Selective Inhibitors of Monoamine Oxidase. 2.[1] Arylamide SAR

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Monoamine oxidase (MAO) exists in two forms distinguishable by substrate specificity. Inhibition of MAO A is believed to be responsible for the antidepressant activity of MAO inhibitors. A group of *N*-arylacetamides are highly specific inhibitors of MAO A, some with IC_{50} values in the 10–100 nM range. The requirements for high activity and specificity include a nearly linear tricyclic aromatic portion but a larger and a smaller central ring component. The amide group, which is best acetamido, is optimally placed para to the smaller central group. The size and shape of the aromatic moiety appear to be the major influence on activity and specificity for MAO A.

Monoamine oxidase (MAO) (EC 1.4.3.4, amine oxidase, flavin containing) consists of two different forms that are distinguishable by their substrate specificity[2] and their amino acid sequence.[3] Ratios of MAO A to MAO B of ca. 1 and 2 for human brain and liver, respectively, have been reported.[4,5] 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,[6] 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, such as moclobemide and brofaromine for MAO A inhibition (MAO A-I) and deprenyl for MAO B-I.[7]

MAO A-I is believed to lead to a clinically useful antidepressant effect, while MAO B-I does not.[8] MAO B-I only serves in this context as a source of possible side effects and therefore is undesirable. An additional safety factor could be provided by having the inhibitor reversibly bound to MAO A and displaceable by tyramine so that tyramine at high concentrations would reactivate the MAO A to add to the tyramine oxidation by MAO B. An inhibitor with this combination of selectivity and displaceability by tyramine was the target of our work. An earlier report[9] mentioned 2-acetamido-9*H*-carbazole (**2**), which was selective for MAO A and had an IC_{50} for MAO A-I of 0.04 μM. Although it met our criteria, **2** was suspected, because of its structure, of being a carcinogen. A literature report indicated no excess of tumors over control values on a 6-month feeding of **2** to rats. However, we found that the Ames test with **2** was strongly positive. Attempts to develop safe and effective inhibitors starting from **2** took three paths. The first, based on the ready availability of a variety of arylamines and nitroarenes easily convertible to the arylamides, was to study SAR in this series, in the hope that this rapidly developable SAR would be transferable to other series. That is the subject of this paper. A second approach was to attempt to remove potential carcinogenicity by structural modification of the arene moiety. Success in removing Ames test mutagenicity has been reported.[9] However, the resulting arylamides were still subject to suspicion because of their structural similarity to known carcinogens. A third line was to study replacement of the amide function by other hydrophilic groups. This will be the subject of a future report.

Results and Discussion

Results given in the tables support the following conclusions for the SAR of MAO inhibitory activity of arylamides.

1. Tricyclic systems are necessary though not sufficient to confer high activity. Thus the selection of varied bicyclic (Table 4) and monocyclic (Table 5) *N*-arylacetamides shows none with appreciable MAO inhibition at or below a 1 μM concentration level. In the examples that we have examined, compounds with IC_{50} values above 1 μM have shown low specificity for MAO A.

2. High activity combined with selectivity in inhibiting MAO A with little effect on MAO B activity is found in both aromatic 6:5:6 (Table 1, compounds **2**, **13**) and 6:6:6 tricyclics (Table 2, compounds **26–30**, **32**, and **37**) with relatively linear structure (but see 5 below).

3. The limited number of angular 6:6:6 compounds in Table 3 show negligible MAO inhibition, with the exception of the benzocoumarin **39**. The reason for this exceptional behavior of **39** is not obvious.

4. The tricyclics in which one outer ring is not aromatic (Table 1, compounds **23** compared with **2**, and **24**) showed low or no MAO I activity.

