(9:1)) in order to prevent artifactual shifts due to DNA-induced pK_a changes.[39] DNA (from calf thymus) was sonicated to reduce the viscosity of the solutions.

Aliquots (0.5 mL) of a stock sonicated DNA solution were freeze-dried, treated with D_2O to exchange the residual water, and relyophilized. A solution of **7b** in D_2O was diluted so that the required amount of product was present in 0.5 mL of solution and this was used to dissolve the DNA. Accuracy of this protocol was ensured by checking the weights of the solutions.

Spectra were run on a Varian XL-200 instrument with presaturation of the residual water peak and the chemical shifts were measured with respect to internal DSS.

Relaxation Experiments. Plasmids were propagated in *Escherichia coli* strain HB101. Plasmid DNA was prepared by conventional procedures.[40] Topoisomerase-I (BRL) treatment in the presence of **7b** or ethidium bromide was performed as described by Peck and Wang.[41] After relaxation, the drug was removed by phenol extraction and the DNAs were precipitated with ethanol and redissolved in 10 mmol L^{-1} TRIS, 1 mmol L^{-1} EDTA, pH = 7.5, and the distributions of topoisomers were resolved by one-dimensional gel electrophoresis. Purified DNAs were electrophoresed through a 1% agarose–TBE gel containing 1.25 $\mu g\ mL^{-1}$ of the intercalator chloroquine phosphate (SIGMA). Electrophoresis was carried out for 20 h at 1.6 V cm^{-1}. After electrophoresis, gels were stained with 0.5 $\mu g\ mL^{-1}$ ethidium bromide and photographed under UV light. Densitometric scans of the negatives were carried out in a Joyce-Loebl densitometer.

Acknowledgment. Financial support from the Comisiõn Interministerial para la Ciencia y la Tecnologia (CICYT Grant No. BT 86-0018) is gratefully acknowledged.

Registry No. **7a**, 103344-05-4; **7b**, 103344-04-3; **7c**, 103344-03-2.

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Nicotinamide Ethers: Novel Inhibitors of Calcium-Independent Phosphodiesterase and [^{3}H]Rolipram Binding

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The synthesis and biological properties of a series of nicotinamide ethers are described. These compounds, structurally novel calcium-independent phosphodiesterase inhibitors, also inhibit the binding of [^{3}H]rolipram to rat brain membranes and reverse reserpine-induced hypothermia in the mouse. Several compounds exhibited potent in vivo activity comparable to the standard agent, rolipram.

In previous papers[1,2] we described catechol ether 2-imidazolidinone derivatives which constitute a very interesting class of calcium-independent phosphodiesterase (CaIPDE) inhibitors. Not only are these agents selective for the particular form of the enzyme responsible for hydrolysis of brain cAMP but they also potently inhibit [^{3}H]rolipram binding.[3] Rolipram is known to inhibit CaIPDE and elevate brain levels of cAMP.[4-8] It also increases norepinephrine (NE) synthesis and metabolism.[9] Increased neuronal cAMP concentrations and NE turnover may overcome some of the neurochemical perturbations associated with depression. Rolipram is active in animal models which detect antidepressant drugs, such as reserpine-induced hypothermia,[10] and has been investigated clinically.[11-14]

We now report a new class of compounds, structurally distinct from rolipram and other catechol ethers, that selectively inhibit CaIPDE and [^{3}H]rolipram binding. These novel agents, the 2-(aryloxy)alkoxynicotinamides were derived from the structure of another known inhibitor of calcium-independent phosphodiesterase, nitraquazone.[15]

Our rationale was to mimic the properties of nitraquazone with a ring-opened structure. We felt that the N-1

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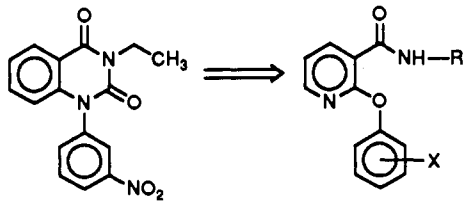


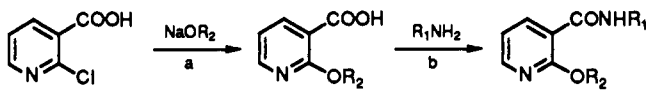
nitraquazone

nitrogen of nitraquazone could be replaced in a ring-opened compound with a nicotinamide ether oxygen and were rewarded with a series of compounds exhibiting not only the anticipated in vitro properties but also activity in reversing reserpine-induced hypothermia. Thus, these substances may be antidepressantlike.

