Selective Inhibitors of Monoamine Oxidase. 3. Structure−Activity Relationship of Tricyclics Bearing Imidazoline, Oxadiazole, or Tetrazole Groups

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Inhibition of monoamine oxidase A (MAO A) is believed to cause antidepressant and possibly antianxiety effects. The previous paper had developed structure−activity relationships (SAR) for in vitro MAO A inhibition by tricyclic *N*-arylamides.[1] It is shown in this paper that the same in vitro SAR can be carried over to tricyclics whose potentially toxic amide function is replaced by an appropriately substituted imidazoline, a 1,2,4- or 1,3,4-oxadiazole, or an alkylated tetrazole moiety. Dialysis of the inhibitor from the enzyme was used as a measure of reversibility which correlates with a low ability to cause a blood pressure rise with ingested tyramine ("cheese effect").

Monoamine oxidase (MAO) (EC 1.4.3.4, amine oxidase, flavin containing) consists of two differing enzyme forms that were first distinguished by their substrate specificity[2] and later by their amino acid sequences.[3] Ratios of MAO A to MAO B of ca. 1 and 2 have been reported[4,5] for human brain and liver, respectively. Serotonin is specifically deaminated by MAO A, while 2-phenethylamine is relatively specifically deaminated by MAO B. Tyramine, which is generally dietary in origin, is deaminated with similar efficiencies by both forms. Early MAO inhibitors were found to have clinically significant antidepressant and antianxiety properties. However, ingestion of foods high in tyramine by some patients treated with early MAO inhibitors led to unacceptible hypertension ("cheese effect").

Inhibition of MAO A is believed to be responsible for the desired antidepressant and antianxiety effects. It therefore seemed desirable to look for an inhibitor which selectively inhibited MAO A, leaving the MAO B to cope with ingested tyramine. Further, since the predominant MAO in human liver and intestine is MAO A, it seemed desirable that any inhibitor be readily displaceable from inhibited MAO A by tyramine, thereby reactivating the MAO A to destroy any tyramine not oxidized by the MAO B.

Our previous paper[1] described use of tricyclic *N*-arylamides, which are potent selective inhibitors of MAO A but potentially carcinogenic, to rapidly develop structure−activity relationships (SAR) which might be applicable to classes of compounds with lower predictable toxicity. An old publication from our group had reported[6] irreversible MAO inhibition by 2-alkoxynaphthylimidazolines. Although that work was done with mouse brain MAO rather than rat brain MAO used in the present work, and the relative proportion of the forms of MAO inhibited by the arylamides was not determined, substitution of the 2-imidazoline moiety for the NHAc group used in our previous work[1] was selected for this early study.

Results and Discussion

This paper reports that the replacement of the "hydrophilic" amide moiety of the *N*-arylamides by the 2-imidazoline function allowed at least moderately successful transfer of SAR for MAO inhibition from the former series to the latter. (Unless otherwise stated, all of the potent tricyclic compounds mentioned are essentially specific for inhibition of MAO A). For example, a tricyclic aryl system seems necessary though not sufficient to lead to inhibition, as was reported in the earlier work. A variety of monocyclic and bicyclic 2-arylimidazolines (not tabulated) was tested, and none showed appreciable potency. An exception to this is the high potency found with the alkoxynaphthylimidazolines mentioned above.[6] This was studied in some detail, and results will be reported elsewhere. The corresponding acetaminonaphthalenes were not studied because of the known carcinogenic activity of members of that group.

Another area of correspondence with the previous work is the high potency and selectivity for inhibition of MAO A found for the thioxanthen-9-ones in Table 1 and of the two monoimidazoline-substituted phenoxathiins in Table 2. Examples of the markedly greater potency of the sulfone rather than the unoxidized form of the heterocyclic sulfur are shown by comparison of activity of **1** and **2** and of **31** to **32**. It is also evident that moving the imidazoline from the 3- to the 4-position (compare **1** and **2** to **25** and **26**) destroys activity. Corresponding to the pronounced maximum potency for the *N*-arylacetamides in the previous study compared to more highly substituted acyl analogs, substitution of a methyl on an imidazoline nitrogen (**71**) or on the 3-carbon (**72**) essentially destroyed activity, as did expansion of the imidazoline to the tetrahydropyrimidine (**73**). Replacement of the imidazoline function by imidazole (**74**) also destroyed activity.

