is performed at pH 9 and 25 °C and requires only minutes to accomplish, significant P–N bond cleavage does not occur during the assay.[11]

Registry No. 1, 74406-94-3; 2·DCHA, 97280-40-5; 3, 82180-41-4; 3·2K, 97280-41-6; 4, 97280-42-7; 5, 97280-42-7; 6, 97293-86-2; 6·2Na, 97293-85-1; 7, 97280-44-9; 8, 14295-48-8; 9, 97280-45-0; 10, 97280-46-1; 11, 97280-47-2; 12, 97280-48-3; 13, 86053-89-6; 13·2Na, 82184-87-0; 14, 97280-49-4; 15, 97280-51-8; 15·2K, 97280-50-7; 16, 76166-63-7; L-alanyl-L-proline benzyl ester hydrochloride, 41591-35-9; diphenyl phosphorochloridate, 2524-64-3; L-alanyl-L-proline *tert*-butyl ester hydrochloride, 41591-35-9; bis(4-nitrophenyl) phosphorochloridate, 6546-97-0; (2-phenylethyl)-phosphonic acid, 4672-30-4; phenyl phosphorodichloridate,, 770-12-7; angiotensin converting enzyme, 9015-82-1.

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Selective Thromboxane Synthetase Inhibitors. 1. 1-[(Aryloxy)alkyl]-1*H*-imidazoles

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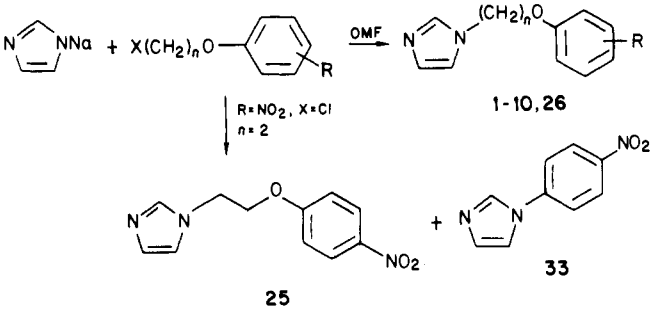
1-(2-Phenoxyethyl)-1*H*-imidazole was found to be an inhibitor of thromboxane (TxA_2) synthetase, but it also inhibited the adrenal cytochrome P-450 enzyme steroid 11β-hydroxylase. The preparation of a series of analogues is described, and activity against TxA_2 synthetase, PGI_2 synthetase, cyclooxygenase, and steroid 11β-hydroxylase is discussed. Potency against TxA_2 synthetase was increased by introduction of a carboxyl group at a suitable distance from the imidazole ring. A distance of 8.1–8.8 Å between N-1 of the imidazole and the carboxyl carbon was found to be optimal. Introduction of a carboxyl group also had the effect of reducing activity against steroid 11β-hydroxylase. The most potent and selective compound was found to be 4-[2-(1*H*-imidazol-1-yl)ethoxy]benzoic acid (14).

During the last decade, two new pathways of prostaglandin endoperoxide metabolism have been elucidated. First, Samuelsson et al.[1,2] reported that an enzyme in blood platelets, thromboxane synthetase, converts the endoperoxide PGH_2 into thromboxane A_2 (TxA_2). TxA_2 was found to be a potent vasoconstrictor and platelet aggregating agent and was thought to be involved in normal hemostasis.[3] In 1976, Vane et al.[4–9] reported the discovery of prostacyclin (PGI_2), which is produced from PGH_2 by an enzyme located in the endothelial lining of blood vessels. PGI_2 is a potent vasodilator and antiaggregatory agent. It was postulated that, under normal conditions, there is a balance between the opposing effects of TxA_2 and PGI_2, but in certain pathological situations the proaggregatory and vasoconstrictor actions of TxA_2 could predominate.[4–9] Thus, a compound that inhibits selectively the formation of TxA_2 may be useful in the treatment or prophylaxis of cardiovascular diseases where vasospasm or thrombosis plays a role. A potential advantage of such a compound would be that accumulated PGH_2 produced by platelet cyclooxygenase may be utilized by vascular PGI_2 synthetase, thereby increasing PGI_2 levels.[10,11] By contrast,

Scheme I



although agents that inhibit cyclooxygenase would also prevent TxA_2 formation, there would normally be no possibility for increased PGI_2 production since no PGH_2 would be produced.

Imidazole has been reported to be a weak inhibitor of TxA_2 synthetase.[12,13] We[14] and other workers[15,16] found that the introduction of a lipophilic 1-substituent increased inhibitory activity, but substitution elsewhere in the imidazole ring was detrimental. Imidazole inhibitors of cytochrome P-450 enzymes such as those present in liver and adrenals follow the same structure–activity trend,[17–22]