5. MAO A inhibition is maximized, and specificity toward MAO A vs MAO B inhibition appears to increase, when the center ring of the tricyclic structure has a larger and a smaller group and the amide function is para to the smaller group. Thus in Table 1, **2**, which has its acetamide moiety para to the single bond (calculated carbon–carbon distance of 1.41 Å) is far more active than **4**, which has its amide function para to the –N– function (calculated C-1–C-8 distance of 2.27 Å). Analogously, in Table 2, **27** (with acetamide function para to the C=O) is more potent than **26** (acetamide para to SO_2) and appears to be more specific

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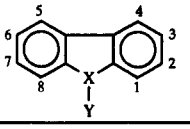
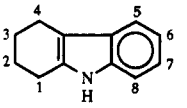
Table 1. Monoamine Oxidase Inhibition in 6:5:6 Systems



compd. no.	X	Y	2	3	other	IC_{50} (μM) or %I at μM MAO A	MAO B	formula	anal.	mp (°C)	recryst solvent[a]
1	N	H	NHCHO[b]			1.1	ND	$C_{13}H_{10}N_2O$	CHN	244[b]	A
2	N	H	NHAc			0.043	38% at 10	c			
3	N	Me	NHAc			0.03	ND	c			
4	N	H	H	NHAc		22% at 10	37% at 10	d			
5	N	H	NHCOPr			36% at 1	ND	$C_{16}H_{16}N_2O$	CHN	258	A
6	N	H	$NHCONH_2$[i]			74% at 1	26% at 1	$C_{13}H_{11}N_3O$	CHN	165–170	A-W
7	N	H	NHAc		6-Br	61% at 1	ND	$C_{14}H_{10}BrN_2O$	CHN	277–278	A
8	N	$(CH_2)_3$[f] MePip	NHAc			80% at 10	ND	$C_{22}H_{30}Cl_2N_4O_3$[f]	CHN	208–211	A-E
9	N	CH_3		CH_2NHAc[g]		16% at 1	30% at 1	$C_{16}H_{18}N_2O$[g]	CHN	177–179	A
10	N	CH_3		$(CH_2)_2NHAc$[h]		50% at 1	0% at 1	$C_{17}H_{18}N_2O$[h]	CHN	118–119	A-E
11	N	H	CHMeNHAc[i]			7% at 1	20% at 1	$C_{16}H_{16}N_2O$	CHN	217–218	A-W
12	O		NHAc[a]			0.3	ND	*j*			
13	C=O		NHAc			0.04	3% at 1	*k*			
14	C=O		NHAc		7-NO_2	1.3	ND	*l*			
15	CHOH		NHAc			0.3	ND	*k*			
16	SO_2			NH_2[m]		1.0	ND	*m,n*			
17	SO_2			NHCHO[m,o]		1.0	ND	$C_{13}H_8NO_3S$	CHN	257–258	DMF-A
18	SO_2			NHAc[m]		0.26	13% at 1	*p*			
19	SO_2			NHCOEt[m]		1.2	ND	$C_{15}H_{13}NO_3S$	CHN	273–274	A
20	SO_2			NHAc[m]	7-NHAc	6.6% at 1	ND	*q*			
21	SO_2			NHMe[m]		34% at 1	ND	$C_{13}H_{12}ClNO_3S$	CHN	263–264	Ac
22	SO_2			NMeAc[m]		0% at 1	ND	$C_{15}H_{15}NO_3S$[r]	CHN	200–201	A
											