The most interesting results displayed in Table 1 show the influence of the substituent in the 7-position on potency. This was also noted in a few examples in the earlier *N*-arylamide paper but is much more evident in Table 1 here, where increases of 30-fold in potency

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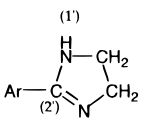
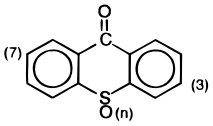
Table 1. MAO Inhibition of 2′-Imidazolines



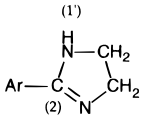
where Ar = thioxanthene-9-one:



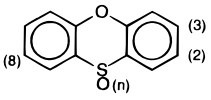
compd no.	1′	2	3[a]	4	6	7	n	IC50 (μM) or % inhib at μM: MAO A	IC50 (μM) or % inhib at μM: MAO B	% reversible on 24 h dialysis[c]	formula	anal.	mp (°C)	recryst solvent[b]
1			Im				0	1	14% at 1		$C_{16}H_{12}N_2OS\cdot H_2O$	CHNS	222−223	A-D-W
2			Im				2	0.3	3% at 0.3	100[d]	*e*			
3			Im			F	2	0.2	12% at 1	62	*e*			
4			Im			Cl	2	0.07	3% at 0.2	60[d]	*e*			
5			Im			Me	2	0.02	0% at 10	26	*e*			
6			Im			Et	2	0.01	27% at 10	11[d]	$C_{18}H_{16}N_2O_3S$	CHN	204	A
7			Im			Pr	2	0.04	0% at 0.3	2	$C_{19}H_{18}N_2O_3S$	CHN	200	M
8			Im			*i*-Pr	2	0.05	0% at 0.1	40	*e*			
9			Im			*t*-Bu	2	0.8	NS[f] at 1	56	$C_{20}H_{20}N_2O_3S$	CHN	224	CH
10			Im			OMe	2	0.01	33% at 1	5	*e*			
11			Im			OEt	2	0.007	12% at 1	4	*e*			
12		OPr			Im		2	0.04[g]	NS at 0.1		*e*	CHN	157−160 dec	A-W
								0.009[h]		2				
13			Im			−NHMe	2	0.023		8	$C_{17}H_{15}N_3O_3S\cdot HCl\cdot 0.7H_2O$	CHN	>300	0.6 N HCl
14			Im			−NHEt	2	0.01	0% at 1	0[d]	*e*	CHN		
15			Im			−NHPr	2	0.02	4% at 0.0	0	*e*			
16			Im			$-NCH_2CH{=}CH_2$	2	0.009	12% at 1	5	e			
17			Im			$-NHC_2H_4NH_2$	2	0.3	11% at 1	20[d]	*e*			
18			Im			$-NHC_2H_4OH$	2	0.04	NS[c] at 1		$C_{18}H_{17}N_3O_4S\cdot 0.5\,H_2O$	CHN	221−222	A−D−W
19			Im			$-NMe_2$	2	0.02	8% at 0.03	21	*e*			
20			Im			−NMeEt	2	0.04	NS[c] at 0.1	20[d]	*e*			
21			Im				2	0.01	4% at 1		*e*			
22			Im				2	0.02	10% at 0.2		$C_{20}H_{19}N_3O_3S_2$	CHN	240−242	A−D−W
23			Im		Me[d,i]		2	53% at 1	5% at 1		$C_{17}H_{14}N_2O_3S$	CHN	189−194	A−W
24			Im		CF_3		2	12% at 1	10% at 1		$C_{17}H_{11}F_3N_2O_3S$	CHN	192−194	A−D−W
25				Im			0	7% at 1	10% at 1		$C_{16}H_{12}N_2OS$	CHN	250.5−251.5	A−D−W
26				Im			2	−5% at 1	−6% at 1		$C_{16}H_{12}N_2O_3S$	CHN	201−202	A−W
27	Me		Im				2	27% at 1	11% at 1		$C_{17}H_{14}N_2O_3S\cdot HCl$	CHN	270−275	A−I
28	Ac		Im			F	2	4% at <1	−11% at <1		$C_{18}H_{13}FN_2O_4S$	CHN	254−257 dec	A−D−W
29	Ac		Im			OPr	2	16% at 0.1	20% at 0.1		$C_{21}H_{20}N_2O_5S$	CHN	172−174	Ac−H
30		Im			Im		2	46% at 1	3% at 1		$C_{19}H_{16}N_4O_3S\cdot 0.25DMSO$	CHN	277−278	A−D

[a] Im = imidazolin-2-yl. [b] Recrystn solvents: A = EtOH; Ac = Me_2CO; D = DMSO; H = hexanes; I = *i*-PrOH; M = MeOH; N = $MeNO_2$; W = H_2O. [c] See text for significance. [d] Values of po ED_{80} ex vivo in rat brain; **2**, 50 mg/kg; **4**, 25 mg/kg; **6**, 25 mg/kg; **14**, 25 mg/kg; **19**, 25 mg/kg; **31**, 25 mg/kg. [e] Harfenist, M.; Joyner, C. T.; Heuser, D. J. *Europ. Patent Appl.* EP 150891 A1 850807. [f] NS = not significant. [g] Isethionate, a relatively soluble salt. [h] Free base. [i] 54% 6-Me + 46% 8-Me by proton NMR.