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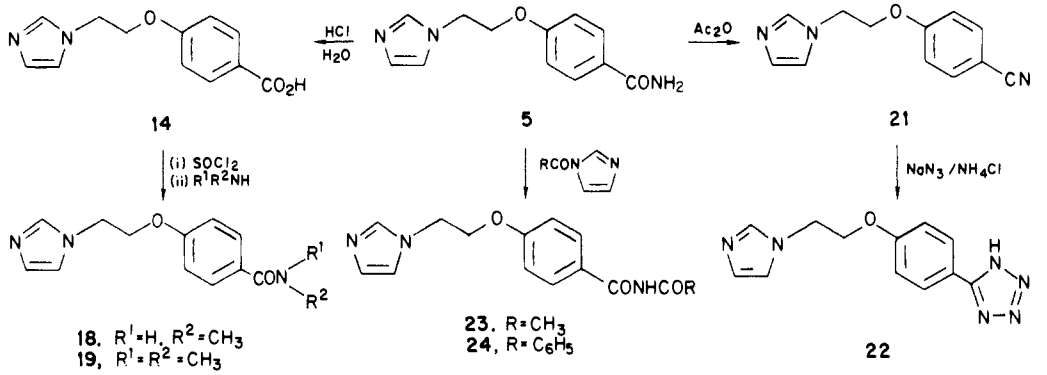
Scheme II



which led to the suggestion that TxA_2 synthetase may be a heme enzyme.[14,15] There is now good experimental evidence that TxA_2 synthetase is in fact a cytochrome P-450 enzyme,[23] and imidazole inhibitors presumably act by coordination of the basic nitrogen at a vacant axial ligand site of a heme unit.

We have reported previously[14] that 3-(1*H*-imidazol-1-ylmethyl)-2-(1-methylethyl)-1*H*-indole (UK-34,787) (**31**) was a potent inhibitor of TxA_2 synthetase without having a significant effect on PGI_2 synthetase or cyclooxygenase. However, further progression of this compound was precluded by the fact that it also inhibited other cytochrome P-450 enzymes. Thus, it was found to inhibit rat adrenal mitochondrial steroid 11β-hydroxylase, and liver enzyme inhibition was indicated by the fact that it prolonged pentobarbitone sleeping time in rats at doses required to cause complete inhibition of TxA_2 synthesis.[24]

1-(2-Phenoxyethyl)-1*H*-imidazole (**1**) was also found to be a more potent inhibitor of TxA_2 synthetase than imidazole, but it strongly inhibited steroid 11β-hydroxylase at 10^{-5} M (Table I). We decided to investigate whether introduction of polar substituents into the phenyl ring would decrease affinity for liver and adrenal cytochrome P-450, while maintaining activity against TxA_2 synthetase.

Chemistry. Several compounds were prepared by treatment of imidazole with NaH in DMF followed by an (aryloxy)alkyl chloride or bromide (Scheme I). This method was used for compounds **1–10**, **25**, and **26**. In the reaction of (4-nitrophenoxy)ethyl chloride a 26% yield of 1-(4-nitrophenyl)-1*H*-imidazole (**33**) was also formed by nucleophilic displacement of the ether side chain. Novel (aryloxy)ethyl chlorides were prepared by treatment of a phenol with NaH in DMF followed by [(toluenesulfonyl)oxy]ethyl chloride except where R = CH_2CONH_2 (**34**) in which case the reaction was carried out in 2-butanone in the presence of K_2CO_3. The latter conditions were also used to prepare **39** from ethyl 4-hydroxybenzoate and 1,3-dibromopropane.

Other compounds were prepared by modification of substituents. Hydrolysis of the esters **2**, **4**, and **8** under acidic conditions gave the acids **11**, **12**, and **17**, respectively. Basic hydrolysis of the esters **6** and **9** gave the acids **16** and **15**. The acid **13** was prepared by basic hydrolysis of the carbonitrile **10**.

The amide **5** was used as a starting material for the preparation of several other analogues (Scheme II). Thus, acid hydrolysis produced the acid **14**, which was then treated with $SOCl_2$ and either methylamine or dimethylamine to give the amides **18** and **19**. Dehydration with acetic anhydride gave the carbonitrile **21**, which gave the tetrazole **22** on reaction with NaN_3 and NH_4Cl. Treatment of **5** with acetyl- or benzoylimidazole gave low yields of the acylamides **23** and **24**.

The amide **20** was prepared by ammonolysis of the ester **8**. Reduction of the nitro analogue **25** with iron and HCl gave the amine **27**, which produced the urea **28** on treatment with KCNO. Chlorosulfonation of **1** followed by treatment with NH_3 gave the sulfonamide **29**.

Results and Discussion

1-(2-Phenoxyethyl)-1*H*-imidazole (**1**) is a more potent inhibitor of TxA_2 synthetase than imidazole (**30**). However, a marked increase in potency occurs on introduction of a 4-carboxyl group into the phenyl ring (**14**). A carboxyl group at the 3-position (**13**) gives no improvement in potency over **1**, and at the 2-position it is detrimental (**11**). Introduction of linking groups such as CH_2 (**16**) or OCH_2 (**17**) between the ring and the 4-carboxyl group or extension of the linkage between the rings (**15**) gives compounds with greater potency than **1** but slightly less than that of **14**. The acetic acid **12** is less potent than **1**, again showing the adverse effect of an acidic substituent at the 2-position. The amide **5** and tetrazole **22** show a substantial increase in potency over that of **1**, but all other substituents examined either have no significant effect or are detrimental.