23			H_2	H_2	7-NHAc	35% at 1	0 at 1	*s*			
24			H_2	HCOMe	7-NHAc	0 at 1	11% at 1	*t*			

ND: inhibition of MAO B was not done. [a] Recrystallization solvents: A = EtOH, Ac = acetone; DMF = $HCONMe_2$; E = Et_2O; W = water. [b] From the amine and methyl formate in MeOH. [c] Sawicki, E. Synthesis and Ultraviolet Absorption Spectra Studies of 2,3- and 3,4-Disubstituted Carbazoles. *J. Am. Chem. Soc.* **1954**, *76*, 664–670. [d] Anderson, G.; Campbell, N. 2- and 4-Nitro-3-aminocarbazole. *J. Chem. Soc.* **1950**, 2904–2905. [e] From the amine with KCNO in HOAc–water. [f] Dihydrochloride dihydrate. MePip = 4-methylpiperazine. [g] Made by reduction of the oxime using Rh/C and H_2 in AcOH in Parr hydrogenator, followed by acetylation in EtOH with Ac_2O of the isolated but uncharacterized amine. [h] Made by $LiAlH_4$ reduction of the 2-nitroethene from $MeNO_2$ and the aldehyde. [i] By reduction with H_2 and Adams' catalyst with added Rh/C in EtOH of the oxime (Kyziol, J. B.; Lyzniak, A. *Tetrahedron* **1980**, *36*, 3017–3019). The resulting amine was dissolved in 2 N HCl and filtered from much unreduced starting material. NaOH liberated the base into Et_2O. Ac_2O converted the base in EtOH to 11. [j] Miller, E. C.; Miller, J. A.; Sandin, R. B.; Brown, R. K. The carcinogenic Activity of Certain Analogs of 2-Acetylaminofluorene in the Rat. *Cancer Res.* **1949**, *9*, 504–509. The formal correct numbering system is 3-acetylamino. [k] Gutmann, H. R.; Kaplan, E. Separation and Identification of Metabolites of the Arylamide N-3-Fluorenylacetamide by High Pressure Liquid Chromatography. *J. Chromatogr.* **1977**, *144*, 136–140. [l] Eckert, A.; Langecker, E. 2-Aminofluorenone. *J. Prakt. chem.* **1928**, *118*, 266. [m] Corresponds to carbazole 2-position. [n] Gilman, H.; Jacoby, A. L.; Pacevitz, H. A. Relative Reactivities of Organometallic Compounds XVIII. Selective Metallations of Dibenzothiophene. *J. Org. Chem.* **1938**, *3*, 120–124. [o] From **16** heated for 1 h under reflux with formic acid. [p] Brown, R. K.; Christiansen, R. G.; Sanoin, R. B. Some Derivatives of Dibenzothiophene. *J. Am. Chem. Soc.* **1948**, *70*, 1748–1749. [q] Courtot, C.; Evain, R. Notes deLaboratoire. *Bull. Soc. Chim. Fr.* **1931**, 527–530. [r] By treating **19** with NaH and MeI in DMF. [s] Edwards, G. A. E.; Plant, S. G. E. Derivatives of Tetrahydrocarbazole. Part III. Amino-compounds. *J. Chem. Soc.* **1923**, 2393–2399. [t] Heating of equivalent amounts of 4-methoxycyclohexanone and 3-nitrophenylhydrazine hydrochloride (neat, 1 h in steam bath) followed by recrystallization from EtOH and then MeOH gave yellow crystals, mp 216–219 °C. Proton NMR showed only one product, 3-methoxy-7-nitro-2,3,4,9-tetrahydro-1*H*-carbazole. Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 8.73; N, 11.37. Found: C, 63.31; H, 8.74; N, 11.43. Reduction (Adams' catalyst/H_2 in EtOH) and addition of the Ac_2O to the filtered solution gave a solid which was recrystallized from EtOH and then had mp 198–200 °C. TLC (Et_2O, silica gel) showed a single spot; IR showed two NH bands. Anal. for *N*-(2,3,4,9-tetrahydro-3-methoxy-1*H*-carbazol-7-yl)acetamide Calcd: C, 69.74; H, 7.03; N, 10.85. Found: C, 69.70; H, 7.08; N, 10.80.

(MAO B inhibition for **27** undetectable at 1 μM whereas it was 39% for **26** at the same concentration). The deviation from structural linearity caused by differing sizes of the central ring's linking functions appears desirable, for while the anthaquinone amide **37** has substantial inhibitory activity, it has lost much specificity, e.g., compared to **27**.

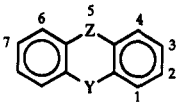
6. When the amide function is held para to the smaller central group, an additional increment of potency appears to be conferred by a fairly small, and in these examples lipophilic, moiety in the other outer ring para to the larger central group. Thus although a 7-methyl (**28**) lowered potency, the 7-ethyl (**29**) and 7-propyl (**30**) were considerably more potent than the 7-H compound (**27**).

7. The *N*-arylacetamides appear to be more potent inhibitors than their lower (Table 1, the formamide **1** compared to **2**) or their higher homologs (**5** compared to **2**). The nonacylated arylamines show negligible MAO inhibition.

