

Notes

Characterization of the Neurotoxic Potential of *m*-Methoxy-MPTP and the Use of Its *N*-Ethyl Analogue as a Means of Avoiding Exposure to a Possible Parkinsonism-Causing Agent

Dennis M. Zimmerman,* Buddy E. Cantrell, Jon K. Reel, Susan K. Hemrick-Luecke, and Ray W. Fuller

Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285.

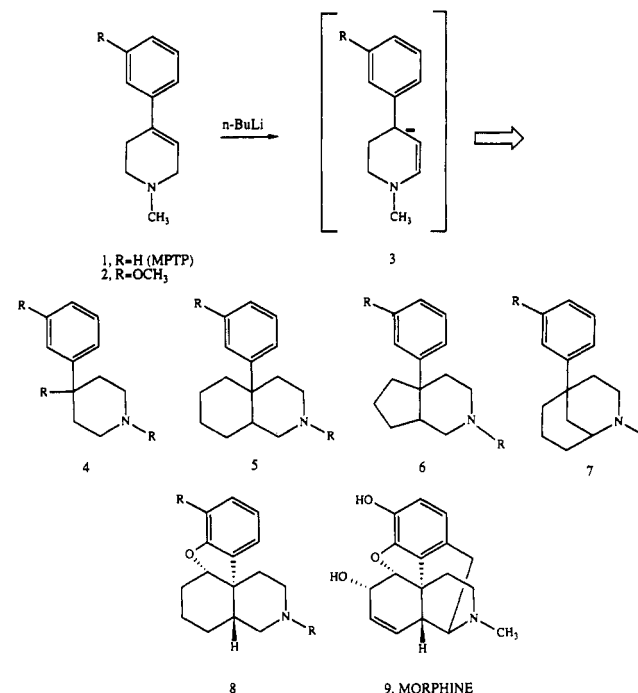
Received September 30, 1985

1-Methyl-4-(3-methoxyphenyl)-1,2,3,6-tetrahydropyridine (**2**) produced persistent depletion of striatal dopamine in mice after four daily injections, although it was less potent than 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP has been implicated as a cause of Parkinsonism in drug abusers who inadvertently self-administered it and in industrial chemists who were exposed to it. Our results suggest that the *m*-methoxy compound has the same neurotoxic potential to cause destruction of nigrostriatal dopamine neurons that would lead to Parkinsonian symptoms in humans. In contrast, 1-ethyl-4-(3-methoxyphenyl)-1,2,3,6-tetrahydropyridine (**11**) had no effects on striatal dopamine in mice, even at doses 8 times those of MPTP. A method of preparing **11** and using it as an intermediate in the synthesis of potential analgesic drugs, thus avoiding a potentially neurotoxic intermediate, is described.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), **1**, has been implicated as a cause of Parkinsonism in human drug abusers who self administered it intravenously.¹ MPTP was present as a contaminating byproduct in a preparation of 1-methyl-4-phenyl-4-propionoxypiperidine, synthesized as a meperidine analogue with narcotic activity but not at the time classified as a controlled substance. Subsequently, MPTP has been shown to produce destruction of nigrostriatal dopamine neurons in primates and lower species and to produce movement disorders in monkeys that resemble closely those of naturally occurring Parkinson's disease in humans.²⁻⁴

MPTP and some of its analogues have been important intermediates in the synthesis of new medicinal agents for some time. There have been two case reports of Parkinson's disease at unusually early ages after extensive exposure to MPTP.^{5,6} Although there is no means of proving that their disease was caused by MPTP, that possibility must be considered. Those cases, along with the knowledge that cumulative doses of MPTP as low as 11 mg are neurotoxic in rhesus monkeys,² require that great caution be exerted by researchers who work with MPTP in chemical syntheses or in biologic experiments. MPTP and related compounds are still frequently used in the synthesis of potential drugs, especially analgesic drugs. For example, both MPTP and *m*-methoxy-MPTP, **2**, have been found to have considerable utility via the metalated enamines, **3**, in the synthesis of potential new nonaddicting analgesics derived from the morphine ring system (Scheme I). The application of this chemistry has led to efficient synthesis of the 4-alkyl-4-arylpiperidines **4**, the *cis*- and *trans*-aryldecahydroisoquinolines **5**, the *cis*- and *trans*-arylpiperidines **6**, and the

