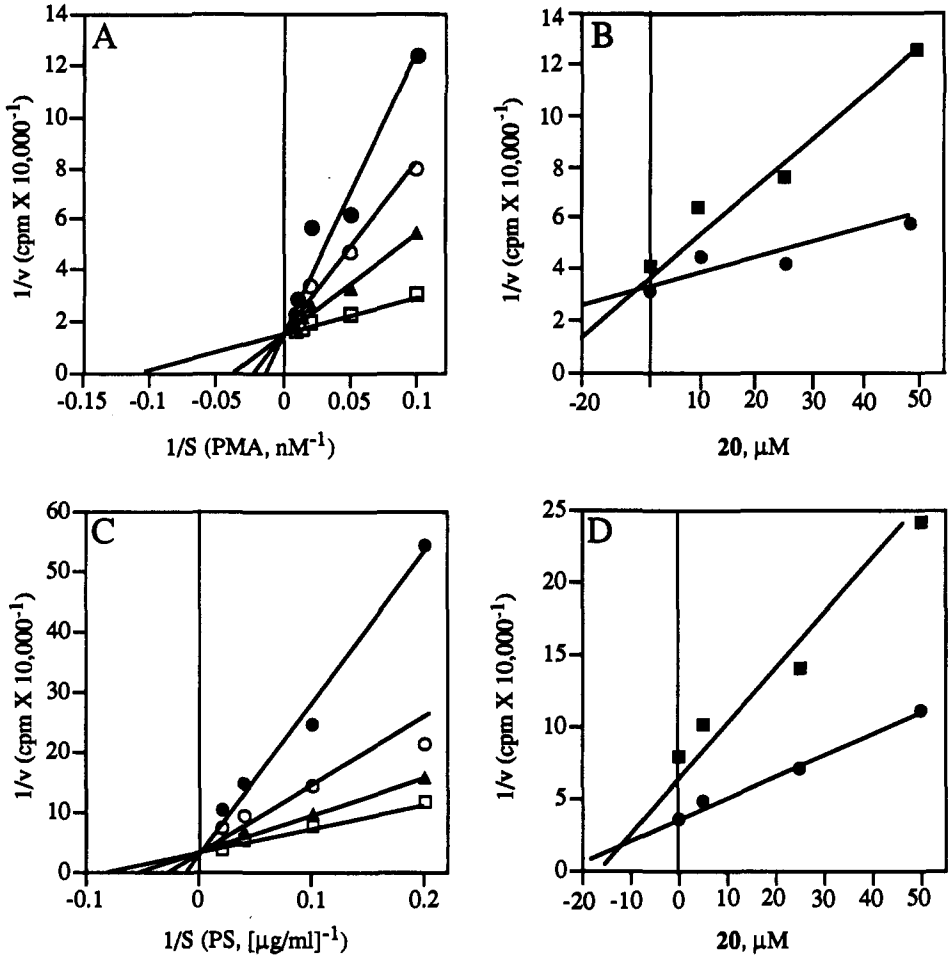


Figure 1. (A) Lineweaver–Burk plot of the effect of PMA on the inhibition of PKC-α by compound **20**. PKC activity was determined in the presence of 400 μM "free calcium", 100 μg/mL PS, 10 μM ATP, and the indicated concentrations of PMA. Compound **20** was present at 0 μM (■), 10 μM (▲), 25 μM (○), and 50 μM (●). (B) Dixon analysis (1/velocity vs concentration of **20**) of the effect of PMA on the inhibition of PKC-α by compound **20**. K_i may be determined from the point of intersection of the two lines.[22] PMA concentrations used were 10 nM (■) and 50 nM (●). (C) Lineweaver–Burk plot of the effect of PS on the inhibition of PKC-α by **20**. PKC activity was determined in the presence of 400 μM "free calcium", 1 μM ATP, and the indicated concentrations of PS. Compound **20** was present at 0 μM (■), 10 μM (▲), 25 μM (○), and 50 μM (●). (D) Dixon analysis (1/velocity vs concentration of **20**) of the effect of PS on the inhibition of PKC-α by compound **20**. PS concentrations were 5 μg/mL (■) and 25 μg/mL (●).

indicate this analogue is a competitive inhibitor with respect to phorbol ester activation (K_i = 5 ± 3 μM).[21] Additionally, phosphatidylserine activation of PKC was competitively inhibited by **20** (K_i = 12 ± 4 μM, Figure 1).[21] Compound **20** also inhibited [^{3}H]phorbol dibutyrate binding (IC_{50} = 23 ± 4 μM) to the enzyme preparation, further suggesting that inhibition of PKC activity by **20** may be the result of interactions with the enzyme's regulatory domain. Recent studies employing recombinant mutant PKC enzymes support evidence that compound **20** interacts with the regulatory domain of the enzyme.[23] Use of the mutant enzymes localized the effect of compound **20** to the N-terminal region of the C-1 domain known to possess the amino acid sequence that includes the pseudosubstrate region and part of the first of the cysteine-rich repeat sequences.