Table 2. MAO Inhibition of 2′-Imidazoles



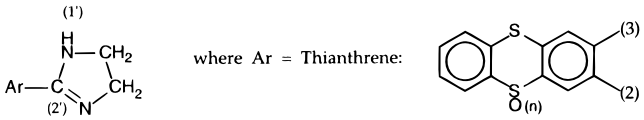
where Ar = Phenoxathiin:



compd no.	1′	2	3	4	6	7	8	n	IC50 (μM) or % inhib at μM: MAO A	IC50 (μM) or % inhib at μM: MAO B	% reversible on 24 h dialysis[c]	formula	anal.	mp (°C)	recryst solvent
31		Im						0	0.1	10	46	$C_{15}H_{12}N_2OS$	CHN	157−158	A−W
32		Im						2	0.04	59% at <1	16	$C_{15}H_{12}N_2O_3S$	CHN	298−300 dec	A−D−W
33		Im					Im	0	1	10		$C_{18}H_{16}N_4OS$	CHNS	293−294.5	BuOH
34		Im					Im	2	0 at 0.1	5 at 0.1		$C_{18}H_{16}N_4O_3S\cdot 0.25H_2O$	CHNS	294−296	A

are evident in compounds **2**−**22** (IC_{50} values as low as 10−20 nM are shown). The great variation of potency with structure despite the lack of direct conjugation of the 7-substituent with the imidazoline function and the increase in potency with small changes in substituent size unrelated to expected electron input (alkyl vs halogen vs OR vs NR) imply that influence of the 7-substituent on the pK_a of the imidazoline moiety is not a major influence on potency but rather that there is a site of binding to the enzyme corresponding to the 7-position which is available when the inhibitor molecule is oriented correctly by the tricyclic system and the "hydrophilic" substituent. Unfortunately, presence of the 7-substituents in this series led to tight binding to MAO A in vitro, so that the most potent such compounds caused inhibition of the enzyme which was not completely reversed after 24 h of dialysis. Data for reversibility are tabulated. Such irreversibility on dialysis can be correlated with an increase in blood pressure in rats given inhibitor and then tyramine po

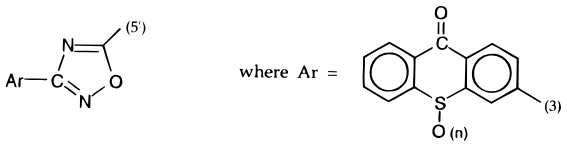
Table 3. MAO Inhibition of 2′-Imidazolines[a]

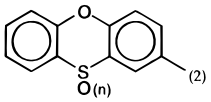


compd no.	1′	2	3	4	6	7	8	*n*	IC50 (μM) or % inhib at μM MAO A	IC50 (μM) or % inhib at μM MAO B	formula	anal.	mp (°C)	recryst solvent
35		Im						0	0.3	31% at 1	$C_{15}H_{12}N_2S_2$	CHN	192.5−193.5	C−H
36		Im						1	14% at 1	14% at 1	$C_{15}H_{12}N_2OS_2$	CHN	178−180	CH_2Cl_2

[a] 75% 2-imidazoline + 25% 3-imidazoline by 100 MHz NMR.

Table 4. MAO Inhibition of 1,2,4-Oxadiazoles



compd. no.	5′	aryl substit	*n*	IC50 (μM) or % inhib at μM MAO A	IC50 (μM) or % inhib at μM MAO B	formula	anal.	mp (°C)	recryst solvent
37	OMe	H	2	0.08	23% at 0.5	$C_{16}H_{10}N_2O_5S$	CHNS	251.6	MeCN
				where Ar = 					
38	Me	H	0	0.2	14% at 1	$C_{15}H_{10}N_2O_2S$	CHN	103.6	H
39	Me	H	2	98% at 1	17% at 1	$C_{15}H_{10}N_2O_4S$	CHN	232.8−235 dec	HOAc−W

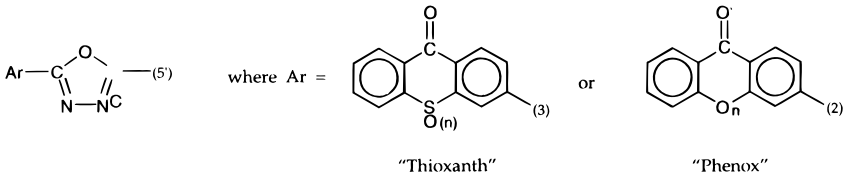
and therefore was used in initial screening as a surrogate for the labor-intensive in vivo determination of the potential of compounds to cause the "cheese effect". Values of percentage of reversibility given in the tables can be interpreted as follows: 0−20% indicates that the binding of the compound to the enzyme is essentially not reversible on dialysis, while a value above 80% shows that binding to the enzyme is readily reversible. Intermediate values, of course, indicate slow or partial dialysis in the time allowed. Even though MAO inhibition by even the tightest bound compounds was greatly decreased or frequently undetectable in the brains of rats 24 h after dosing, indicating that binding had been noncovalent, irreversibly-bound analogs would be anticipated to be clinically unacceptable. Ex vivo values for inhibition of brain MAO A of rats sacrificed 2 h after administration of the compounds po are footnoted below the tables for a small number of compounds. It is instructive to compare **2** with **6**: Compound **2** has an IC_{50} of 0.2 μM and a K_i of 0.08 μM, and binding to the enzyme is competitive with serotonin, while **6** has an IC_{50} of 0.01 μM and a K_i of 0.004 μM, and binding to the enzyme is not affected detectably by increases in serotonin levels.