Scheme I



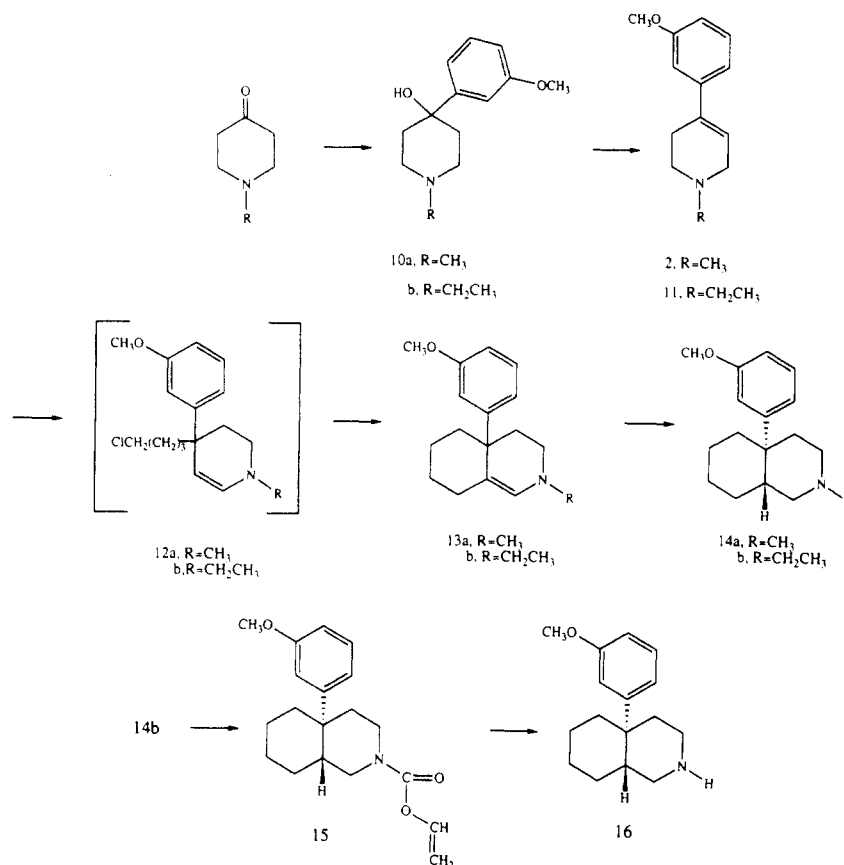
arylmorphans **7**.^{7,8} Further application of this chemistry has subsequently led to the synthesis of the octahydrobenzofuroisoquinolines **8**⁹ and to a synthesis of (±)-morphine itself.¹⁰ The synthesis of the *trans*-aryldecahydroquinoline **14a** (Scheme II) is illustrative of the efficiency of this approach for the synthesis of new morphine-based analgesics.

Relatively few structure-activity studies have been reported with MPTP as a dopaminergic neurotoxin as yet. Here we describe studies showing that *m*-methoxy-MPTP

- (1) Langston, J. W.; Ballard, P.; Tetrud, J. W.; Irwin, I. *Science (Washington, D.C.)* **1983**, *219*, 979-980.
- (2) Burns, R. S.; Chiu, C. C.; Markey, S. P.; Ebert, M. H.; Jacobowitz, D. M.; Kopin, I. J. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4546-4550.
- (3) Wallace, R. A.; Boldry, R.; Schmittgen, T.; Miller, D.; Uretsky, N. *Life Sci.* **1984**, *35*, 285-291.
- (4) Heikkilä, R. E.; Hess, A.; Duvoisin, R. C. *Life Sci.* **1985**, *36*, 231-236.
- (5) Langston, J. W.; Ballard, P. A. *N. Engl. J. Med.* **1983**, *309*, 310.
- (6) Markey, S. P.; Burns, R. S. *Chem. Eng. News* **1984**, *2*.