8. Some bulk is acceptable and indeed may be desirable at the larger central group (compare **3** to **2**), but a great deal of bulk appears undesirable (compare **8**).

9. Both relatively "electron-rich" (e.g., the carbazole system of **1–3**) and "electron-poor" (e.g., the thioxanthen-9-one 10,10-dioxide system of **27–30**) tricyclics can serve as tricyclic scaffolding of active and selective MAO inhibitors, as can the phenoxathiin 10,10-dioxide moiety

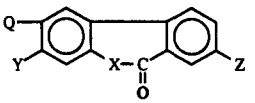
Table 2. Monoamine Oxidase Inhibition in 6:6:6 Linear Systems

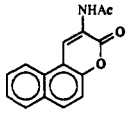


compd. no.	Z	Y	2	3	other	IC_{50} (μM) or %I at μM MAO A	MAO B	formula	anal.	mp (°C)	recryst solvent[a]
25	SO_2	CH_2		NHAc[b]		17% at 1	49% at 1	$C_{15}H_{13}NO_3S$	CHN	120–121	A–W
26	SO_2	C=O	NHAc			0.12	39% at 1	$C_{15}H_{11}NO_4S$[c]	CHN	236	Ac_2O–AcOH
27	SO_2	C=O		NHAc		0.06	−1% at 1	$C_{15}H_{11}NO_4S$[c]	CHN	282.5	AcOH–W
28	SO_2	C=O		NHAc	7-Me	0.7	0% at 1.2	$C_{16}H_{13}NO_4S$[c]	CHN	227.8	AcOH–W
29	SO_2	C=O		NHAc	7-Et	0.01	15% at 0.3	$C_{17}H_{15}NO_4S$[c]	CHN	248.2	AcOH–W
30	SO_2	C=O		NHAc	7-Pr	0.02	−7% at 10	$C_{16}H_{17}NO_4S$[c]	CHN	217.5	AcOH–W
31	SO_2	C=O		$N(CHO)_2$[d]		18% at 10	3% at 10	$C_{16}H_9NO_5S$[c]	CHN	259	AcOH
32	SO_2	O		NHAc		0.014	0% at 0.1	*e*			
33	SO_2	NH		NHAc[f]		74% at 10	22% at 10	$C_{14}H_{12}N_2O_3S$	CHNS	>250	DMF–W
34	SO_2	NMe		NHAc[g]		60% at 1	18% at 1	$C_{15}H_{14}N_2O_3S$	CHNS	202–204	A
35	SO_2	NMe		NHAc[h]	7-NHAc	2% at 1	7% at 1	$C_{17}H_{17}N_3O_4S$	CHN[h]	>300	A
36	C=O	C=O			1-NHAc	12% at 1	ND	*i*			
37	C=O	C=O	NHAc			0.16	3.5	*j*			
38	NH	CO		NHAc		72% at 1	21% at 1	*k*			