The two thianthrenes in Table 3 illustrate another 6:6:6 system with potential. Thus **35**, the unoxidized compound, was comparable in potency to the sulfide forms of the thioxanthone and phenoxathiin **1** and **31**, respectively. The sulfoxide mixture **36** was of low potency. The mixture could not be separated, so NMR was used to determine the proportion of the 2- to 3-isomer. The low activity of sulfoxides was explored in other series and found to be a general phenomenon.

The high activity and selectivity for MAO A of these 2-arylimidazolines led us to prepare the analogous thioxanth-9-one and phenoxathiin analogs with the imidazoline replaced by the other 5-membered heterocycles shown in Tables 4−6. In contrast to the imidazolines of Table 1 (note **27**−**29**), the 1,2,4-oxadiazoles of Table 4 and the 1,3,4-oxadiazoles of Table 5 tolerated small aliphatic substituents in the 5′-position. IC_{50} values of 1−2 nM for MAO A inhibition are liberally sprinkled throughout Table 5, while inhibition of MAO B was negligible. Similarly, the 5′-aryltetrazoles in Table 6 include a number of extremely potent and selective entries, discussed below. The persistence of activity with the 5′-methoxy of **37** in Table 4 and of **47** in Table 5 is clear. Perhaps this is an example of the SAR dictum that oxygen in an aliphatic chain is frequently a "null" element. However, other substituents larger than methyl or methoxy (CF_3 in **43**, Et in **44**) diminish or eliminate activity. The SAR is even more restricted for the tetrazoles of Table 6. 1-Unsubstituted tetrazoles, perhaps not surprisingly since they are protic acids, are inactive. While 2′-methyltetrazoles such as **51**−**54** are extremely potent, 1′-methyl analogs such as **50** are of lower potency, and the 2′-ethyltetrazoles such as **55**, **57**, and **58** are far less potent. Fortunately, the major product of methylation with methyl iodide of the sodium salt of the 2-aryltetrazoles is the 2′-methylated product. None of the few xanthone−tetrazoles tested (**57**, **60**) was active, nor was the dibenzothiophene dioxide **61**.

Compound **2** was selected for further study. **2** initially seemed free of pharmacological behavior other than that associated with antidepressant activity, and tyramine administered at a threshold dose for blood pressure rise in control rats (15 mg/kg po) caused no increase in blood pressure in rats pretreated with **2**, while pargyline pretreatment led to a maximal bp rise under the same conditions. However, high oral dosage during chronic toxicity testing in rats led to some tissue

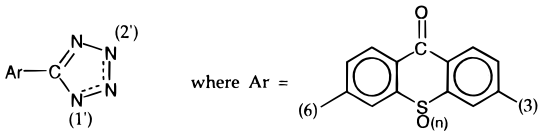
Table 5. MAO Inhibition of 1,3,4-Oxadiazoles



compd no.	5′	aryl substit	*n*	IC$_{50}$ (μM) or % inhib at μM MAO A	MAO B	% reversible on 24 h dialysis	formula	anal.	mp (°C)	recryst solvent
40	Me	2-phenox	2	0.002	NS at 0.001	12[a]	$C_{15}H_{10}N_2O_4S$	CHN	231.4	HOAc−W
41	Me	3-thioxanth-7-Me	2	0.002	NS at 0.001	10	$C_{17}H_{12}N_2O_4S$	CHN	243.4	HOAc−W
42	Me	3-thioxanth-7-OMe	2	0.001	0.1	14	$C_{17}H_{12}N_2O_5S$	CHN	260.4	HOAc−W
43	CF_3	3-thioxanth	2	7% at 0.5	7% at 0.1		$C_{16}H_7F_3N_2O_4S$	CHN	205.1	HOAc−W
44	Et	3-thioxanth	2	0.5	23% at 1		$C_{17}H_{12}N_2O_4S$	CHN	191.9	HOAc−W
45	Me	3-thioxanth	2	0.002	NS at 1	33[b]	$C_{16}H_{10}N_2O_4S$	CHN	248−250	DMF
46	Me	3-thioxanth-7-OPr	0	0.06	1	10	$C_{19}H_{16}N_2O_3S$	CHN	213.6	HOAc−W
47	OMe	3-thioxanth	2	0.08	14% at 1		$C_{16}H_{10}N_2O_5S$	CHNS	211.9	MeCN

[a] At 20 mg/kg (rat, po) MAO A was selectively inhibited 100% in brain. [b] At 25 mg/kg (rat, po) MAO A was selectively inhibited 100% in brain.