- (7) Evans, D. A.; Mitch, C. H.; Thomas, R. C.; Zimmerman, D. M.; Robey, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 5955-5956.
- (8) Zimmerman, D. M.; Robey, R. L. U.S. Patent 4 236 009, 1980; *Chem. Abstr.* **1981**, *94*, 121345t.
- (9) Shenvi, A. B.; Ciganek, E. J. *Org. Chem.* **1984**, *49*, 2942-2947.
- (10) Evans, D. A.; Mitch, C. H. *Tetrahedron Lett.* **1982**, 285-288.

Scheme II



produces persistent depletion of striatal dopamine and its metabolites in mouse striatum as does MPTP itself. Though the *m*-methoxy compound is slightly less potent, it could still pose a considerable hazard to those handling it, especially if it is used over a long period of time. Because of the possible further applications of the metalated enamine chemistry and other uses of MPTP-related intermediates, we wanted to alert medicinal chemists to the neurotoxicity of *m*-methoxy-MPTP and also to indicate a less toxic alternative compound that could be used in the place of MPTP. We have found that the *N*-ethyl analogue of the *m*-methoxy-MPTP is not neurotoxic, even at higher doses, and propose the synthetic strategy of using *N*-ethyl or larger *N*-alkyl analogues of MPTP or similar compounds as a means of avoiding the known neurotoxic intermediates. Because often in medicinal chemistry, especially with morphine-based analgesics, it is necessary to replace the *N*-alkyl substituent with other substituents, we demonstrate, using the *trans*-phenylisoquinoline structure as a prototypic molecule, that the *N*-ethyl group can be removed easily and in high yield using vinyl chloroformate.¹¹

Results

Chemistry. Methoxy-MPTP and the *N*-ethyl analogue were synthesized by addition of the aryllithium to the *N*-alkyl-4-piperidone followed by phosphoric acid catalyzed dehydration of the resulting carbonol (Scheme II). The *N*-ethyl-*trans*-arylisoquinoline **14b** was synthesized via lithiation of the tetrahydropyridine **11** followed by alkylation with 1-bromo-4-chlorobutane to give **12a**. The halo enamine **12a**, when heated in the presence of sodium iodide, gave the bicyclic enamine **13a** in approximately 70% yield. Catalytic hydrogenation of **13b** with platinum oxide in ethanol afforded the *trans*-isoquinoline **14b** ex-

clusively in near-quantitative yield. *N*-Dealkylation of **14b** was accomplished in 80% yield using vinyl chloroformate (Scheme II).

Pharmacology. Table I (experiment 1) compares the effects of MPTP and *m*-methoxy-MPTP on striatal concentrations of dopamine and its major metabolites, (3,4-dihydroxyphenyl)acetic acid (DOPAC) and homovanillic acid (HVA), measured 1 week after the last of four daily doses of each compound. MPTP itself at the 20 mg/kg sc dose caused 71% depletion of striatal dopamine and slightly less depletion of DOPAC and HVA, in agreement with earlier results.¹² The *m*-methoxy compound at this dose caused no significant effect on the concentrations of dopamine or its metabolites in striatum. Higher doses of *m*-methoxy-MPTP, however, significantly decreased dopamine, DOPAC, and HVA as did MPTP itself. These results indicate that *m*-methoxy-MPTP is neurotoxic to nigrostriatal dopamine neurons in mice, being about one-third as potent as MPTP.

The *N*-ethyl analogue of *m*-methoxy-MPTP was compared at equal doses (Table I, experiment 2). In this experiment, *m*-methoxy-MPTP produced 87% depletion of dopamine, 80% depletion of DOPAC, and 58% depletion of HVA in mouse striatum. The *N*-ethyl analogue had no significant effect.

Considering the possibility that the *N*-ethyl compound might have neurotoxic potential but be less potent even than *m*-methoxy-MPTP, we gave it at twice the dose used in the first experiment also including MPTP itself as a positive control. Table I (experiment 3) further shows that *m*-methoxy-EPTP (**11**), even at 160 mg/kg, did not significantly affect striatal concentrations of dopamine or its metabolites. In contrast, MPTP caused the usual depletion of these substances in this experiment.