ND: inhibition of MAO B was not done. [a] Recrystallization solvents: A = EtOH, AcOH = acetic acid, Ac_2O = acetic anhydride, DMF = $HCONMe_2$, W = water. [b] Compound **25** made by reduction (PtO_2/H_2 in EtOH) and then acetylation (Ac_2O in EtOH) of 3-nitrothioxanthene 10,10-dioxide (anal. CHN, mp 161–162 °C; recrystallized from 1 EtOH:10 EtOAc) made from thioxanthene 10,10-dioxide in $HOAc/H_2SO_4$/fuming HNO_3 at 0 °C. [c] Preparation detailed in the Experimental Section. [d] Heating 3-aminothioxanthen-9-one 10,10-dioxide at 80 °C for 10 min in excess formic acid and 0.03 volumes of 70% $HClO_4$ and then pouring into ice–water gave yellow crystals of 3-(*N*,*N*-diformylimino)thioxanthen-9-one 10,10-dioxide, **31**. [e] Nobis, J. F.; Blardinelli, A. J.; Blaney, D. J. Nitration of Phenoxanthiin and some New Amino Derivatives *J. Am. Chem. Soc.* **1953**, *75*, 3384–3387. [f] Nitration (HOAc, catalytic H_2SO_4, 2 equiv of HNO_3 at 0 °C) of *N*-acetylphenothiazine 5,5-dioxide gave after ice–water quench 3-nitrophenothiazine 5,5-dioxide. This was reduced (Parr, PtO_2/H_2, EtOH), and the resulting amine was acetylated without isolation. [g] Nitration of 1-methylphenothiazine in HOAc at room temperature gave the 3-nitro 5-oxide (compare Schmalz, A. C.; Burger, A. The Action of Hydrochloric and Nitric Acids on Some Derivatives of Phenothiazine. *J. Am. Chem. Soc.* **1954**, *76*, 5455–5499). This was oxidized to the 5,5-dioxide (H_2O_2, 1 N HOAc) and the nitro group reduced (Parr, PtO_2/H_2). The resulting 3-amino-5,5-dioxide was acetylated in EtOH with Ac_2O. [h] From 10-methyl-3,7-dinitrophenothiazine 5-oxide as in footnote *g*. N: calcd, 11.69; found, 11.21. [i] Graebe, C.; Blumenfeld, S. Concerning Some 1-Derivatives of Anthraquinones and Anthracene (title translated). *Chem. Ber.* **1897**, *30*, 1115–1119. [j] Ullman, F.; Madenwald, R. Concerning 2-Aminoanthraquinone (title translated). *Chem. Ber.* **1913**, *46*, 1798–1809. [k] Tanasescu, I.; Ramontianu, E. On the Acridones. (VI). The Structure of the So-called N-Oxyacridones and Acridoles (title translated). *Bull. Soc. Chim. Fr.* **1934**, *1* (5), 547–561.

Table 3. Monoamine Oxidase Inhibition in 6:6:6 Non-Linear Systems



compd. no.	X	Y	Z	Q	IC_{50} (μM) or %I at μM MAO A	MAO B	formula	anal.	mp (°C)	recryst solvent[a]
39	O	NHAc	H	H	0.04	8% at 1	*a*			
40	O	H	H	NHAc	10% at 0.1	23% at 0.1	*b*			
41	NH	H	NHAc	H	55% at 1	0% at 1	$C_{15}H_{13}N_2O_2$	CHN	324–329	AcOH
42	NH	H	H	NH_2	0% at 10	ND	c			
43	NH	H	H	NHAc	0% at 1	11% at 1	$C_{15}H_{12}N_2O_2 \cdot {}^1/_2H_2O$	CHN	346–350	EtOH–W
44					10% at 1	12% at 1	*d*			

ND: inhibition of MAO B was not done. [a] Pan, H.-L.; Fletcher, T. L. Derivatives of Fluorene. IX. 4-Hydroxy-2-fluorenamine; New 3,4-Benzocoumarin Derivatives. *J. Org. Chem.* **1960**, *25*, 1106–1109. [b] Kenner, G. W.; Murray, M. A.; Taylor, C. M. B. Oxidative Cyclization of Diphenyl-2-carboxylic acid. *Tetrahedron* **1957**, *1*, 259. [c] Migachev, G. I.; Terent'ev, A. M. Studies on Phenanthridone and Dioxotetrahydrodiazapyrene. 4-Synthesis of Amino-substituted Derivatives of phenanthridone and Dioxotetrahydrodiazapyrene. *Khim. Geterotsikl. Soedin.* 1981, No. 3, 394–7. [d] Chakravarti, D.; Das, R. Synthesis of 3-Substituted Coumarins. *J. Indian Chem. Soc.* **1971**, *48*, 371–374.

of **32**, which is anticipated to be of intermediate "electron availability".

Our working hypothesis is that these *N*-arylamides are MAO inhibitors due to interaction with the FAD prosthetic group of the enzyme. A possible explanation for the wide range of aromatic tricyclics that support activity is that the activity only requires the polarizability of the aromatic systems to allow them to stack with the flavin.[10]

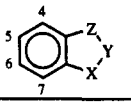
The tabulated results suggest that the carbazole, the thioxanthen-9-one 10,10-dioxide and the phenoxathiin 10,10-dioxide systems would be of special interest when other groups replace the amide function. The applicability of these observations to replacement of the amide function by other hydrophilic substituents will be the subject of a future publication.