Table 6. MAO Inhibition of Tetrazoles



compd no.	aryl substit	tetrazole position	tetrazole substit	*n*	IC$_{50}$ (μM) or % inhib at μM MAO A	MAO B	% reversible on 24 h dialysis	formula	anal.	mp (°C)	recryst solvent
48[a]	H	3	H	2	9% at 10	1% at 10					
49	7-*i*-Pr	3	H	0	24% at 1	5% at 1		$C_{17}H_{14}N_4O_3S$	CHN	233.5−234.5	A−D−W
50	H	3	1′-Me	2	53% at 0.1	1% at 0.1		$C_{15}H_{10}N_4O_3S$	CHN	270−271°	C−H
51	H	3	2′-Me	2	0.01	10% at 1	100	$C_{15}H_{10}N_4O_3S$	CHN	206.8−207.5	HOAc
52	7-*i*-Pr	3	2′-Me	2	0.005	−30% at 0.1	0	$C_{18}H_{16}N_4O_3S$	CHN	219−221	A−D−W
53	2-OMe	6	2′-Me	2	0.002	0.06	29	$C_{16}H_{12}N_4O_4S$	CHN	247.4	DMF−W
54	2-OPr	6	2′-Me	2	0.002[a]	−13% at 0.003	0	$C_{18}H_{16}N_4O_4S$	CHN	165−167	A−H
55	H	3	2′-Et	2	36% at 1	45% at 1		$C_{16}H_{12}N_4O_3S$	CHN	153.3−155.9	A−W
	where Ar = Phenoxathiin										
56	H	2	2′-Me	2	0.002	10% at 50		$C_{14}H_{10}N_4O_3S$	CHN	253−255	HOAc
	where Ar = Xanthen-9-one										
57	7-OH	3	2′-Et	2	0.1			$C_{16}H_{12}N_4O_3$	CHN	251−252	DMF
58	7-OEt	3	2′-Et	6% at <1	32% at <1			$C_{18}H_{16}N_4O_3$	CHN	186−189	HOAc
59	2-OC_2H_4OH	6	2′-Me	64% at 1	21% at <1			$C_{17}H_{14}N_4O_4$	CHN	220−222	A−D−W
60	7-OC_2H_4OH	3	2′-HOC_2H_4	29% at <1	18% at <1			$C_{18}H_{16}N_4O_5$	CHN	206−208	A−D−W
61	(2)				9% at 1	0 at 1		$C_{13}H_8N_4O_2S$	CHN	275 dec	

[a] Doxantrazole.

damage, so **2** was judged an unsatisfactory clinical candidate. Rats given oxadiazoles or a potent 2-methyltetrazole po showed no inhibition of rat brain MAO ex vivo. It was not ascertained whether this was due to poor absorption, binding by nontarget tissue, or rapid metabolism. Work on this group of compounds was abandoned in favor of other series.

Experimental Section

Chemistry. Melting points below 305 °C were determined using a Thomas Hoover heated oil bath and those above that temperature with a block (MEL-TEMP Laboratory Devices). All are uncorrected. All compounds showed a single spot on TLC and had proton NMR spectra in accord with their anticipated structure.

Preparation of 3-cyanothioxanth-9-ones and the corresponding 10,10-dioxides required to make the imidazolines of Table 1, the amidoximes antecedent to the 1,2,4-oxadiazoles of Table 4 and the tetrazoles of Table 6 followed literature[7] methods outlined in Scheme 1. This nitrile preparation involved the displacement by a thiolate of an aromatic nitro group activated by cyano groups.[8] Although direct cyclization of the resulting nitrile to the thioxanthone could be done, hydrolysis to the

Table 7. MAO Inhibition of Some Inactive Tricyclic Compounds with 2-Imidazoline Substitution at Numbered Position

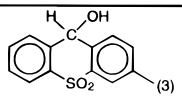
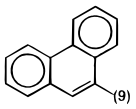
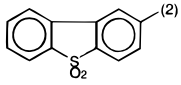
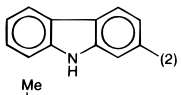
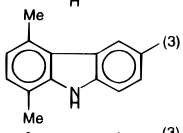
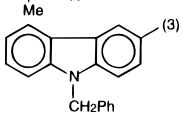
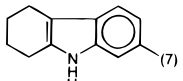
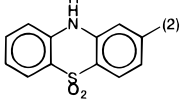
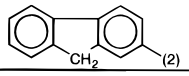
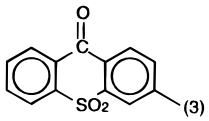
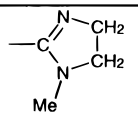
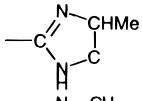
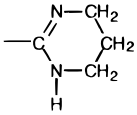
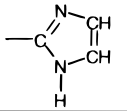
cmpd. no.	Aryl Substitution	IC_{50} (μM) or % I @ μM MAO A I	MAO B I	formula	anal.	mp (°C)	recryst solvent
62		20% @ 1	10% @ 1	$C_{16}H_{13}N_2O_3S \cdot H_2SO_4 \cdot H_2O$	CHN	239°	A-W
63		30% @ 1	n.d.	$C_{17}H_{14}N_2 \cdot C_7H_8O_3S$	CHN	212.5-213°	A-W
64		41% @ 1	16% @ 1	$C_{15}H_{12}N_2O_2S$	CHN	128-130°	A-1N NaOH
65		33% @ 3	38% @ 3	$C_{15}H_{13}N_3 \cdot HCl \cdot H_2O$		194° (dec)	M-EA
66		62% @ 1	10% @ 1	$C_{17}H_{17}N_3 \cdot HCl$	CHN	>380°	A-W
67		20% @ 10	61% @ 10	$C_{22}H_{19}N_3 \cdot HCl \cdot H_2O$	CHN	334°	6N-HCl
68		20% @ 1	9% @ 1	$C_{15}H_{17}N_3 \cdot C_2H_6O$	CHN	82°	A
69		23% @ 1	12% @ 1	$C_{15}H_{13}N_3O_2S$	CHN	290-295	A-D-W
70		59% @ 1	11% @ 1	$C_{16}H_{14}N_2$	CHN	200.7-201.8	A-W