(11) Olofson, R. A.; Schnur, R. C.; Bunes, L.; Pepe, J. P. *Tetrahedron Lett.* 1977, 1567.

(12) Fuller, R. W.; Steranka, L. *Life Sci.* 1985, 36, 243.

Table I. Dopamine and Its Metabolites in Mouse Brain 1 Week after Four Daily Injections of MPTP and Its Analogues

expt	compd injected ^a	dose, mg/kg	concn in striatum, ^b nmol/g ^a		
			concn dopamine	DOPAC	HVA
1	none	20	94.1 ± 4.1	5.59 ± 0.24	8.88 ± 0.40
	MPTP (1)		27.4 ± 0.9*	2.32 ± 0.04*	4.71 ± 0.14*
			(-71%)	(-59%)	(-47%)
	2	20	84.8 ± 1.9	5.09 ± 0.04	8.07 ± 0.21
	2	40	38.1 ± 2.0*	2.93 ± 0.14*	5.66 ± 0.21*
2			(-59%)	(-48%)	(-36%)
	2	80	15.9 ± 4.1*	1.38 ± 0.22*	2.97 ± 0.28*
			(-83%)	(-75%)	(-67%)
	none	80	58.9 ± 1.4	4.41 ± 0.18	5.75 ± 0.19
	2		7.9 ± 1.3*	0.90 ± 0.10*	2.41 ± 0.15*
3			(-87%)	(-80%)	(-58%)
	11	80	62.7 ± 1.4	4.60 ± 0.14	5.46 ± 0.17
	none	20	81.7 ± 2.3	6.01 ± 0.23	6.33 ± 0.22
	MPTP (1)		26.0 ± 1.5*	2.52 ± 0.15*	3.97 ± 0.12*
			(-68%)	(-58%)	(-37%)
	11	160	82.1 ± 2.0	5.55 ± 0.13	6.38 ± 0.22

^a Mice were killed 1 week after the last of four daily doses of each compound. MPTP (1), *m*-methoxy-MPTP (2), and *m*-methoxy-EPTP (11) were all injected subcutaneously. ^b Mean values ± standard errors for 6 mice/group are shown. Asterisks indicate statistically significant differences ($P < 0.05$). Percentage changes are shown where there were significant drug effects.

The inability of the *N*-ethyl compound to cause persistent depletion of striatal dopamine and its metabolites is compatible with the findings of Heikkilä et al.⁴ that the *N*-ethyl analogue of MPTP itself is a poor substrate for monoamine oxidase relative to MPTP. Their *in vivo* results showed that MPTP was metabolized at a rate nearly 8 times faster than that of the *N*-ethyl compound. Inadequate conversion of the *N*-ethyl compound to a pyridinium metabolite *in vivo*¹³ might account for its lack of neurotoxicity toward striatal dopamine neurons in mice.

Discussion

We have shown that *m*-methoxy-MPTP, like MPTP, is highly neurotoxic as measured by its ability to cause persistent depletion of striatal dopamine in mice. Though the compound is less potent than MPTP, it would appear to be a very hazardous compound considering the very low doses of MPTP that have been shown to be neurotoxic in primates. MPTP and *m*-methoxy-MPTP have been in the past and will likely be in the future important intermediates in the synthesis of new medicinal agents. We have found *N*-ethyl-*m*-methoxy-MPTP not to be neurotoxic in mice even at doses 8 times those of MPTP which produce a 70% depletion of striatal dopamine. If the lack of persistent depletion of striatal dopamine in mice is predictive of lack of neurotoxic potential in humans, *N*-ethyl-*m*-methoxy-MPTP would be a safe alternative to *m*-methoxy-MPTP as a chemical intermediate. Using the metalated enamine approach for the synthesis of the aryldecahydroquinolines, we have found the *N*-ethyl analogue to be a suitable replacement compound for the normally used *N*-methyl derivative. We propose the synthetic strategy of using the *N*-ethyl analogue of MPTP as a means of reducing potential neurotoxicity hazards.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance (¹H NMR) spectra were taken on a Bruker WM-270 spectrometer, and mass spectra were recorded from a Varian MAT CH-5 spectrometer. NMR and mass spectra data were consistent with proposed structures. Microanalytical data were provided by the Physical Chemistry Department of the Lilly Research Laboratories; only symbols of elements analyzed are given and they were within 0.4% of theoretical values unless indicated

otherwise. Vinyl chloroformate was purchased from Aldrich Chemical Co., Milwaukee, WI.