Synthesis of a novel series of selective PKC inhibitors should provide useful tools for elucidating the physiological role of PKC in various cellular processes. The development of these and related agents for therapeutic evaluation in cellular and living systems is currently in progress.

Barry G. Shearer,* James P. Sullivan, J. Paul Carter
Rose M. Mathew, Phillip Waid, Jane R. Connor
Raymond J. Patch, Ronald M. Burch
Nova Pharmaceutical Corporation
6200 Freeport Center
Baltimore, Maryland 21224
Received June 10, 1991

A Selective, Reversible, Competitive Inhibitor of Monoamine Oxidase A Containing No Nitrogen, with Negligible Potentiation of Tyramine-Induced Blood Pressure Rise

Monoamine oxidase (MAO) (EC 1.4.3.4, amine oxidase, flavin containing) consists of two different forms distinguishable by their substrate specificity[1] and their amino acid sequences.[2] Ratios of MAO A to MAO B of ca. 1 and

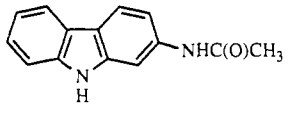
(22) Segal, I. H. *Enzymes Kinetics*, 1st ed.; Wiley and Sons: New York, 1975; pp 109–111.
(23) Sullivan, J. S.; Connor, J. R.; Tiffany, C.; Shearer, B. G.; Burch, R. M. NPC 15437 Interacts With The C1 Domain of Protein Kinase C; An Analysis Using Mutant PKC Constructs. *FEBS Lett.* 1991, in press.
(1) Johnston, J. P. Some Observations Upon a New Inhibitor of Monoamine Oxidase in Brain Tissue. *Biochem. Pharmacol.* 1968, *17*, 1285–1297. Squires, R. F. Additional Evidence for the Existence of Several Forms of Mitochondrial Monoamine Oxidase in the Mouse. *Biochem. Pharmacol.* 1968, *17*, 1401–1409.

0022-2623/91/1834-2931$02.50/0

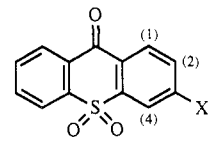
2 for human brain and liver, respectively, have been reported.[3] Serotonin (5-HT) is specifically deaminated by MAO A, while 2-phenethylamine is a relatively specific substrate for MAO B. Tyramine is deaminated by both forms with similar efficiencies. Early MAO inhibitors were found to have clinically significant antidepressant and antiphobic properties. However, ingestion of tyramine-containing foods by patients taking early MAO inhibitors led to the "cheese effect", a significant and sometimes serious increase in blood pressure apparently due to displacement of norepinephrine by undestroyed tyramine,[4] leading to vasoconstriction. Clinical use of MAO inhibitors therefore became severely limited.

More recently, understanding of the differing activities of these two forms has led to selective inhibitors of each form of MAO such as moclobemide and brofaromine for MAO A inhibition (MAO A-I) and deprenyl for MAO B-I.[5]

MAO A-I is believed to lead to a clinically useful antidepressant effect, while MAO B-I does not.[6] An MAO inhibitor that inhibits only MAO A would leave the MAO B to oxidize ingested tyramine. An additional safety factor could be provided by having the inhibitor competitively and reversibly bound to the MAO A and therefore displaceable by high tyramine concentrations, so the MAO A would be reactivated to oxidize tyramine not destroyed by the MAO B present. An inhibitor with this combination of properties was the target of our work. An earlier report[7] mentioned 2-acetamido-9*H*-carbazole (1), which was pos-



1



2: X = $NHC(O)CH_3$
3: X = $C(O)NHCH_3$

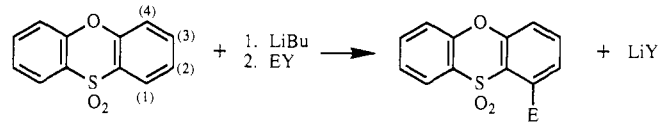
itive in the Ames bacterial mutagenicity test, and related 3-substituted 9*H*-thioxanthen-9-one 10,10-dioxides 2, which were negative. Compounds 2, however, were deemed too similar in structure to 1 to be viable drug candidates. More recently the 3-*N*-methyl carboxamide of 9*H*-thioxanthen-9-one-10,10-dioxide 3 met our criteria[8] but was reported to cause liver enzyme abnormalities in Phase 1 trials. The nonbasicity of the nitrogens in these compounds led us to question whether the nitrogen moiety was essential to their MAO I.