Table 8. Thioxanth-9-one 10,10-Dioxides 3-Substituted by Congeners of 2-Imidazoline



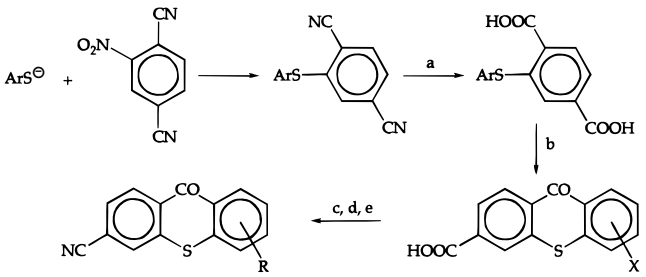
cmpd. no.	Aryl Substitution	IC_{50} (μM) or % I @ μM MAO A I	MAO B I	formula	anal.	mp (°C)	recryst solvent
71		27% @ 1	18% @ 1	$C_{17}H_{14}N_2O_3S \cdot HCl$	CHN	270-275°	A
72		36% @ 10	49% @ 10	$C_{17}H_{14}N_2O_3S$	CHN	204°	A
73		37% @ 1	5% @ 1	$C_{17}H_{14}N_2O_3S$	CHN	243°	A-W
74		44% @ <5	10% @ <	$C_{16}H_{10}N_2O_3S \cdot HCl$	CHN	339° (dec)	A-W

acid, cyclization of that, and reconversion of the resulting thioxanthone carboxylic acid to the nitrile as shown was preferred since it gave a better yield of a purer product.

The imidazolines in Table 1 and others containing electron-withdrawing groups were generally made by methoxide-catalyzed addition of ethylenediamine to the corresponding nitrile in methanol without isolation of the intermediate iminoether (Scheme 3). Although base-catalyzed cleavage of thioxanth-9-one 10,10-dioxides to give xanthenones and other products is reported in the literature, no problem was encountered in our work in isolating pure imidazolines. An example, the synthesis of **6**, is given below. In this group of compounds, the Pinner reaction or the Oxley and Short variation[9] showed no advantage for imidazoline synthesis.

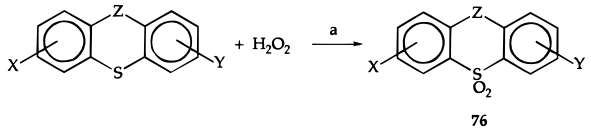
Scheme 4 shows the synthesis of the 1,2,4-oxadiazoles of Table 4 from the nitriles by way of the amidoximes and their *O*-acyl derivatives. A synthesis of **39** from **38**, as well as an alternate method, is given in greater detail below.

Scheme 1[a]



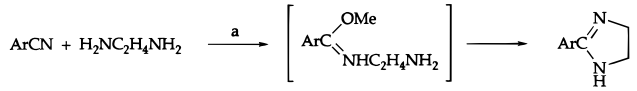
[a] (a) Aqueous NaOH, then H^+; (b) PPA or H_2SO_4; (c) $SOCl_2$; (d) 28% aq NH_3; (e) $SOCl_2$ (DMF).

Scheme 2[a]



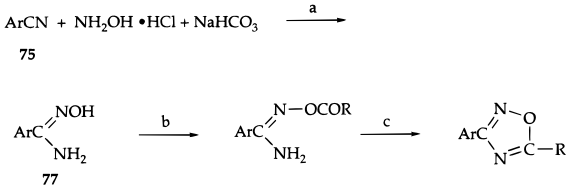
[a] (a) HOAc, 80−90 °C.

Scheme 3[a]



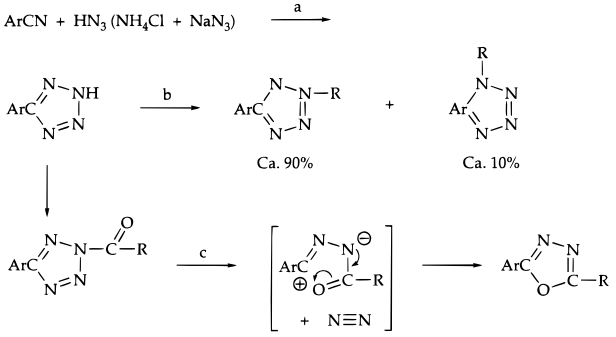
[a] (a) NaOMe in MeOH.

Scheme 4[a]



[a] Stir in PrOH at room temperature; (b) $(RCO)_2O$ or EtOCOCl, EtOAc; (c) $(RCO)_2O$, reflux.