Since the data reported in this paper are for inhibition of enzyme preparations *in vitro*, these data cannot lead to an estimate of activity in preventing a tyramine-induced blood pressure rise.

Experimental Section

Chemistry. Melting points below 305 °C were determined by using a Thomas-Hoover heated oil bath; those above that temperature by using a block (Mel-temp Laboratory Devices).

Table 4. Monoamine Oxidase Inhibition in Bicyclic Compounds



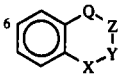
compd no.	X	Y	Z	other	IC_{50} (μM) or %I at μM MAO A I	MAO B I	formula	anal.	mp (°C)	recryst solvent[a]
45	CH_2	CH_2	CH_2	5-NHAc	13% at 1	37% at 1	*a*			
46	N-Ac	CH_2	CH_2	6-NHAc	37% at 1	-27 at 1	*b*			
47	NH	CH	CH	5-NHAc	0 at 1	0 at 1	*c*			
48	NH	CMe	CMe	5-NHAc	<1% at 1	42% at 1	*d*			
49	NAc	N	CH	5-NHAc	4% at 1	27% at 1	*e*			
50	NH	CNHAc	N	-	8% at 10	-2% at 10	*f*			
51	NH	CNHAc	N	5,6-$(Me)_2$	50% at 10	18% at 10	*g*			
52	N	CNHAc	S	5,6-$(Me)_2$	54% at 10	38% at 10	$C_{11}H_{12}N_2OS$	CHN	239	A
										
53	O	CH_2	O	CH_2	6-NHAc	42% at 10	22% at 10	*h*		
54	O	CO	CHNHAc	CH	-	5% at 1	53% at <10	*i*		

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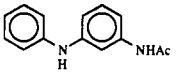
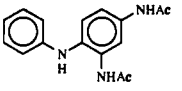
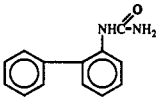
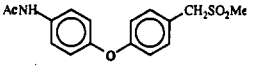
Table 5. Monoamine Oxidase Inhibition of Some Miscellaneous Arylamides

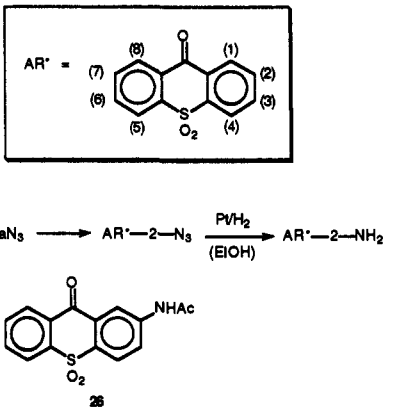
compd no.	structure	A	B	footnotes
55		44% at 10	ND	
56		4% at 10	4% at 10	*a*
57		22% at 10	13% at 10	*b*
58		31% at 1	25% at 1	*c*

[a] Phillips, M. A. The Formation of 1-Substituted Benziminazoles. *J. Chem. Soc.* **1929**, 2820–2828. ND, inhibition of MAO B was not done. [b] Kurzer, F. Cyanamides. Part II. The Influence of Substituents in the Synthesis of Arylsulfonylarylcyanamides. *J. Chem. Soc.* **1949**, 3029–3033. [c] Gorvin, J. H.; Harfenist, M. Diphenyl Ethers and Their Use for Treating Liver Infections in Mammals. German Offen. DE 2921824. 6 Dec 1979.

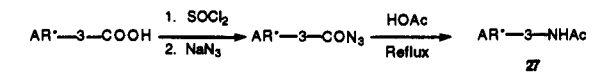
All are uncorrected. All compounds showed a single spot on TLC on UV-fluorescent silica gel plates (MK6F, Whatman International, Ltd.); developing solvents were Et_2O or EtOAc–hexanes unless otherwise specified. Spots were visualized with 254-μm ultraviolet light.