Table I. Metabolites of 4a and Their Inhibition of Rat Brain MAO A in Vitro and ex Vivo

	E	MAO Aa[a] IC_{50}, μM	ED_{80}, mg/kg, or % MAO inhibn at mg/kg[a]
4a	CH_2CH_3	0.035	20
4b	$CH(OH)CH_3$	0.4	30
4c	CH_2CH_2OH	0.02	55 at 20
4d	$CH(OH)CH_2OH$	1.2	20
4e	$C(O)CH_3$	2.0	70 at 30
4f	$CH{=}CH_2$	0.04	20
4g	CH_2COOH	Ca. 30	NS at 20[a]

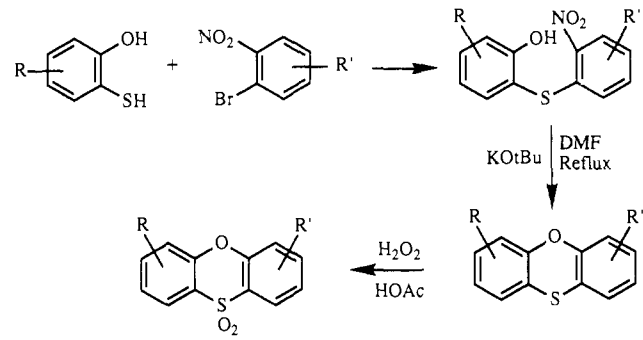
[a] No significant inhibition of MAO B was observed for any of these compounds at the highest concentration tested (generally 10 μM or at the solubility limit).

Scheme I



4a - 4g

Scheme II



We report here that a group of variously substituted phenoxathiin 10,10-dioxides, which do not contain nitrogen, are extremely potent MAO inhibitors and are selective for MAO A. In contrast to the classical MAO inhibitors such as phenelzine and tranylcypromine and the propargylic amines, which are too tightly bound to be dialyzable from their complex with the enzyme, **4a** and many of its close analogues are dialyzable even after preincubation with the enzyme despite IC_{50} values in the 10–100 nanomolar range. Compound **4a** (1370U87), 1-ethylphenoxathiin 10,10-dioxide, was selected for clinical development on the basis of potency in vitro (K_i for rat and human MAO A-I = 10 nM) and in vivo against rat brain MAO A (ED_{50} = 8 mg/kg), negligible MAO B-I, low toxicity in all species tested, and no significant blood pressure change when tyramine was administered orally at 15 mg/kg to rats that had been pretreated with **4a** at a dose which produced 80% inhibition of MAO A in the brain. In addition to its in vitro enzyme inhibiting activity, **4a** potentiated 5-HT effects in rats and mice and was active in the Porsolt test in rats, both standard antidepressant tests. Compound **4a** also showed activity in a Rhesus model of borderline personality disorder.[9] Neither **4a** nor its metabolites

(2) Bach, A. W. J.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S.-W.; Seeburg, P. H.; Shih, J. C. cDNA cloning of Human Liver Monoamine Oxidase A and B: Molecular Basis of Differences in Enzymatic Properties. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4934–4938.

(3) O'Carroll, A. M.; Anderson, M. C.; Tobbia, I.; Phillips, J. P.; Tipton, K. F. Determination of the Absolute Concentrations of Monoamine Oxidase A and B in Human Tissues. *Biochem. Pharmacol.* **1989**, *38*, 901–905.

(4) Meszaros, L. A. The Biochemistry of Stress. *CHEMTECH* **1988**, *18*, 22–25.

(5) Kyburz, E. New Developments in the Field of MAO Inhibitors. *Drug News Perspect.* **1990**, *3*, 592–599.