Pharmacology. Male CRL/CFW mice from Charles River Breeding Laboratories, Wilmington, MA, received daily sc injections of MPTP hydrochloride, compound 2, or compound 11 for 4 days. Seven days after the last injection, the mice were killed. Brains were removed rapidly; striata were dissected, frozen on dry ice, and stored at -15 °C prior to analysis. Dopamine, DOPAC, and HVA were measured by liquid chromatography with electrochemical detection.^{14,15}

1-Ethyl-4-hydroxy-4-(3-methoxyphenyl)piperidine (10b). Under a nitrogen atmosphere, a solution of 247.5 mL (0.40 mol) 1.6 M *n*-butyllithium was added slowly to 214 g (1.14 mol) of *m*-bromoanisole in 300 mL of tetrahydrofuran cooled to -78 °C. The reaction temperature was held below -70 °C. After the addition was complete, the reaction mixture was stirred at -78 °C for 30 min, then allowed to warm to -20 °C. To this mixture was added 1-ethyl-4-piperidone (42.0 g, 0.33 mol) in 100 mL of tetrahydrofuran in a dropwise manner. Following the completion of the addition, the reaction mixture was stirred for 2 additional hours and was then quenched with 200 mL of saturated aqueous sodium chloride solution. The aqueous solution was extracted several times with diethyl ether, and the ethereal extracts were combined, dried over K₂CO₃, and concentrated to dryness. The resulting solid was slurried with 400 mL of *n*-hexane at 0 °C, filtered, and dried *in vacuo* to yield 10b (48.0 g, 62%): mp 75–77 °C. Anal. (C₁₄H₂₁NO₂) C, H, N.

1-Methyl-4-hydroxy-4-(3-methoxyphenyl)piperidine (10a). By a procedure similar to that described for 10b, 10a was obtained in 85% yield: mp 109–111 °C. Anal. (C₁₃H₁₉NO₂) C, H, N.

1-Ethyl-4-(3-methoxyphenyl)-1,2,3,6-tetrahydropyridine (11). To a stirred solution of 192 mL of 85% phosphoric acid preheated to 60 °C was added portionwise 10b (48.0 g, 0.20 mol). During the addition the reaction temperature was held near 60 °C, after which the mixture was heated between 70 and 80 °C for 3 h. To this mixture was added 190 mL of water followed by 400 mL of ammonium hydroxide solution (maintaining the temperature at 60 °C). The desired product was extracted into three 100-mL portions of *n*-hexane at 60 °C. The extracts were washed, dried over K₂CO₃, concentrated under vacuum, and vacuum distilled to yield 39.8 g (90%): bp 92–96 °C, (0.1 mm). Anal. (C₁₄H₁₉NO) C, H, N.

1-Methyl-4-(3-methoxyphenyl)-1,2,3,6-tetrahydropyridine (2). Following the procedure described for 11, 2 was synthesized in 82% yield: bp 120–128 °C (0.1 mm). Anal. (C₁₃H₁₇NO₂) C, H, N.

2-Ethyl-4a-(3-methoxyphenyl)-2,3,4a,5,6,7,8-octahydroisoquinoline (13b). To a stirred cold (0 °C) solution of 11 (13.5

(13) Markey, S. P. (*London*); Johannessen, J. N.; Chiueh, C. C.; Burns, R. S.; Herkenham, M. A. *Nature (London)* 1984, 311, 464–467.

(14) Fuller, R. W.; Perry, K. W. *Biochem. Pharmacol.* 1977, 26, 2087.