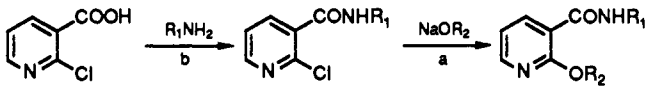
Chemistry

The nicotinamide ethers (Table I) were prepared by either of two straightforward synthetic routes, methods A and B. According to method A, the requisite alcohol or

Method A[a]



Method B[a]



[a] (a) DMF/reflux; (b) *N*-methylmorpholine/isobutyl chloroformate/THF/−10–25 °C.

phenol is first converted to its sodium salt (NaH, DMF) and then reacted with 2-chloronicotinic acid at elevated temperature to afford the alkoxy nicotinic acid derivative in modest yield (ca. 20–50%). The acid is then coupled with an amine under standard mixed anhydride conditions (*N*-methylmorpholine, isobutyl chloroformate, THF, −10 °C) to give the amide generally in good yield. Method B illustrates that the order of steps can be reversed: 2-chloronicotinic acid is first converted to the appropriate amide which is in turn reacted with phenoxide or alkoxide to give the final product.

Pharmacology

Our work on nicotinamide ethers emanated from our earlier studies with rolipram and related imidazolidinones.[1,2] Rolipram has been extensively investigated by many laboratories including our own, where it has been shown to be an inhibitor selective for CaIPDE over many other phosphodiesterases, including calcium-dependent PDE, calmodulin-sensitive PDE, and cGMP PDE.[1,5,16,17] Following the observation by Schneider et al.[3] that rolipram exhibited potent stereoselective binding to brain tissue, we conducted a detailed autoradiographic study of [^{3}H]rolipram binding in mouse brain and found a discrete and reproducible distribution with highest levels in the limbic system, frontal cortex, and molecular layer of the cerebellum.[18,19] These data prompted us to determine whether this binding activity was related to either the CaIPDE inhibitory property of rolipram and related structures and/or in vivo behavioral effects.[1] The specificity and potency of the binding also suggested to us that it represented a pharmacologically relevant phenomenon. As described below, the observation that a series of nicotinamide ethers distinct from the imidazolidinones also exhibited CaIPDE inhibitory activity led us to determine the effects of these compounds on [^{3}H]rolipram binding and rodent behavior.

Table II presents the biological properties of the nicotinamide ethers. All of the compounds listed are modestly potent inhibitors of CaIPDE, with relative potencies 5–10-fold weaker than the previously described catechol ethers.[1,2] The CaIPDE enzyme preparation is believed to be a mixture of several isoenzymes with similar high affinity for cAMP.[20] Inhibition of calcium-dependent PDE was also determined.[4] In all cases, the inhibition of calcium-dependent PDE was 10–20% at a test compound concentration of 10^{-5} M; thus, IC_{50} values were consistently $\gg 10$ μM. These results show that the nicotinamide ethers exhibit selectivity for the CaIPDE enzyme preparation. These agents are also good inhibitors of [^{3}H]rolipram binding, once again, weaker as a class than the catechol ethers.

Certain in vitro structure–activity relationships are readily apparent. With regard to the nature of the 3-carboxamide group, a wide variety of N-substitution is tolerated (e.g., benzyl, substituted benzyl, phenyl, isopropyl; cf. compounds 1, 2, 8, and 11). The in vitro SAR is far more sensitive to modification of the ether linkage. The most potent binding inhibitor was the *m*-carbomethoxyphenyl compound 4. However, certain meta substitution (cf. compounds 9, 13, and 14) caused a decrease in in vitro potency. In general, ortho substitution was not tolerated (compounds 16 and 18); the origin of this effect is unknown.