Thus, most of the substituents that have a beneficial effect on potency are acidic, and the results suggest that there is an optimum distance between the imidazole ring and the carboxyl group. In the case of compound **14** the distance seems to be optimal as a reduction in potency occurs on increasing and, more markedly, on reducing the distance. Other workers have also noted the importance of the distance between the imidazole and carboxyl group for high activity against TxA_2 synthetase,[16,25,26] and an optimal distance of 8.5–9.0 Å between N-1 of the imidazole and the carboxyl carbon was suggested on the basis of examination of Dreiding models of a series of inhibitors.[26]

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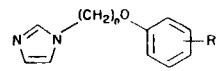
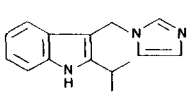
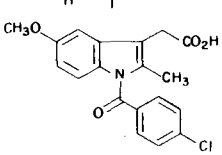
Table I. [(Aryloxy)alkyl]-1*H*-imidazoles



no.	R	*n*	mp, °C	yield, %	recrystn solvent[a]	formula[b]	IC_{50}, M: TxA_2 synthetase	PGI_2 synthetase	cyclooxygenase	11β-hydroxylase
1	H	2	86–87[c]	62	$CHCl_3$/PE		6.6×10^{-7}	$>10^{-4}$	6.7×10^{-5}	72% at 10^{-5}
2	2-$CO_2C_2H_5$	2	89–90.5	69	*i*-PrOH	$C_{14}H_{16}N_2O_3 \cdot C_4H_4O_4$[d]	4.4×10^{-6}	$>10^{-4}$		
3	2-$CONH_2$	2	147–148	38	2-butanone	$C_{12}H_{13}N_3O_2$	2.2×10^{-5}			
4	2-$CH_2CO_2C_2H_5$	2	129.5–130.5	46	*i*-PrOH/EtOAc	$C_{15}H_{18}N_2O_3 \cdot HCl$	2.0×10^{-6}			
5	4-$CONH_2$	2	148–149	51	EtOAc/MeOH	$C_{12}H_{13}N_3O_2$	5.6×10^{-8}	$>10^{-4}$	$>10^{-4}$	1.0×10^{-5}
6	4-$CH_2CO_2C_2H_5$	2	101.5–103	17.5	EtOAc	$C_{15}H_{18}N_2O_3 \cdot C_4H_4O_4$[e]	2.9×10^{-6}	$>10^{-4}$		
7	4-CH_2CONH_2	2	147–149	71	2-butanone	$C_{13}H_{15}N_3O_2$	1.8×10^{-6}			
8	4-$OCH_2CO_2C_2H_5$	2	97–98	55	EtOAc	$C_{15}H_{18}N_2O_4 \cdot C_4H_4O_4$[e,f]	6.1×10^{-7}			
9	4-$CO_2C_2H_5$	3	112–114	83	EtOH/Et_2O	$C_{15}H_{18}N_2O_3 \cdot HCl$	3.0×10^{-6}			
10	3-CN	2	126–128	56	*i*-PrOH/EtOAc	$C_{12}H_{11}N_3O \cdot HCl$	3.5×10^{-6}			
11	2-CO_2H	2	138–139.5	58	*i*-PrOH	$C_{12}H_{12}N_2O_3 \cdot HCl$	$>10^{-5}$			
12	2-CH_2CO_2H	2	146–147	85	*i*-PrOH	$C_{13}H_{14}N_2O_3 \cdot HCl$	$>10^{-5}$	$>10^{-4}$		
13	3-CO_2H	2	232–233	85	AcOH	$C_{12}H_{12}N_2O_3 \cdot HCl$	5.3×10^{-7}	$>10^{-4}$		$>10^{-4}$
14	4-CO_2H	2	239–241	87	AcOH	$C_{12}H_{12}N_2O_3 \cdot HCl$	3.0×10^{-9}	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
15	4-CO_2H	3	225–227	70.5		$C_{13}H_{14}N_2O_3$	2.7×10^{-8}			
16	4-CH_2CO_2H	2	163–164	57	EtOH	$C_{13}H_{14}N_2O_3$	3.2×10^{-8}			
17	4-OCH_2CO_2H	2	162–164	44	CH_3CN	$C_{13}H_{14}N_2O_4 \cdot HCl$	9.5×10^{-8}	$>10^{-4}$		$>10^{-4}$
18	4-$CONHCH_3$	2	132–133	74.5	EtOAc	$C_{13}H_{15}N_3O_2$	8.6×10^{-7}	$>10^{-4}$	$>10^{-4}$	
19	4-$CON(CH_3)_2$	2	113–115	43	EtOAc	$C_{14}H_{17}N_3O_2 \cdot C_4H_4O_4$[e]	1.1×10^{-6}	$>10^{-4}$	$>10^{-4}$	
20	4-OCH_2CONH_2	2	123–124	42	2-butanone	$C_{13}H_{15}N_3O_3$	8.5×10^{-7}			$>10^{-4}$
21	4-CN	2	112–114	49	EtOH/Et_2O	$C_{12}H_{11}N_3O \cdot C_4H_4O_4$[g]	1.3×10^{-5}			
22	4-CHN_4[h]	2	196–197	34	EtOH/H_2O	$C_{12}H_{12}N_6O$	1.5×10^{-8}	$>10^{-4}$		
23	4-$CONHCOCH_3$	2	164–165	13	2-butanone	$C_{14}H_{15}N_3O_3$	6.3×10^{-7}			
24	4-$CONHCOC_6H_5$	2	152–154	10	MeOH/EtOAc	$C_{19}H_{17}N_3O_3$	1.8×10^{-7}			
25	4-NO_2	2	56–57	36.5[i]	EtOAc/PE	$C_{11}H_{11}N_3O_3$	$>5 \times 10^{-5}$			
26	4-$NHCOCH_3$	2	168–170	62	EtOAc	$C_{13}H_{15}N_3O_2$	4.0×10^{-6}	$>10^{-4}$		
27	4-NH_2	2	91–93	69	EtOAc/PE	$C_{11}H_{13}N_3O$	$>5 \times 10^{-5}$			
28	4-$NHCONH_2$	2	199–201	82.5	H_2O	$C_{12}H_{14}N_4O_2$	8.4×10^{-7}			
29	4-SO_2NH_2	2	147.5–148.5	19	EtOH	$C_{11}H_{13}N_3O_3S$	$>5 \times 10^{-5}$			
30	imidazole						6.7×10^{-4}[j]	4.0×10^{-3}[j]	$>10^{-2}$	
31	 (UK-34.787)						1.5×10^{-8}[k]	$>10^{-4}$[j]	$>10^{-4}$	2.0×10^{-6}
32	 (indomethacin)						$>10^{-4}$		1.5×10^{-7}	