Most of the arylamines required for acylation to the amides were either purchased or made by reduction of the nitro compounds. Reduction of nitroarenes free of other reducible groups was generally done using Adams' catalyst and hydrogen in ethanol or, where solubility dictated this, in acetic acid. The nitro compounds for which this procedure seemed inapplicable were reduced with excess iron powder in hot ethanol containing a little aqueous HCl, or where solubility in ethanol of the product was low, in hot acetic acid. Compound **26** was made (Scheme 1) from 2-chlorothioxanthen-9-one 10,10-dioxide by conversion to the azide and catalytic reduction of that to the amine. Methods used to make (acetylamino)thioxanthen-9-one 10,10-dioxides **27**–**30** are shown in Schemes 2 and 3. Preparation of **27** by acid-catalyzed "diaza Schmidt reaction"[11] of the tetrazole doxantrazole (**59**)[12] is shown in Scheme 4. The arylamides were made by acylation of the corresponding amines. The procedure used in most cases involved adding excess acetic anhydride to an ethanolic solution of the amine and allowing the reaction to remain at room temperature at least 10 min. The reaction was then heated

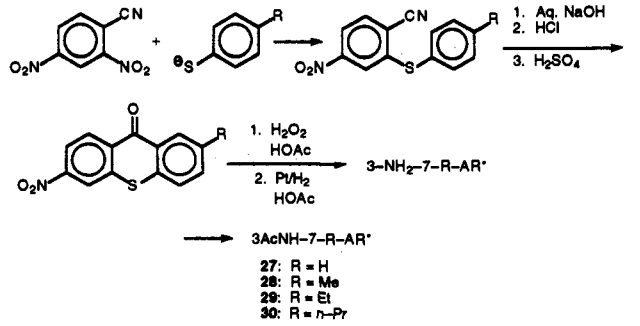
Scheme 1



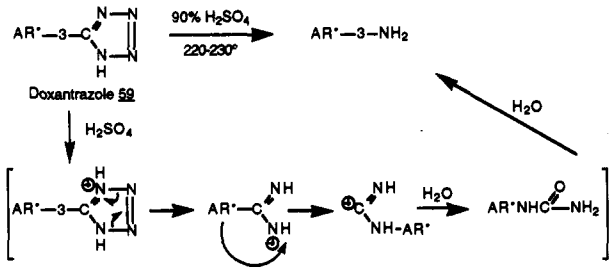
Scheme 2



Scheme 3



Scheme 4



to the boiling point, and water was added to incipient turbidity. This procedure caused destruction of excess acyl anhydride and led to crystallization of the acylamino product, usually analytically pure. Amines from electron-poor arenes such as the aminoanthraquinones **36** and **37** were best acetylated with acetic anhydride in acetic acid using a little sulfuric acid as catalyst. However, the extremely non-basic amines in the thioxanthen-9-one 10,10-dioxide series **27–30** were best acetylated under reflux in acetic acid with excess of acetic anhydride and a small amount of perchloric acid. (*Warning: Mixtures of organic materials with anhydrous perchloric acid must be considered potentially explosive.*)

2-Azidothioxanthen-9-one 10,10-Dioxide. A mixture of 5.6 g (0.02 mol) of 2-chlorothioxanthen-9-one 10,10-dioxide, 1.30 g of NaN_3 (0.02 mol), and 100 mL of dried DMSO was heated under reflux overnight. Cooling and addition of water led to the product, which was recrystallized from ethanol and then was 5.4 g, mp 217 °C. TLC (Et_2O) showed one spot. Anal. ($C_{13}H_7N_3O_3S$) C, H, N.

2-Acetamidothioxanthen-9-one 10,10-Dioxide (26). The above azide was reduced in ethanol using Adams' catalyst in a Parr shaker overnight, replacing the hydrogen twice during the reduction. The resulting amber solution was filtered to remove catalyst, and solvent was removed in vacuo. The residue was not characterized but was treated with 20 mL of Ac_2O and heated on a steam bath for 30 min. Fifty milliliters of ethanol was then added, and heating continued for 1 h. Cooling overnight led to a first crop of 0.4 g of **26**. It was recrystallized from HOAc by addition of water near the bp. The mp of the yellow solid was 236 °C. TLC (Et_2O) showed one spot with R_f 0.18. Anal. ($C_{15}H_{11}NO_4S$) C, H, N.