Scheme 5[a]



[a] (a) DMF, 100−140 °C, *in hood*; (b) RX + K2CO3, DMF; (c) (RCO)2O, reflux.

Syntheses of both the 1,3,4-oxadiazoles of Table 5 by thermolysis of the 5-aryl-2-acyltetrazoles and of the N-alkylated tetrazoles of Table 6 by alkylation of the corresponding tetrazoles preferably by alkyl iodides are shown in Scheme 5. In the alkylations, about 10% of the 1-methylated tetrazole and 90% of the 2-methyl isomer were formed with methyl iodide. A preparation of **42** and preparations of **50** and **51** are given below.

2-Ethyl-6-(2-imidazolinyl)thioxanthen-9-one 10,10-Dioxide (6). In a 500 mL 3-necked flask fitted with a stirrer, condenser, and nitrogen demand system was placed 15.5 g (0.052 mol) of 3-cyano-7-ethylthioxanthen-9-one 10,10-dioxide[10] followed by 31.33 g (0.52 mol) of ethylenediamine, 200 mL of methanol, and 0.9 g of sodium methoxide. The reaction mixture was stirred and heated under reflux until no starting nitrile was visible by TLC (silica gel, EA:EtOH:TEA = 4:1: trace; R_f of nitrile ca. 0.74, of intermediate ca. 0.63, and of desired product ca. 0.23). This required ca. 6 h. The reaction mixture was then cooled, turning from blue to yellow. The solid was removed by filtration and rinsed with 30 mL of cold MeOH, leaving 10.7 g, mp 203−205 °C.

2-(2-Phenoxathiinyl)-5-methyl-1,2,4-oxadiazole 10,10-Dioxide (39). a. Phenoxathiin 2-Amidoxime (77). A mixture of 15.78 g (0.07 mol) of crude 2-cyanophenoxathiin (**75**, Ar = phenoxathiin), 6 g (0.086 mol) of hydroxylamine hydrochloride, 7.4 g (0.088 mol) of powdered sodium bicarbonate, and 200 mL of 2-propanol were stirred at room temperature for 4 days. Samples partitioned between chloroform and water showed nearly complete lack of starting nitrile by TLC after 3.6 days (254 μm fluorescing silica gel, 1 EA:9 hexanes, R_f ca. 0.69). The reaction mixture was partitioned between chloroform and 200 mL of water twice, and each chloroform layer was back-washed with water and dried over $MgSO_4$. Removal of solvent at 80 °C in vacuo left 19.9 g from the first extract and 0.7 g from the second of yellow oils. These were crystallized and recrystallized from toluene/hexane, mp 91.6 °C. Anal. ($C_{13}H_{10}N_2O_2S$) Calcd: C, 60.45; H, 3.90; N, 10.84. Found: C, 60.56; H, 3.90; N, 10.68. TLC (silica gel, Et_2O:Hex = 1:1) showed a faint spot at the origin and a major spot with R_f 0.14. The starting nitrile had R_f 0.78.

b. 2-(2-Phenoxathiinyl)-5-methyl-1,2,4-oxadiazole (38). In a 100 mL flask equipped with an air condenser topped with a nitrogen demand system were placed 20.35 g (0.0788 mol) of phenoxathiin 2-amidoxime (**77**, Ar = phenoxathiin) and 100 mL of acetic anhydride. A few SiC chips and a small piece of dry ice were added, and the reaction mixture was heated to the reflux point over 1.5 h and under reflux for an additional 1.5 h. A sample still had a faint spot corresponding to starting material on TLC (silica gel, ether). Evaporation on steam/water aspirator left 21.9 g of a dark oil which on chromatography using 15:85 CH_2Cl_2:petroleum ether gave 0.62 g of nitrile **76**, with the calculated elemental analysis and melting point, and 5.16 g of crystals, identified by elemental analysis after recrystallization from hexanes as **38**. **39** was made from **38** by oxidation with excess 30% hydrogen peroxide in acetic acid warmed to ca. 80 °C overnight followed, **after testing for absence of peroxides**, by concentration on a water bath at reduced pressure to ca. one-fourth its volume and addition of water to the hot solution and suspended solid. The solid product was filtered from the cooled solution and recrystallized as tabulated.

Compound **39** was also made from 2-cyanophenoxathiin 10,-10-dioxide (**76**, Ar = phenoxathiin 10,10-dioxide). Anal. ($C_{13}H_7NO_3S$) Calcd: C, 60.69; H, 2.74; N, 5.45. Found: C, 60.63; H, 2.75; N, 5.42. Mp 253−254.8 °C. This compound was made by oxidation of 2-cyanophenoxathiin using H_2O_2 in hot AcOH by the procedure used to oxidize **38** to **39**. The resulting 2-cyanophenoxathiin 10,10-dioxide was converted to the amidoxime using NH_2OH− HCl and $NaHCO_3$ in 2-propanol. Anal. ($C_{13}H_{10}N_2O_4S$) Calcd: C, 53.78; H, 3.47; N, 9.65. Found: C, 53.73; H, 3.49; N, 9.53. Mp 194.5 °C.