The 2-(aryloxy)alkoxynicotinamides were also tested for their ability to reverse reserpine-induced hypothermia. Compounds 2, 4, 8, and 11 were the most potent agents in this assay, each exhibiting a minimum effective dose of 0.001 mg/kg. The most potent CaIPDE inhibitor, compound 1 (IC_{50} = 0.22 μM), did not show comparable in vivo activity (MED = 1.0 mg/kg). In general, in vitro potency proved to be a poor predictor of activity in the reserpine test, possibly due to differences in bioavailabiilty and pharmacokinetics. Furthermore, as we noted in an earlier paper, the relationship of [^{3}H]rolipram binding to in vivo activity is unclear and, in many instances, binding and enzyme inhibition do not appear to correlate.[1] In fact, compounds 15 and 16 are moderately potent CaIPDE inhibitors (IC_{50} values = 3.1 and 10.0 μM, respectively), both of which exhibit a MED of 0.01 mg/kg in the reserpine test despite being very poor displacers of [^{3}H]rolipram binding.

In conclusion, we have described the preparation and biological properties of a new structural class of CaIPDE inhibitors which exhibit good in vivo central nervous system activity. Further studies of these agents are in progress.

Experimental Section

Chemistry. Reagents, starting materials, and solvents were purchase from common commercial suppliers and were used as received or distilled from the appropriate drying agent. Melting points were obtained on a Büchi 510 apparatus and are uncorrected. NMR spectra were recorded on either a Varian VT-300 (299.9 MHz), a Bruker WM-250 (250 MHz), or a Varian EM 390 spectrometer (90 MHz). Mass spectra were obtained with an A.E.I MS-30 mass spectrometer. Elemental analyses were performed by the Pfizer Central Research Analytical Department.

Method A. General Method for the Preparation of 2-(Aryloxy)alkoxynicotinamides via 2-(Aryloxy)alkoxynicotinic Acids. *N*-Benzyl-2-(3-methylphenoxy)nicotinamide (9). A sodium hydride dispersion (60% by weight, 2.54

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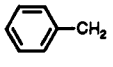
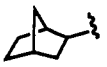
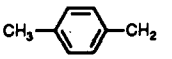
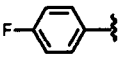
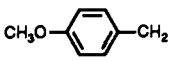
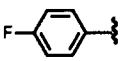
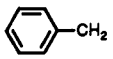
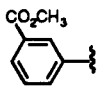
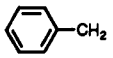
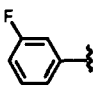
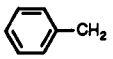
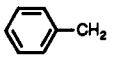
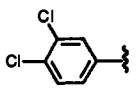
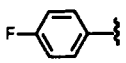
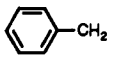
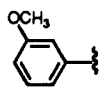
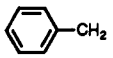
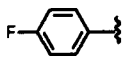
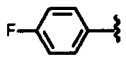
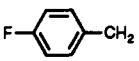
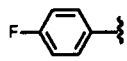
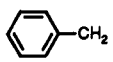
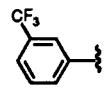
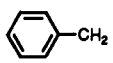
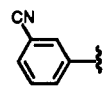
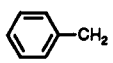
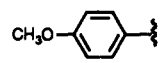
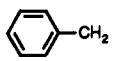
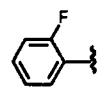
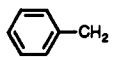
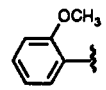
Table I