[a] PE, petroleum ether; bp 60–80 °C. [b] All compounds were analyzed for C, H, and N. [c] Lit.[32] mp 87–88 °C. [d] Maleate. [e] Fumarate. [f] C: calcd, 56.15; found, 55.65. [g] Hemifumarate. [h] 5-Tetrazolyl. [i] 1-(4-Nitrophenyl)-1*H*-imidazole (26%) also formed. [j] Result obtained by bioassay method.[14] [k] Previously reported bioassay figure: 1.7×10^{-8} M.[14]

Preferred conformations of 14 were calculated by using the CHEMLAB molecular modelling system,[27] and N-1 to carboxyl carbon distances were measured for conformers within 3 kcal of the minimum. In this case a range of 8.1–8.8 Å was found. There is presumably a specific binding site near the active site of the enzyme to accommodate the carboxyl group of the PGH_2 substrate, and it seems reasonable to suppose that the carboxyl group of 14 interacts with this site.

None of the compounds examined had any significant effect on PGI_2 synthetase at 10^{-4} M. Compound 14 and the amide derivatives 5, 18, and 19 also had no effect on cyclooxygenase at 10^{-4} M, although the unsubstituted compound 1 showed some activity at 10^{-5} M. Activity against cyclooxygenase has been reported previously for imidazoles with *N*-alkyl groups containing greater than six carbons.[16] Compound 1 also caused marked inhibition of rat adrenal mitochondrial steroid 11β-hydroxylase at 10^{-5} M, but introduction of a carboxylic acid substituent as in 13, 14, and 17 virtually abolished this activity. The amide 5 retained activity against 11β-hydroxylase, but activity was reduced in 20.

Because of its potency and selectivity, compound 14 (UK-37,248, dazoxiben) was selected for further evaluation. Fifteen minutes after intravenous administration of 0.3 mg/kg to anaesthetized rabbits, thromboxane production was inhibited by 75%.[28] The same dose of the acids 15 and 17 and the tetrazole 22 produced less than 30% inhibition after the same time. Oral administration of 3 mg/kg of 14 to conscious dogs produced a high level of inhibition of thromboxane production for up to 6 h,[28] and a similar picture was seen after oral administration of 100- or 200-mg doses to human volunteers.[29] Unlike 31, there is no evidence for inhibition of other cytochrome P-450 enzymes in vivo.

In summary, we have found that in a series of *N*-[(aryloxy)alkyl]-1*H*-imidazoles inhibitory activity against thromboxane synthetase can be increased by introduction of a carboxylic acid group at a suitable distance from the imidazole ring. In all the cases examined, the carboxyl group has the additional advantage of reducing activity against other cytochrome P-450 enzymes such as adrenal steroid 11β-hydroxylase. None of the substituted [(aryloxy)alkyl]imidazoles examined had significant activity against either PGI_2 synthetase or cyclooxygenase.

Experimental Section

Enzyme Assays. (a) Cyclooxygenase. Cyclooxygenase was prepared from ram seminal vesicle microsomes (RSVM) by the method of Takeguchi et al.[30] Test compounds or solvents were preincubated with RSVM (50–100 μg) protein and 50 mM sodium phosphate buffer (pH 7.8) for 5 min prior to addition of 0.1 mM arachidonic acid (AA) ammonium salt (total volume 0.2 mL). After incubation for a further 1 min at 22 °C, the PGH_2 formed was determined by bioassay using a rabbit aorta spiral to measure contraction.[14]

(b) Thromboxane Synthetase. TxA_2 synthetase was prepared from human platelets by the method of Needleman et al.[3] Pretreatment of the platelet microsomes with indomethacin (30 μM) for 30 min at 0 °C was carried out to inactivate the cyclooxygenase also present. The substrate PGH_2 was generated immediately prior to each assay by incubating 0.1 mM AA with RSVM for 1 min at 22 °C and rapidly cooling the incubate to 0 °C. Compounds or solvents were preincubated for 2 min in ice with the indomethacin-pretreated microsomes (100–200 μg of protein) and 20 mM sodium phosphate buffer (pH 7.8). The generated PGH_2 (0.05 mL) was added (total volume 0.105 mL), and incubation was continued for 1 min in ice before termination by addition of 0.5 mL of EtOH.

