oxopyrimidin-5-yl)butyl]benzoyl]-L-glutamic acid (7-DM-DDATHF) (Taylor, E. C.; Harrington, P. M.; Shih, C. *Heterocycles* **1989**, *28*, 1169), an open-chain analogue of DDATHF and 5-DATHF (Taylor, E. C.; Hamby, J. M.; Shih, C.; Grindey, G. B.; Rinzel, S. M.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1989**, *32*, 1517), which possesses structural features similar to 5-DACTHF reported by us (Kelley, J. L.; McLean, E. W.; Cohn, N. K.; Edelstein, M. P.; Duch, D. S.; Smith, G. K.; Hanlon, M. H.; Ferone, R. *J. Med. Chem.* **1990**, *33*, 561).