2-(7-Methoxy-9-oxothioxanth-3-yl)-5-methyl-1,3,4-oxadiazole 10,10-Dioxide (42). Twenty milliliters of acetic anhydride, 2.485 g (0.068 mol) of 2-methoxy-6-(5-tetrazolyl)-thioxanthen-9-one 10,10-dioxide, and a few silicon carbide boiling chips were heated under reflux for 0.5 h and let cool overnight. The product was diluted with 30 mL of 30% Et_2O in hexanes, and the orange platelets were filtered off and rinsed with hexanes, mp 260.5 °C. It was then recrystallized from HOAc−water, yielding 1.18 g of orange platelets with unchanged melting point and single faint spot on TLC (fluorescent silica gel, Et_2O, visualized at 254 μm, R_f 0.50).

7-Isopropyl-9-oxo-3-(1*H*-tetrazol-5-yl)thioxanthene 5,5-Dioxide (49). A mixture of 20 g (0.064 mol) of 3-cyano-7-isopropylthioxanthen-9-one 10,10-dioxide (**76**), 4.37 g (0.067 mol) of sodium azide, 3.76 g (0.07 mol) of ammonium chloride, and 300 mL of DMF was stirred at 125 °C. **Hood!** TLC (silica gel, EtOAc: hexanes = 2:3) showed no starting nitrile and a major product with R_f 0.63. The reaction mixture was cooled, and poured into 400 mL of 2 N aqueous NaOH, and extracted with 300 mL of chloroform twice. The aqueous phase was

poured into excess of iced HCl, and the resulting yellow precipitate was filtered off, washed with water, dried, and recrystallized from EtOH−DMSO−water giving 12.4 g (55% of theoretical) of a yellow solid with appropriate proton NMR.

3-(2-Methyl-2*H*-tetrazol-5-yl)thioxanthen-9-one 10,10-Dioxide (51) and 3-(1-Methyl-1*H*-tetrazol-5-yl)thioxanthen-9-one 10,10-Dioxide (50). A suspension of 62.5 g (0.2 mol) of doxantrazole (**48**) in 700 mL of sieve-dried DMF was treated first with 24.8 g (0.22 mol) of potassium *tert*-butoxide and then, with cooling, with 43 g (0.3 mol) of methyl iodide. The reaction mixture was stoppered and stirred. The solution became homogeneous with the addition of the KO-*t*-Bu but deposited a little yellow solid after some time. After 20 h a sample was found to show pH of 7 with wetted test paper, and the reaction mixture was poured into 2500 mL of water containing 4 g of $NaHCO_3$ and warmed to ca. 50 °C. The yellow solid was filtered off, washed with water, and dried. TLC (silica gel, $CHCl_3$) revealed two spots with R_f values of 0.38 for the major and 0.20 for the lesser spot. Recrystallization, best from AcOH, gave the major material as an off-white solid, mp 206.8−209 °C. TLC (silica gel, 5% EtOAc in CH_2Cl_2) showed a single spot with R_f 0.60. The 2′-position of the methyl group was confirmed by X-ray diffraction. The mother liquor of such a recrystallization was evaporated to dryness in vacuo, and the residual solid was chromatographed on silica gel using 5% EtOAc in CH_2Cl_2 to give **50**, mp 270−271°C. TLC (silica gel, 5% EtOAc in CH_2Cl_2) showed a single spot with R_f 0.30.

Biological Methods: MAO Assays. MAO A and B were assayed by a radiometric procedure described earlier:[10] Rat brain mitochondrial MAO extract 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 μM (5 Ci/mol) and 10 μM (3 Ci/mol), respectively, in a total volume of 300 μL. Incubation was continued for 20 min. Pargyline at 2 mM was included in blank assays to inhibit all MAO activity. Reactions were 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 programmed for double-label counting. Assays were performed in triplicate at each concentration of inhibitor and are expressed as mean percent inhibiton with SEM within 5% of the mean. IC_{50} values were extrapolated from plots of mean percent inhibition vs log of inhibitor concentration. The above substrate concentrations are ca. 2 times the K_m values for serotonin and phenethylamine at MAO A and B sites, respectively, and at these concentrations the activity with each substance was independent of the other. This product 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 in vitro inhibitory potencies.

Dialysis. The reversibility of the MAO inhibition was determined by dialysis. EDTA-treated Spectra/Por (Fisher Scientific) membrane tubing with a 12 000-14 000 MW cut-off was used for this purpose. Mixtures (in duplicate) of buffer and MAO extract with and without test compound were preincubated at 37 °C for 15 min, and then a 1 mL portion of each mixture was dialyzed at 4 °C with shaking vs 40 mL of outer buffer (0.1 M potassium phosphate, pH 7.4, 5% sucrose, 1 mM dithiothreitol). Outer buffer was replaced with fresh buffer at ca. 3 and 19 h after the start of dialysis, and dialysis was terminated after 24 h. Undialyzed portions of each mixture were maintained at 4 °C over the same time period. Dialyzed and undialyzed mixtures were assayed for MAO activity at the same time in order to evaluate reversibility of the inhibition.

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