The incubation mixtures were centrifuged for 2 min, aliquots of the supernatant were diluted (1:20) with Isogel Tris buffer (10 mM Tris/140 mM NaCl/0.1% w/v gelatin, pH 7.4), and 0.1-mL aliquots of the diluted samples were assayed in triplicate for TxB_2 with a specific RIA.[27]

Incubations were carried out at different concns of test compounds to enable construction of dose–response curves.

(c) PGI_2 Synthetase. PGI_2 synthetase was prepared from porcine aorta by the method of Moncada et al.[4] The PGH_2 synthetase present was deactivated with indomethacin as described in (b). Incubations were carried out at 22 °C and pH 7.5, and incubation with PGH_2 was carried out for 30 s before termination with EtOH. Otherwise the procedure was identical with that described in (b). After centrifugation and dilution of the supernatant (1:50), 0.1-mL aliquots were assayed in triplicate for 6-keto $PGF_{1\alpha}$ with a specific RIA.[27]

(d) Steroid 11β-Hydroxylase. The adrenals of three male rats (Charles River strain) were homogenized in 33 vol of medium (10 mM HEPES/250 mM sucrose/2 mM EGTA, pH 7.4), and the homogenate was centrifuged at 900*g* for 10 min. The pellet was resuspended in the same volume of medium and centrifuged as before at 2–4 °C. The combined supernatants were centrifuged at 10000*g* for 15 min at 2–4 °C. The mitochondrial pellet was resuspended in the original volume of homogenizing medium and recentrifuged at 10000*g* for 15 min. The washed mitochondria were suspended in 20 vol of homogenizing medium and used within 30 min.

The incubation mixture consisted of 10 mM HEPES, pH 7.4, 250 mM sucrose, 1 mM $MgCl_2$, 5 mM KH_2PO_4, 4 mM sodium malate, 50 μM 4-[^{14}C]-11-deoxycorticosterone (sp act. 0.1 μCi/μM), and test drug in a total volume of 1 mL. After a 5-min preincubation at 37 °C in a shaking water bath, mitochondria suspension (100 μL) was added and the mixture was incubated for 10 min after which 5 mL of CH_2Cl_2 was added. Unlabeled 11-deoxycorticosterone and corticosterone were added (20 μg), and the contents were transferred to capped tubes with 0.5 mL of H_2O and 1 mL of CH_2Cl_2. The tubes were rotated for 20 min and then centrifuged at 1000*g* for 5 min at room temperature. The lower CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated under N_2. The residue was redissolved in 75 μL of CH_2Cl_2 and applied to silica TLC plates that were developed in $CHCl_3$/MeOH (97:3 v/v). The areas corresponding to substrate and product were scraped into scintillation vials, and after addition of 1 mL of MeOH and 10 mL of toluene Omnifluor scintillant, the radioactivity in the samples was counted.

Chemistry. All melting points are uncorrected and were obtained on an Electrothermal capillary melting point apparatus. The structures of all compounds were confirmed by their IR and 60-MHz NMR spectra. The IR spectra were recorded on a Perkin-Elmer 197 or 237 spectrophotometer, and the ^{1}H NMR spectra were obtained on a Perkin-Elmer R12B spectrometer using Me_4Si as internal standard.

[4-(2-Chloroethoxy)phenyl]acetamide (34). A mixture of (4-hydroxyphenyl)acetamide (98.8 g, 0.65 mol), [(toluenesulfonyl)oxy]ethyl chloride (150.5 g, 0.65 mol), anhydrous K_2CO_3 (98.0 g, 0.7 mol), and 2-butanone (2 L) was heated under reflux with stirring for 16 h and then cooled and filtered. The filtrate was evaporated to give an oily solid that was stirred with a small volume of ether, and the mixture was filtered. The insoluble residue from the reaction mixture was washed with water, combined with the other solid, and crystallized from ethanol to give 34: yield 70.0 g (55%); mp 173–175 °C. Anal. ($C_{10}H_{12}ClNO_2$) C, H, N.

3-(2-Chloroethoxy)benzonitrile (35). Sodium hydride (2.40 g, 0.05 mol of 50% dispersion in mineral oil) was added portionwise to a stirred solution of 3-cyanophenol (5.95 g, 0.05 mol) in dry DMF (50 mL), and the mixture was warmed to 100 °C for 10 min and then cooled. [(Toluenesulfonyl)oxy]ethyl chloride (11.75 g,

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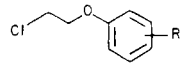
Table II. Physical Data for (2-Chloroethoxy)aryl Intermediates



no.	R	mp or bp, °C	% yield	mol formula[a]
36	2-$CO_2C_2H_5$	167–71/12mm	51	$C_{11}H_{13}ClO_3$
37	4-$CH_2CO_2C_2H_5$	70–72[b]	66	$C_{12}H_{15}ClO_3$
38	4-$OCH_2CO_2C_2H_5$	138–40/0.1mm	37	$C_{12}H_{15}ClO_4$

[a] All compounds were analyzed for C and H. [b] From 60–80 °C petroleum ether.

0.05 mol) was added, and the mixture was heated on a steam bath for 3 h and then cooled and poured into water (ca. 250 mL). The mixture was extracted several times with ether, and the combined extracts were washed well with water and dried (Na_2SO_4). Filtration and evaporation of the solvent gave an oil that was chromatographed on silica gel. Elution with hexane gave mineral oil, and further elution with ether/hexane (1:1) gave pure product. The product-containing fractions were combined and evaporated, and the residue was distilled to give **35**: yield 4.14 g (46%); bp 141–143 °C (0.6 mm); mp 42–44 °C. Anal. (C_9H_8ClNO), C, H, N.