(15) Perry, K. W.; Fuller, R. W. *Soc. Neurosci. Abstr.* 1979, 5, 348.

g, 0.062 mol) in 200 mL of tetrahydrofuran was added dropwise over 30 min 43.6 mL of 1.6 M *n*-butyllithium (0.070 mol) in hexane under a nitrogen atmosphere. Following completion of the addition, the solution was stirred for 10 min at 0 °C and then cooled to -30 °C. The cold solution next was added via cannulation over a 20-min period to a stirred solution of 1-bromo-4-chlorobutane (36.0 g, 0.21 mol) in 130 mL of ether chilled to -50 °C. Following completion of the addition, the reaction mixture was warmed to -20 °C and quenched with 150 mL of saturated sodium chloride solution chilled to 0 °C.

During the workup the various extracts were kept as cool as possible. The organic layer was separated, washed with water, and the desired product was extracted therefrom with three 400-mL portions of 1 N hydrochloric acid. The aqueous acidic layer was washed with diethyl ether and the ether extracts were discarded. The acidic aqueous solution was then made alkaline by the dropwise addition of 50% aqueous sodium hydroxide. The resulting alkaline solution was extracted several times with diethyl ether, and the ethereal extracts were combined, washed with water, and dried over K₂CO₃. Evaporation of the solvent under reduced pressure at 10 °C afforded the chloro enamine **12b** as an oil which was dissolved in 1100 mL of acetonitrile containing 23 g (0.15 mol) of sodium iodide and 17 g of K₂CO₃ (0.12 mol). The reaction mixture was heated at reflux temperature with stirring under nitrogen for 20 h. The reaction mixture was filtered, and the solvent was removed by evaporation under reduced pressure. The crude product thus formed was dissolved in a mixture of 480 mL of 1 N sodium hydroxide and 1000 mL of diethyl ether, and the mixture was stirred vigorously for 45 min. The ethereal layer then was separated, washed with saturated aqueous sodium chloride, and dried over K₂CO₃. Removal of the solvent by evaporation under reduced pressure afforded the product as an oil, which, upon bulb-to-bulb distillation, provided 12.0 g of **13b** (71%). The perchlorate salt was prepared from the free base and the product obtained crystallized from ethanol/isopropyl ether (1:1): mp 152-153 °C. Anal. (C₁₈H₂₆NO₃Cl) C, H, N.

2-Ethyl-4a-(3-methoxyphenyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline (14b). A solution of **13b** (10.5 g, 0.039 mol)

in 138 mL of ethanol containing 1.1 g of platinum oxide was stirred at room temperature for 16 h under a hydrogen atmosphere of 60 psi. The hydrogenation mixture was filtered to remove the catalyst and the filtrate evaporated to dryness and bulb-to-bulb distilled at 180 °C (0.1 mm) to yield **14b**, 9.9 g (93%). Anal. (C₁₈H₂₇NO) C, H, N. The hydrochloride salt was prepared from the free base and recrystallized from ethyl acetate: mp 142-144 °C. Anal. (C₁₈H₂₈NOCl) C, H, N.

4a-(3-Methoxyphenyl)-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline (16). A solution of **14b** (5.5 g, 0.02 mol) in 50 mL of 1,2-dichloroethane was added dropwise to vinylchloroformate (9 mL, 0.04 mol) and 8.6 g (0.06 mol) of proton sponge in 150 mL of 1,2-dichloroethane at 0 °C under a nitrogen atmosphere. The reaction was allowed to warm to room temperature then refluxed for 2 h. The mixture was filtered, and the solvent was removed by evaporation under vacuum. The residue was taken up in ether and washed with cold 1 N HCl and water. The ether layer was dried over potassium carbonate, filtered, and evaporated to dryness. The resulting residue (4.9 g), comprising the vinyl carbamate, was refluxed for 1 h in 200 mL of ethanol and 200 mL of ethanol saturated with gaseous hydrochloric acid. The mixture was evaporated to dryness and the residue partitioned between ether and 1 N sodium hydroxide. The ether layer was washed with water, dried over K₂CO₃, and evaporated to dryness. The resulting oil was purified by bulb-to-bulb distillation at 190 °C (0.1 mm) to yield 4.3 g (88%). Anal. (C₁₈H₂₃NO) C, H, N. The hydrochloride salt was prepared from the free base and recrystallized from isopropyl ether/ethanol (1:1): mp 181-183 °C. Anal. (C₁₈H₂₄NOCl) C, H, N.