compd	R_1	R_2	method[a]	% overall yield	mp, °C	formula[b]	anal.
1			B	32.5	200 (HCl salt)	$C_{20}H_{22}N_2O_2 \cdot HCl$	C, H, N
2			B	37.1	64.5–66	$C_{20}H_{17}N_2O_2F$	C, H, N
3			B	27.3	76.5–78	$C_{20}H_{17}N_2O_3F$	C, H, N
4			B	12.8	119–120	$C_{21}H_{18}N_2O_4$	C, H, N
5			A	7.0	86–86.5	$C_{19}H_{15}N_2O_2F$	C, H, N
6			B	25.3	86–87	$C_{19}H_{16}N_2O_2$	C, H, N
7			A	11.6	108–110	$C_{19}H_{14}N_2O_2Cl_2$	C, H, N
8			B	19.5	133–135	$C_{18}H_{13}N_2O_2F$	C, H, N
9			A	38.5	102–103.5	$C_{20}H_{18}N_2O_3$	C, H, N
10			B	21.4	102–103	$C_{19}H_{15}N_2O_2F$	C, H, N
11			B	10.4	104–106	$C_{15}H_{15}N_2O_2F$	C, H, N
12			B	20.9	136.5–138	$C_{19}H_{14}N_2O_2F_2 \cdot {}^1/_3H_2O$	C, H, N
13			A	32.0	90–91	$C_{20}H_{15}N_2O_2F_3 \cdot {}^2/_3H_2O$	C, H, N
14			A	18.2	117–118	$C_{20}H_{15}N_3O_2$	C, H, N
15			B	32.0	111–113	$C_{20}H_{18}N_2O_3$	C, H, N
16			B	7.4	129–130	$C_{19}H_{19}N_2O_2F \cdot {}^1/_3H_2O$	C, H, N
17			B	23.6	164–167	$C_{20}H_{18}N_2O_3 \cdot {}^1/_3H_2O$	C, H, N

[a] Refers to general method of synthesis; see details in the Experimental Section. [b] All compounds were fully characterized spectroscopically (^1H NMR, ^{13}C NMR, HRMS).

g, 63.47 mmol) was added to 32 mL of DMF under a nitrogen atmosphere. 3-Methoxyphenol (3.94 g, 31.73 mmol) was then added portionwise with stirring over a 5-min period; an exothermic reaction was observed and gas evolved. The reaction mixture was stirred for an additional 5 min and 2-chloronicotinic acid (5.00 g, 31.73 mmol) was added portionwise. After gas evolution ceased, the mixture was heated at reflux for 20 h, cooled to room temperature, and quenched with 300 mL of water. The reaction mixture was washed with ether (2 × 200 mL; discarded), and the aqueous phase was adjusted to acidic pH with glacial acetic acid and then stirred for 18 h. The resultant precipitate was collected by filtration, washed with water (3 × 150 mL), and dried in vacuo to afford 3.95 g (50.8%) of the nicotinic acid ether as a light tan, crystalline solid: mp 166.5–168 °C.

The nicotinic acid ether from above was dissolved in 20 mL of THF under a nitrogen atmosphere at room temperature and *N*-methylmorpholine (0.41 g, 4.08 mmol) was added. The solution was cooled to −10 C and isobutyl chloroformate (0.56 g, 4.08 mmol) added dropwise while the temperature was maintained below 0 °C. The reaction mixture was stirred at −10 °C for 30 min and then benzylamine (0.45 g, 4.49 mmol) was added dropwise; again a temperature of less than 0 °C was maintained. The mixture was slowly warmed to room temperature, stirred for 3 h, quenched with 100 mL of water, and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with 2 N sodium hydroxide solution (2 × 100 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to yield an oil. Trituration with 1:1 ether/pentane afforded 1.03 g (75.7%) of **9** as a white solid: mp 102–103.5 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 8.66 (dd, 1 H, J = 8, 2 Hz), 8.23 (dd, 2 H, J = 5, 2 Hz), 7.36–7.1 (m, 7 H), 6.84–6.68 (m, 3 H), 4.72 (d, 2 H), 3.76 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.45, 160.85, 160.19, 153.60, 150.11, 142.46, 138.26, 130.15, 128.72, 127.48, 127.42, 119.50, 116.92, 113.90, 111.43, 107.90, 55.45, 43.95; HRMS calcd for $C_{20}H_{18}N_2O_3$: 334.1319; found 334.1318. Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