Other novel (2-chloroethoxy)aryl intermediates were prepared similarly, and physical data are summarized in Table II.

Ethyl 4-(3-Bromopropoxy)benzoate (39). A mixture of ethyl 4-hydroxybenzoate (8.80 g, 0.05 mol), 1,3-dibromopropane (15.14 g, 0.075 mol), anhydrous K_2CO_3 (15 g), and 2-butanone (150 mL) was heated under reflux with stirring for 72 h. The mixture was cooled and filtered, and the residue was washed well with 2-butanone. The combined filtrate and washings were evaporated to give an oil that was dissolved in ethyl acetate. The solution was washed with 1 N NaOH and water and dried (Na_2SO_4). Filtration and evaporation gave an oil that was distilled to give **39**: yield 5.69 g (40%); bp 128–132 °C (0.1 mm). Anal. ($C_{12}H_{15}BrO_3$) C, H.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzamide (5). Sodium hydride (14.4 g, 0.3 mol of 50% dispersion of mineral oil) was added portionwise to a stirred solution of imidazole (20.4 g, 0.3 mol) in dry DMF (100 mL), and the mixture was warmed to 100 °C for 10 min. A solution of 4-(2-chloroethoxy)benzamide (60.0 g, 0.3 mol) in the minimum volume of dry DMF was added, and the mixture was heated on a steam bath for 5.5 h. The solvent was evaporated, and the residue was poured into water. The mixture was extracted several times with chloroform, and the combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was triturated with a little ether to remove mineral oil, and the solid was crystallized from ethyl acetate containing a trace of methanol to give **5**: yield 35.2 g (51%); mp 148–149 °C. Anal. ($C_{12}H_{13}N_3O_2$), C, H, N.

This method was used for the preparation of compounds **1–4**, **6–10**, **25**, and **26**. In most cases the crude product was purified by chromatography (silica gel/$CHCl_3$).

4-[2-(1*H*-Imidazol-1-yl)ethoxy]nitrobenzene (25). Treatment of imidazole (6.80 g, 0.1 mol) with NaH (4.8 g of 50% dispersion, 0.1 mol) followed by 4-(2-chloroethoxy)nitrobenzene (20.15 g, 0.1 mol) in dry DMF (100 mL) by the above method gave a crude product that was chromatographed on silica gel. Elution with EtOAc first gave mineral oil followed by a solid that was crystallized from MeOH to give **33**: yield 4.90 g (26%); mp 205–206 °C (lit.[31] mp 204–205 °C). Anal. ($C_9H_7N_3O_2$), C, H, N.

Further elution with EtOAc gave a solid that was crystallized from EtOAc/PE to give **25**: yield 8.50 g (36.5%); mp 56–57 °C. Anal. ($C_{11}H_{11}N_3O_3$), C, H, N.

2-[2-(1*H*-Imidazol-1-yl)ethoxy]benzoic Acid Hydrochloride (11). A solution of **2** (5.10 g, 0.02 mol) in 5 N HCl (30 mL) was heated on a steam bath for 8 h and then evaporated. The residue was crystallized from 2-propanol to give 11·HCl: yield 3.08 g (58%); mp 138–139 °C. Anal. ($C_{12}N_{12}N_2O_3$·HCl), C, H, N.

This method was used to prepare compounds **12** and **17** (Table I) from the esters **4** and **8**, respectively.

4-[3-(1*H*-Imidazol-1-yl)propoxy]benzoic Acid (15). A mixture of **9** (free base) (0.49 g, 0.0018 mol), KOH (0.20 g), ethanol (1 mL), and water (5 mL) was heated under reflux for 2 h and then evaporated to small bulk. The solution was filtered, and the filtrate was acidified with acetic acid. The precipitate was filtered off, washed with water, and dried to give **15**: yield 0.31 g (70.5%); mp 225–227 °C. Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

This method was also used for the preparation of **16** from the ester **6**.

3-[2-(1*H*-Imidazol-1-yl)ethoxy]benzoic Acid (13). A mixture of compound 10·HCl salt (2.0 g, 0.008 mol) and 5 N NaOH (15 mL) was heated on a steam bath for 6 h. The solution was made acidic with dilute HCl and evaporated. The residue was extracted with hot acetic acid, and the solution was filtered and evaporated. The residue was crystallized from acetic acid to give 13·HCl: yield 1.82 g (85%); mp 232–233 °C. Anal. ($C_{12}H_{12}N_2O_3$·HCl) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzoic Acid Hydrochloride (14). A solution of **5** (4.50 g, 0.019 mol) in 5 N HCl (20 mL) was heated on a steam bath for 2 h and then allowed to cool. The solid was filtered off and crystallized from acetic acid to give 14·HCl: yield 4.55 g (87%); mp 239–241 °C. Anal. $C_{12}H_{12}N_2O_3$·HCl C, H, N.