(6) Murphy, D. L.; Lipper, S.; Pickar, D.; Jimerson, D.; Cohan, R. M.; Garrick, N. A.; Alterman, I. S.; Campbell, I. C. Selective Inhibition of Monoamine Oxidase Type A: Clinical Antidepressant Effects and Metabolic Changes in Man. In *Monoamine Oxidase Inhibitors—The State of the Art*; Youdin, M., Paykel, E., Eds.; John Wiley & Sons: New York, 1981, pp 189–205.

(7) Harfenist, M. Prevention of Ames Test Mutagenicity by Chemical Modification in a Series of Monoamine Oxidase Inhibitors. *J. Med. Chem.* **1980**, *23*, 825–827.

(8) White, H. L.; Harfenist, M.; Beek, O.; Soroko, F.; Cooper, B. R.; Maxwell, R. A. BW A616U-A Selective Inhibitor of MAO-A With Negligible Tyramine-potentiating Activity and Potential Antidepressant Activity. *Soc. Neurosci. Abstr.* **1985**, *11*, 442.

(9) White, H. L.; Harfenist, M.; Beek, O.; Kraemer, G. W.; Cooper, B. R. BW 1370U87—A Novel, Reversible, Selective Inhibitor of MAO-A with Negligible Tyramine-Potentiating Activity. *American College of Neuropsychopharmacology*, Puerto Rico, Dec 10–14, 1990; Abstract, p 133.

(Table I) caused a significant inhibition of MAO B, either in vitro or in rat brain after oral dosing.

A number of metabolites of **4a** were isolated from rat, dog, and monkey urine. Of these, the 1-(1-hydroxyethyl) (**4b**), 1-(2-hydroxyethyl) (**4c**), and 1-(1-vinyl) (**4f**) analogues had high MAO A-I, as shown in Table I, and the 1-(1,2-dihydroxyethyl) (**4d**) and 1-(1-acetyl) (**4e**) analogues had moderate potency. 1-Acetic acid **4g**, however, had essentially no MAO inhibition. None had detectable MAO B-I.

Our working hypothesis is that the inhibitory activity of these compounds is due to noncovalent binding to the flavin prosthetic group of the enzyme. Quantum mechanical calculations for a similar interaction have been published.[10]

Chemistry

Phenoxathiins were oxidized to the sulfones with hydrogen peroxide in acetic acid at or over a temperature of 95 °C. 1-Substituted derivatives were prepared by lithiation, generally using butyl lithium in tetrahydrofuran at temperatures below −30 °C and treating the lithio compound with the appropriate electrophile (Scheme I). Compound **4a** prepared by this procedure using ethyl iodide was contaminated with 1,9-diethyl homologue as well as smaller amounts of other impurities (possibly 4- or 6-ethylated), which required extensive chromatography to yield pure product. This compound therefore was best made by a two-step process involving reaction of the lithio compound with acetaldehyde to yield **4b**, followed by removal of the hydroxy group by means of Pearlman's $Pd(OH)_2$ on carbon catalyst[11] and hydrogen.

A general synthetic method (Scheme II) for preparing phenoxathiins with various substitution patterns involved formation of the diaryl sulfide from an *o*-nitroaryl halide and an *o*-hydroxythiophenol, followed by cyclization with displacement of the nitro function by the phenoxide oxygen. Various bromophenoxathiins were made by this method and converted by way of lithium-for-bromine exchange followed by electrophilic substitution to alkyl derivatives, and via boronates to hydroxy compounds. Migration to the 4-position was not observed. The products could then be oxidized to the 10,10-dioxides (after protection of any free hydroxyl function).

Biological Methods

MAO activities were determined by a double-label assay using tritiated serotonin and [^{14}C]-2-phenethylamine.[12] Ex vivo MAO inhibition was determined after removing rat brains 3 h after oral administration of the substance to be tested. Portions of a washed mitochondrial preparation from rat brain were incubated at 37 °C with [^{3}H]serotonin (0.19 mM, 5 Ci/mol) and [^{14}C]-β-phenethylamine (10 μM, 3 Ci/mol) in a total volume of 0.30 mL of 30 mM, pH 7.4, potassium phosphate buffer for 20 min. At these substrate concentrations, which are approximately twice the K_m concentrations at MAO A and MAO B sites, the activity with each substrate was independent of the other. Therefore it was possible to evaluate MAO A-I and MAO B-I simultaneously. This procedure gives IC_{50} values that approximate twice the K_i values for competitive inhibitors and has provided a reliable way to rank inhibitory potencies.[12] The 5-hydroxytryptophan potentiation test, the Porsolt test for antidepressant activity, and a mother-reared/peer-deprived Rhesus monkey test were performed following published methods.[13-15]

In summary, 1-ethylphenoxathiin 10,10-dioxide (**4a**, 1370U87) is a potent monoamine oxidase inhibitor selective for MAO A. It is reversibly bound to and displaceable from the enzyme. Compound **4a** gives positive results in standard antidepressant tests in rats and in a personality disorder paradigm in Rhesus monkeys. A negligible rise in blood pressure is found in rats ingesting tyramine after pretreatment with this compound. A number of other phenoxathiin dioxides also inhibit MAO A selectively, including five of the six isolated metabolites of this compound. This series is unique in having potent MAO inhibitors that contain no nitrogen.