Registry No. 2, 73224-22-3; **10a**, 73224-20-1; **10b**, 102573-71-7; **11**, 102538-16-9; **12b**, 102538-17-0; **13b**, 102538-18-1; **13b**-perchlorate, 102538-19-2; **14b**, 102538-20-5; **14b**-hydrochloride, 102538-21-6; **15**, 102538-22-7; **16**, 102538-23-8; **16**-hydrochloride, 102538-24-9; *m*-bromoanisole, 2398-37-0; 1-ethyl-4-piperidone, 3612-18-8; 1-methyl-4-piperidone, 1445-73-4; 1-bromo-4-chlorobutane, 6940-78-9; vinyl chloroformate, 5130-24-5.

Analogues of 1,3-Dipropyl-8-phenylxanthine: Enhancement of Selectivity at A₁-Adenosine Receptors by Aryl Substituents

J. W. Daly,* W. L. Padgett, and M. T. Shamim

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Laboratory of Bioorganic Chemistry, Bethesda, Maryland 20205. Received September 13, 1985

The effect of a variety of aryl substituents on the potency and selectivity of 19 analogues of 1,3-dipropyl-8-phenylxanthine as antagonists at A₁- and A₂-adenosine receptors in brain tissue was determined. The 4-sulfamoylphenyl and 4-carbamoylphenyl analogues are potent and somewhat selective for the A₁ receptor. None of the dihydroxyphenyl analogues are remarkably potent, but all are selective for the A₁ receptor. 1,3-Dipropyl-8-(2-hydroxy-4-methoxyphenyl)xanthine is the most selective A₁ antagonist of the analogues with a A₁/A₂ potency ratio of about 90.

8-Phenyltheophylline is the parent member of a variety of potent adenosine receptor antagonists. Originally discovered as a result of screening xanthines and other heterocycles as adenosine antagonists in fibroblasts,¹ 8-phenyltheophylline has been employed as a potent antagonist of adenosine-elicited responses in many biochemical¹⁻¹⁰ and physiological¹¹⁻²² studies. Three considerations

have prompted the preparation and investigation of further 8-phenylxanthines as adenosine antagonists. These are (i)

- (1) Bruns, R. F. *Biochem. Pharmacol.* **1981**, *30*, 325.
- (2) Smellie, F. W.; Davis, C. W.; Daly, J. W.; Wells, J. N. *Life Sci.* **1979**, *24*, 2475.
- (3) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547.
- (4) Wu, P. H.; Phillis, J. W.; Nye, M. J. *Life Sci.* **1982**, *31*, 3857.
- (5) Fredholm, B. B.; Persson, C. G. A. *Eur. J. Pharmacol.* **1982**, *8*, 673.
- (6) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2077.

- (7) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. J. *J. Med. Chem.* **1985**, *28*, 487.
- (8) Fredholm, B. B.; Jonzon, B.; Lindren, E.; Lindstrom, K. J. *Neurochem.* **1982**, *39*, 165.
- (9) Ukena, D.; Furler, R.; Lohse, M. J.; Engle, G.; Schwabe, U. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *326*, 233.
- (10) Munshi, R.; Hansske, F.; Baer, H. P. *Eur. J. Pharmacol.* **1985**, *111*, 107.
- (11) Griffith, S. G.; Meghji, P.; Moody, C. J.; Burnstock, G. *Eur. J. Pharmacol.* **1981**, *75*, 61.
- (12) Hillyard, P. A.; Nials, A. T.; Skidmore, I. F.; Vardey, C. J. *Br. J. Pharmacol.* **1984**, *83*, 337.
- (13) Burnstock, G.; Hills, J. M.; Hoyle, C. H. V. *Br. J. Pharmacol.* **1984**, *81*, 533.
- (14) Corradetti, R.; Moroni, F.; Passani, M. B.; Pepeu, G. *Eur. J. Pharmacol.* **1984**, *103*, 177.