***N*-Methyl-4-[2-(1*H*-imidazol-1-yl)ethoxy]benzamide (18).** Thionyl chloride (1.0 mL) was added dropwise to a stirred mixture of 14·HCl (1.0 g, 0.0037 mol) and dry DMF (5 mL) at room temperature. The solution was stirred for 5 h and then added dropwise to a stirred solution of 40% aqueous methylamine (20 mL). The mixture was evaporated, the residue was dissolved in water, and the solution was made just basic with $NaHCO_3$ and then reevaporated. The residue was extracted with hot ethyl acetate, and the extract was filtered and evaporated. The residue was crystallized from ethyl acetate to give **18**: yield 0.68 g (74.5%); mp 132–133 °C. Anal. ($C_{13}H_{15}N_3O_2$) C, H, N.

Compound **19** (Table I) was prepared similarly.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzonitrile (21). A solution of **5** (1.0 g, 0.0043 mol) in acetic anhydride (5 mL) was heated under reflux for 3 h and then evaporated. The residue was dissolved in ethyl acetate, and the solution was washed with $NaHCO_3$ solution and then dried (Na_2SO_4). Evaporation of the solvent gave an oil that was chromatographed on silica gel. Elution with $CHCl_3$ first gave some impurity followed by pure **21** as an oil: yield 0.45 g (49%); IR 2220 cm^{-1} (C≡N). A portion of the oil was dissolved in a small volume of EtOH, and an excess of an ether solution of fumaric acid was added. The solid was filtered off and crystallized from EtOH/Et_2O to give **21**·$^1/_2C_4H_4O_4$, mp 112–114 °C. Anal. ($C_{12}H_{11}N_3O_2$·$^1/_2C_4H_4O_4$) C, H, N.

5-[4-[2-(1*H*-Imidazol-1-yl)ethoxy]phenyl]-1*H*-tetrazole (22). A mixture of **21** (2.1 g, 0.0092 mol), NaN_3 (3.25 g, 0.05 mol), and NH_4Cl (2.67 g, 0.02 mol) in dry DMF (25 mL) was heated on a steam bath for 22 h and then evaporated. The residue was extracted with $CHCl_3$, and the extract was evaporated. The residue was crystallized from aqueous EtOH to give **22**: yield 0.81 g (34%); mp 196–197 °C. Anal. ($C_{12}H_{12}N_6O$) C, H, N.

***N*-Acetyl-4-[2-(1*H*-imidazol-1-yl)ethoxy]benzamide (23).** Acetic acid (1.80 g, 0.03 mol) was added to a solution of 1,1′-carbonylbis(1*H*-imidazole) (4.86 g, 0.03 mol) in dry DMF (10 mL), and the solution was stirred for 5 min. **5** (4.60 g, 0.02 mol) was added, and the solution was heated under reflux for 3 h and then evaporated. The residue was treated with $NaHCO_3$ solution, and the mixture was extracted several times with ethyl acetate. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated to give an oil that was chromatographed on silica gel. Elution with $CHCl_3$ gave some impurity, and further elution with $CHCl_3$/MeOH (50:1) gave a solid that was crystallized from 2-butanone to give **23**: yield 0.90 g (13%); mp 164–165 °C. Anal. ($C_{14}H_{15}N_3O_3$) C, H, N.

This method was also used for the preparation of **24** using benzoic acid in place of acetic acid.

[4-[2-(1*H*-Imidazol-1-yl)ethoxy]phenoxy]acetamide (20). A mixture of **8** (1.0 g, 0.0034 mol), ethanol (10 mL), and concentrated ammonia solution (20 mL) was allowed to stand at room temperature for 20 h, and the resulting solution was evaporated. The residue was crystallized from 2-butanone to give **20**: yield 0.38 g (42%); mp 123–124 °C. Anal. ($C_{13}H_{15}N_3O_3$) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzeneamine (27). Iron powder (6.0 g) was added portionwise to a warm solution of **25**

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(3.70 g, 0.016 mol) in 5 N HCl (60 mL), and the mixture was warmed on a steam bath for 20 min. It was then cooled and made basic with dilute NaOH solution, and the mixture was shaken with $CHCl_3$ and filtered. The $CHCl_3$ layer of the filtrate was separated, dried (Na_2SO_4), and evaporated. The residue was crystallized from ethyl acetate/petroleum ether (bp 60–80 °C) to give **27**: yield 2.25 g (69%); mp 91–93 °C. Anal. ($C_{11}H_{13}N_3O$) C, H, N.

[4-[2-(1*H*-Imidazol-1-yl)ethoxy]phenyl]urea (28). A solution of KCNO (0.30 g, 0.0037 mol) in water (1 mL) was added to a solution of **27** (0.60 g, 0.0029 mol) in 1 N HCl (3.0 mL). The mixture was allowed to stand at room temperature for 15 min, and the precipitate was filtered off, washed with water, and crystallized from water to give **28**: yield 0.60 g (82.5%); mp 199–201 °C. Anal. ($C_{12}H_{14}N_4O_2$) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzenesulfonamide (29). PCl_5 (2.08 g, 0.01 mol) was added cautiously to chlorosulfonic acid (2.91 g, 0.025 mol), and the mixture was cooled to 0 °C. **1** (1.88 g, 0.01 mol) was added portionwise, and then the mixture was heated on a steam bath for 10 min and cooled. It was poured onto a mixture of crushed ice and excess concentrated NH_3 solution, and the gummy solid was filtered off and sucked dry. The solid was chromatographed on silica gel. Elution with $CHCl_3$/MeOH (4:1) gave a gum that crystallized on trituration with a little EtOH. The solid was crystallized from EtOH to give **29**: yield 0.50 g (19%); mp 147.5–148.5 °C; 60-MHz ^1H NMR (Me_2SO-d_6) 4.37 (m, 2 H, CH_2), 4.45 (m, 2 H, CH_2), 6.95 (s, 1 H, imidazole H^5), 7.14 (d, J = 8.9 Hz, 2 H, benzene CH), 7.28 (s, 2 H, SO_2NH_2), 7.30 (s, 1 H, imidazole H^4), 7.74 (s, 1 H, imidazole H^2), 7.79 (d, J = 8.9 Hz, 2 H, benzene CH). Anal. ($C_{11}H_{13}N_3O_3S$) C, H, N.