(10) Sanchez-Marin, J.; Orti, E.; Aullo, J. M.; Tomas, F. Molecular Associations Between Lumiflavine and Some Beta-Carbolines. *Theochem.* **1985**, *21*, 21-27.
(11) Pearlman, W. M. Noble Metal Hydroxides on Carbon Nonpyrophoric Dry Catalysts. *Tetrahedron Lett.* **1967**, 1663-1664.
(12) White, H. L.; Glassman, A. T. Multiple Binding Sites of Human Brain and Liver Monoamine Oxidase: Substrate Specificities, Selective Inhibitions, and Attempts to Separate Enzyme Forms. *J. Neurochem.* **1977**, *29*, 987-997.
(13) Smith, L. M.; Peroutka, S. J. Differential Effects of 5-Hydroxytryptamine$_{1a}$ Selective Drugs on the 5-HT Behavioral Syndrome. *Pharmacol. Biochem. Behav.* **1986**, *24*, 1513-1519.
(14) Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. Behavioral Despair in Rats: A New Model Sensitive to Antidepressant Treatments. *Eur. J. Pharmacol.* **1978**, *47*, 379-391.
(15) Kraemer, G. W.; Ebert, M. H.; Schmidt, D. E.; McKinney, W. T. A Longitudinal Study of the Effect of Different Social Rearing Conditions on Cerebrospinal Fluid Norepinephrine and Biogenic Amine Metabolites in Rhesus Monkeys. *Neuropsychopharmacology* **1989**, *2*, 175-189.

† Division of Organic Chemistry.
‡ Current address: Sandoz Crop Protection, Zoecon Research Institute.
§ Division of Pharmacology.

Morton Harfenist,*,† Daniel P. C. McGee†,‡ Helen L. White*,§
Division of Organic Chemistry
Division of Pharmacology
Burroughs Wellcome Co.
Research Triangle Park
North Carolina 27709
Received May 28, 1991

Nitroheterocycle Reduction as a Paradigm for Intramolecular Catalysis of Drug Delivery to Hypoxic Cells

Bioreductive activation is a well-established concept for achieving selective toxicity of antitumor agents to hypoxic cells.[1-3] The reduction of nitroheterocycles such as nitroimidazoles[2] and nitroacridines[3] provides a widely ex-

(1) (a) Sartorelli, A. Therapeutic attack of hypoxic cells of solid tumors: Presidential address. *Cancer Res.* **1988**, *48*, 775-778. (b) See the collected papers in *Biochem. Pharmacol.* **1986**, *35*, 1-122.
(2) (a) Stratford, I. J.; O'Neill, P.; Sheldon, P. W.; Silver, A. R. J.; Walling, J. M.; Adams, G. E. RSU 1069, a nitroimidazole containing an aziridine group. *Biochem. Pharmacol.* **1986**, *35*, 105-109. (b) Mulcahy, R. T.; Carminati, A.; Barascut, J. L.; Imbach, J. L. Aerobic and hypoxic toxicity of a new class of mixed-function drugs associating nitroimidazoles and chloroethylnitrosourea in nitrosourea-sensitive (Mer$^-$) and -resistant (Mer$^+$) human tumor cells. *Cancer Res.* **1988**, *48*, 798-801. (c) Kirkpatrick, D. L. The development of hypoxic tumor cell cytotoxic agents. *Pharmacol. Ther.* **1989**, *40*, 383-399.
(3) Denny, W. A.; Atwell, G. J.; Anderson, R. F.; Wilson, W. R. Hypoxia-selective antitumor agents. 4. Relationships between structure, physicochemical properties, and hypoxia-selective cytotoxicity for nitracrine analogues with varying side chains: The "iminoacridan hypothesis". *J. Med. Chem.* **1990**, *33*, 1288-1295.

0022-2623/91/1834-2933$02.50/0