3-Aminothioxanthen-9-one 10,10-Dioxide from 3-(5-Tetrazolyl)thioxanthen-9-one 10,10-Dioxide (Doxantrazole, 59).[12] A mixture of 20 g of **59** and 250 mL of 96% sulfuric acid (90% acid gave a poorer yield of purer amine) was heated at 220 °C under N_2 until bubbling ceased (1 h.) and an additional half hour. It was then cooled and poured into 1.5 L of iced water. The resulting precipitate weighed 15.88 g and was a mixture by TLC of the desired amine compound (R_f = 0.41), some starting material (R_f = 0.08), and material giving a faint spot at R_f = 0.59. The TLC system was 5:5:1:15 EtOH:$CHCl_3$:HOAc:hexanes by volume. The amine was not purified but was converted to **27**.

3-Acetamido-7-(methylthio)xanthen-9-one 10,10-Dioxide (28) by Curtius Rearrangement of the Azide. 3-Carboxythioxanthen-9-one 10,10-dioxide was converted to the acid chloride using thionyl chloride.[12] A mixture of 3.29 g (0.0103 mol) of this acid chloride and 50 mL of acetone was stirred while 0.9 g (0.0138 mol) of NaN_3 in 3 mL of hot water was added dropwise over 5 min. The reaction mixture was cooled in ice and stirred an additional 2 h. Addition of 100 mL of iced water gave a precipitate which was filtered off and washed with water. The crude yellow solid azide was dried and used without purification. It was heated under reflux with 100 mL of glacial acetic acid ($CaCl_2$ tube) for 1 h and cooled, and the yellow precipitate was removed by filtration. This weighed 1.6 g with mp ca. 339–344 °C; it was not further investigated. Dilution of the filtrate with water to faint turbidity near the bp, and cooling, led to a solid mp 221–234 °C. This was recrystallized from HOAc–H_2O and then had mp 221–234 °C, unchanged on recrystallization. Two repreparations, however, gave products with mp 252–255 °C and 253–255 °C, with correct elemental analysis, and single TLC spots: 1:9 MeOH:$CHCl_3$, R_f = 0.55; 3:7 $CHCl_3$:EtOAc, R_f = 0.22. Anal. ($C_{16}H_{13}NO_4S$) C, H, N.

Biological Methods. MAO A and B were assayed using a radiometric procedure with [^{3}H]serotonin and [^{14}C]phenethylamine as substrates for MAO A and B, respectively.[13] Rat brain mitochondrial MAO extract, prepared as described earlier,[14] was preincubated in the absence or presence of inhibitors for 15 min at 37 °C in 50 mM potassium phosphate buffer (pH 7.4). Substrates [^{3}H]serotonin or [^{14}C]phenethylamine were then added to give final concentrations of 0.2 mM (5 Ci/mol) and 10 μM (3 Ci/mol), respectively, in a total volume of 300 μL. Incubation at 37 °C was continued for 20 min. Blank assays contained 2 mM pargyline to inhibit all MAO activity. The reaction was terminated with 0.2 mL of 2 N HCl, and products were extracted with 6 mL of ethyl acetate/toluene (1:1). A 4-mL aliquot of the organic layer was counted in 10 mL of Scintiverse-BD (Fisher Scientific Co.) in a scintillation spectrometer. Assays at each concentration of inhibitor were performed in triplicate and were expressed as the mean of three assays with SEM within 5% of the mean. IC_{50} values were extrapolated from plots of mean percent inhibition vs log of inhibitor concentration. At the above substrate concentrations, which are approximately twice the K_m concentrations for serotonin and phenethylamine at MAO A and MAO B sites, respectively, the activity with each substrate was independent of the other. This procedure gives IC_{50} values that are 2 or 3 times the K_i values for competitive inhibitors and has provided a reliable way to rank inhibitory potencies.

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