Method B. General Method for the Preparation of 2-(Aryloxy)alkoxynicotinamides via 2-Chloronicotinamides. *N*-Benzyl-2-phenoxynicotinamide (6). 2-Chloronicotinic acid

Table II

compd	IC_{50},[a] μM CaIPDE	IC_{50},[a] μM [^{3}H]rolipram binding	reserpine hypothermia, MED, mg/kg po, mouse
1	0.22	20, 27	1.0
2	0.54 ± 0.27 (3)	19, 25	0.001
3	0.73	26	3.2
4	0.97, 1.4	6.2, 6.6	0.001
5	1.8	29	0.01
6	1.8	39, 49	0.32
7	2.2	19	>3.2
8	2.2	43	0.001
9	2.5	23	0.01
10	2.9	25, 37	0.01
11	3.1	26	0.001
12	4.0	51	0.01
13	6.5	54	3.2
14	9.8	22	0.1
15	3.1	~100	0.01
16	10.0	>100	0.01
17	>10	>100	>3.2
nitraquazone	1.9 ± 1.0 (4)	14 ± 1 (5)	

[a] IC_{50} values were determined from dose–response curves of three log concentrations of the test compounds, each concentration in triplicate. The mean ± standard deviation for (*N*) separate determinations was obtained for 2 and nitraquazone.

(25 g, 0.159 mol) was dissolved in 800 mL of THF under a nitrogen atmosphere and cooled to 0 °C, and *N*-methylmorpholine (16.05 g, 0.159 mol) added. The mixture was chilled to −10 °C and isobutyl chloroformate (21.67 g, 0.159 mol) was added dropwise while the temperature was maintained below 0 °C. The reaction mixture was stirred at −10 °C for 30 min, and then benzylamine (18.70 g, 0.175 mol) was added; the temperature was kept below 0 °C. The mixture was slowly warmed to room temperature, stirred for 18 h, quenched with 1 N HCl (300 mL), and extracted with ethyl acetate (2 × 350 mL). The combined organic extracts were washed with an additional portion of 1 N HCl (300 mL) and then 12% sodium hydroxide solution (2 × 300 mL) and dried over anhydrous magnesium sulfate. Filtration and evaporation of the solvent afforded a white paste which crystallized upon trituration with ether. The solid thus obtained was collected by filtration and washed with ether (2 × 65 mL). Concentration of the filtrates and retrituration with fresh ether afforded a second crop of product. The combined yield of white crystalline *N*-benzyl-2-chloronicotinamide was 28.11 g (71.8%, mp 120 °C).

Sodium hydride dispersion (50% by weight, 0.39 g, 8.11 mmol) was added to 80 mL of DMF under a nitrogen atmosphere. Phenol (0.84 g, 8.92 mmol) was added and the mixture stirred for 20 min. *N*-Benzyl-2-chloronicotinamide (2.00 g, 8.11 mmol) was then added and the reaction was heated at reflux for 1 h. The mixture was then cooled to room temperature and quenched with 200 mL of water, and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with 2 N sodium hydroxide solution (2 × 200 mL) and water (200 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to yield an oil. The crude product was purified by flash column chromatography on silica gel using diethyl ether/hexane (2:1) as the eluant to yield 0.87 g (35.2%) of **6** as a white solid: mp 86–87 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 8.51 (dd, 1 H, *J* = 15, 1 Hz), 8.05 (dd, 1 H, *J* = 10, 3 Hz), 7.43–6.86 (m, 12 H), 4.54 (d, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.49, 160.29, 152.57, 150.01, 142.46, 138.28, 129.79, 128.72, 127.48, 127.41, 125.71, 121.87, 119.43, 116.85, 43.95; HRMS calcd for $C_{19}H_{16}N_2O_2$ 304.1219, found 304.1211. Anal. ($C_{19}H_{16}N_2O_2$) C, H, N.