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Registry No. 1, 30170-83-3; **2** maleate, 75912-61-7; **3**, 75912-58-2; **4**·HCl, 75912-62-8; **5**, 75912-57-1; **6** fumarate, 75912-66-2; **7**, 75912-59-3; **8** fumarate, 75912-68-4; **9**, 74226-04-3; **9**·HCl, 97315-33-8; **10**·HCl, 75922-17-7; **11**·HCl, 75912-72-0; **12**·HCl, 75912-73-1; **13**·HCl, 75912-75-3; **14**·HCl, 74226-22-5; **15**, 75912-95-7; **16**, 97315-34-9; **17**·HCl, 75922-18-8; **18**, 75912-78-6; **19** fumarate, 75912-80-0; **20**, 75912-99-1; **21**, 75912-76-4; **21** $^1/_2$ fumarate, 97315-35-0; **22**, 75912-77-5; **23**, 75912-90-2; **24**, 75912-91-3; **25**, 75912-69-5; **26**, 75912-70-8; **27**, 75912-83-3; **28**, 75912-85-5; **29**, 75912-82-2; **30**, 288-32-4; **33**, 2301-25-9; **34**, 97315-36-1; **35**, 97315-37-2; **36**, 97315-38-3; **37**, 97315-39-4; **38**, 97315-40-7; **39**, 74226-00-9; $4\text{-}HOC_6H_4CH_2CONH_2$, 17194-82-0; $4\text{-}CH_3C_6H_4SO_2O(CH_2)_2Cl$, 80-41-1; $3\text{-}HOC_6H_4CN$, 873-62-1; $4\text{-}HOC_6H_4CO_2C_2H_5$, 120-47-8; $Br(CH_2)_3Br$, 109-64-8; $4\text{-}Cl(CH_2)_2OC_6H_4CONH_2$, 36616-30-5; $4\text{-}Cl(CH_2)_2OC_6H_4NO_2$, 3383-72-0; CH_3NH_2, 74-89-5; $(CH_3)_2NH$, 124-40-3; CH_3CO_2H, 64-19-7; $C_6H_5CO_2H$, 65-85-0; NH_3, 7664-41-7; $ClSO_3H$, 7790-94-5; $2\text{-}HOC_6H_4CO_2C_2H_5$, 118-61-6; $4\text{-}HOC_6H_4CH_2CO_2C_2H_5$, 17138-28-2; $4\text{-}HOC_6H_4OCH_2CO_2C_2H_5$, 20872-28-0; TxA_2 synthetase, 60832-04-4; PGI_2 synthetase, 65802-86-0; cyclooxygenase, 39391-18-9; steroid 11β-hydroxylase, 9029-66-7.

Studies on the 1-Methyl-4-phenyl-2,3-dihydropyridinium Species 2,3-MPDP$^+$, the Monoamine Oxidase Catalyzed Oxidation Product of the Nigrostriatal Toxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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The nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is biotransformed by brain monoamine oxidase (MAO) to an unstable dihydropyridinium intermediate that reacts with cyanide ion to form an α-cyano-tetrahydropyridine adduct and, in the absence of cyanide ion, undergoes disproportionation to the 1-methyl-4-phenylpyridinium species MPP$^+$ and MPTP. Comparison of the HPLC retention times, diode array UV, and chemical ion mass spectral characteristics of these products with those of synthetic standards led us to propose the 1-methyl-4-phenyl-2,3-dihydropyridinium species 2,3-MPDP$^+$ and 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine as tentative structure assignments for the dihydropyridinium metabolite and the cyano adduct, respectively. Results presented in this paper confirm the first assignment and establish that, although the proposed 6-cyano adduct is initially formed, the product that was isolated from the mitochondrial incubation mixtures of MPTP and sodium cyanide actually is the isomeric 2-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. On the basis of the selective incorporation of deuterium into these products, we provide rational mechanistic interpretations of the disproportionation reaction and the rearrangement of the cyano adducts. These results establish that the MAO-catalyzed bioactivation of MPTP leads to the formation of a variety of reactive molecules that are potentially cytotoxic to nigrostriatal cells.

The cyclic allylamine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**) causes the selective degeneration of the dopaminergic nigrostriatal system in a number of species including mice,[1-3] monkeys,[4,5] and humans[6,7] and leads to a Parkinsonian-like syndrome in monkeys and humans. Both in vivo[8] and in vitro[9] studies have led to the characterization of the 1-methyl-4-phenylpyridinium species MPP$^+$ (**3**$^+$) as a principal brain metabolite of the

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