***N*-Benzyl-2-(3-carbomethoxyphenoxy)nicotinamide (4).** The requisite carboxylic acid precursor was prepared according to method B in 35.0% yield (two steps): mp 157–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (dd, 1 H, *J* = 8, 2 Hz), 8.19 (dd, 1 H, *J* = 5, 2 Hz), 8.11 (m, 1 H), 7.98 (dt, 1 H, *J* = 6, 2 Hz), 7.84 (t, 1 H, *J* = 2 Hz), 7.52 (t, 1 H, *J* = 8 Hz), 7.38–7.16 (m, 7 H), 4.71 (d, 2 H, *J* = 6 Hz). The acid (1.00 g, 2.87 mmol) was then slurried in 12 mL of methanol at 0 °C under a nitrogen atmosphere and acetyl chloride (0.57 mL) was added. The mixture was slowly warmed to room temperature, stirred for 18 h, and quenched with 50 mL of 2 N sodium hydroxide solution. The reaction mixture was extracted with ethyl acetate (2 × 50 mL), and the combined extracts were washed with 2 N sodium hydroxide solution (2 × 100 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to yield a pasty solid which crystallized upon trituration with diethyl ether. The isolated yield of white crystalline 4 was 0.38 g (36.5%).

Biology. Measurement of Phosphodiesterase Activity.[14,21] CaIPDE activity was determined in a final volume of 0.1 mL with a solution containing 50 mM Tris-HCl/5 mM $MgCl_2$ (pH 7.5) buffer and [^{3}H]cAMP (NEN NET-275).[1] The finai cAMP concentration was 1.0 μM (400 000 dpm of [^{3}H]cAMP). Vehicle or compound solution (10 μL) and 10 μL of fresh calcium-independent or calcium-dependent PDE (or the respective boiled enzyme) were added to 89 μL of substrate in the Tris-HCl/$MgCl_2$ buffer. The enzymes were prepared from rat brain according to the method of Davis.[4] The hydrolysis reaction was carried out in triplicate at 37 °C for 8 min and stopped by immersing the reaction tube in a boiling water bath for 2 min. Carrier 5′-AMP (0.5 mL of 5 mM 5′-AMP in 0.1 M Hepes/0.1 M NaCl buffer, pH 8.5) was added and the 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 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 radioactive 5′-AMP content.

Measurement of [^{3}H]Rolipram Binding. This assay is based upon that described by Schneider.[3] 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 (Polytron PT-10, Brinkman Instruments, setting 6.5). The resultant homogenate was centrifuged at 15 000 rpm for 20 min at 4 °C. The pellet was resuspended in 20 volumes of Tris buffer (0.5 mg protein/mL) and respun as before. The binding assay was carried out in triplicate on 1.0 mL of this tissue suspension (added last) incubated with 0.1 mL of [^{3}H]rolipram and 0.02 mL of nicotinamide ether at various concentrations. Ro 20-1724 (10 μM) was used to determine nonspecific binding. After 30 min at 25 °C the contents of the incubation tubes were filtered through a Whatman GF/B glass-fiber strip in a Brandel cell harvester. The membranes were washed three times with 3 mL of ice-cold buffer, and radioactivity on the separated filter disks was determined in a liquid-scintillation counter. IC_{50} values were estimated from semilog plots of percent inhibition versus concentration.

Reserpine Hypothermia Test. In accordance with the method of Askew,[22] as modifed by Koe,[23] mice were individually housed at 20 °C in plastic chambers with cardboard bottoms. The animals were injected with reserpine (2 mg/kg, sc) and retained at 20 °C for 18 h. Rectal temperatures were then measured at time 0, after which the animals were treated with either saline or test drug solution. Rectal temperatures were again determined, typically at 0.5, 1, 2, and 4 h. Reserpine-pretreated mice exhibit rectal temperatures of 20–21 °C after injection with vehicle, whereas antidepressant-treated animals experience a temperature increase of several degrees.

Supplementary Material Available: Analysis data for 1–17 and physical data for intermediates (4 pages). Ordering information is given on any current masthead page.

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(22) Askew, B. M. *Life Sci* **1963**, *4*, 725.
(23) Koe, B. K.; Weissman, A.; Welch, W. M.; Browne, R. G. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 686.