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BENZO-2,1,5-OXADIAZOLES—A NOVEL CLASS OF HETEROCYCLIC MONOAMINE OXIDASE INHIBITORS

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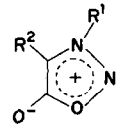
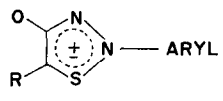
Abstract—The *in vitro* inhibition of monoamine oxidase (MAO) by a series of oxazoles, oxadiazoles, benzoxadiazoles and related heterocycles was tested. An increase in inhibitory activity was observed from the monocyclic oxadiazole, through the bicyclic benzoxadiazole to tricyclic compounds such as naphthofurazan and furoxanobenzofuroxan. Furoxanobenzofuroxan was the most potent inhibitor of the series, with a K_i of 4×10^{-7} M.

REPORTS by Cameron and Wiseman[1,2] of the monoamine oxidase (MAO) inhibitory properties of heterocyclic mesoionic compounds corresponding to the structures I and II, prompted us to describe the *in vitro* MAO inhibitory properties of five-membered heterocycles embraced by the general formulae III and IV.



(I)



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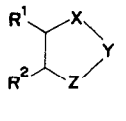

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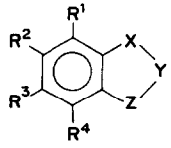


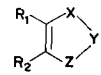
(IV)

The heterocyclic systems thus defined includes the oxazoles (III, X = O, Y = C, Z = N), thiazoles (III, X = S, Y = C, Z = N), isoxazoles (III, X = C, Y = O, Z = N), isothiazoles (III, X = C, Y = S, Z = N), 2,1,5-oxadiazoles (III, X = Z = N, Y = O) and 2,1,5-thiadiazoles (III, X = Z = N, Y = S) and their corresponding benzo derivatives (IV).

EXPERIMENTAL

All compounds were prepared by known methods, references to which are provided in Tables 1 and 2; or were obtained from commercial sources.

TABLE 1.



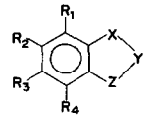
No.	X	Y	Z	R_1	R_2	Ring system	Ref.	K_i(M)
1	O	CH	N	$COCH_3$	CH_3	oxazole	9	6×10^{-3}
2	O	CH	N	$CONH_2$	CH_3	oxazole	10	3×10^{-3}
3	O	CH	N	$\left\{\begin{matrix}CH_3\\C{=}NNHTOS^*\end{matrix}\right\}$	CH_3	oxazole	11	6×10^{-5}
4	O	C—CH_3	N	$COCH_3$	CH_3	oxazole	9	4×10^{-4}
5	O	C—CH_3	N	CH_3	$COCH_3$	oxazole	12	3×10^{-3}
6	O	C—CH_3	N	CH_3	$C(NOH)CH_3$	oxazole	12	4×10^{-5}
7	O	C—CH_3	N	$\begin{matrix}CH_3\\C{=}NNHTOS^*\end{matrix}$	CH_3	oxazole	11	1×10^{-5}
8	O	C—CH_3	N	$\left\{\begin{matrix}CH_3\\C{=}NNHPh\end{matrix}\right\}$	CH_3	oxazole	11	1×10^{-5}
9	O	N	CH	H	H	isoxazole	13	8×10^{-3}
10	O	N	C—CH_3	CH_3	H	isoxazole	14	3×10^{-3}
11	O	N	C—CH_3	CH_3	CH_3	isoxazole	15	6×10^{-4}
12	O	N	C—CH_3	CH_3	NO_2	isoxazole	14	2×10^{-4}
13	S	CH	N	H	H	thiazole	16	1×10^{-2}
14	S	C—CH_3	N	H	C—CH_3	thiazole	16	1×10^{-2}
15	S	C—CH_3	N	Ph	Ph	thiazole	17	2×10^{-5}
16	S	C—NH_2	N	Ph	H	thiazole	17	2×10^{-4}
17	S	N	CH	H	H	isothiazole	16	3×10^{-3}
18	S	N	C—CH_3	H	H	isothiazole	16	2×10^{-3}
19	S	N	CH	H	CH_3	isothiazole	16	2×10^{-3}
20	S	N	CH	CH_3	H	isothiazole	16	1×10^{-3}
21	S	N	CH	H	Br	isothiazole	16	2×10^{-3}
22	S	N	CH	H	NO_2	isothiazole	16	4×10^{-4}
23	N	O	N	H	H	oxadiazole	18	4×10^{-3}
24	N	O	N	CH_3	CH_3	oxadiazole	17	7×10^{-5}

* TOS = *p*-toluene sulphonyl.

MAO preparation. The enzyme source was a washed mitochondrial fraction from rat liver, prepared as described by Hogeboom,[3] from the livers of male Sprague–Dawley rats. The enzyme oxidised 0·019 μmoles of tryptamine per min per mg protein under the assay conditions (substrate concentration 5×10^{-5} M). The protein content (0·31 mg/ml) was measured by the method of Lowry *et al.*,[4] using bovine serum albumin (Sigma Chemical Co.) as standard. The enzyme preparation was stored at −20° for up to 2 months without loss of activity.

MAO assay. MAO activity was measured by the method of Wurtman and Axelrod[5] using ^{14}C-tryptamine (New England Nuclear Corporation 10·7 mCi/mM) diluted with unlabelled tryptamine (Sigma Chemical Co.) as substrate. Assays contained 4 mg enzyme protein, substrate in the concentration range 5×10^{-5} to 5×10^{-6}, and potassium phosphate buffer, (0·1 M, pH 7·4) in a total volume of 2 ml. The primary reaction product was indoleacetaldehyde[6] which was extracted into acidified toluene. Radioactivity in the toluene extract was measured in a Packard Tri-Carb Liquid Scintillation Spectrometer using Packard Scintillants.

TABLE 2.



No.	X	Y	Z	R_1	R_2	R_3	R_4	Ring system	Ref.	K_i(M)
25	O	CH	N	H	H	H	H	benzoxazole	17	5×10^{-5}
26	O	C—CH_3	N	H	CH_3	H	H	benzoxazole	17	2×10^{-4}
27	O	C—SH	N	H	Cl	H	H	benzoxazole	17	6×10^{-5}
28	N	O	C—CH_3	H	H	H	H	anthranil*	19	5×10^{-6}
29	N	O	C—CH_3	H	NO_2	H	H	anthranil*	20	1×10^{-5}
30	S	CH	N	H	H	H	H	benzthiazole	17	1×10^{-3}
31	S	C—CH_3	N	H	NH_2	H	H	benzthiazole	17	2×10^{-3}
32	S	C—NH_2	N	H	H	H	H	benzthiazole	17	2×10^{-4}
33	S	C—NH_2	N	H	Cl	H	H	benzthiazole	17	4×10^{-4}
34	S	C—SH	N	H	H	H	H	benzthiazole	17	1×10^{-4}
35	N	S	CH	H	H	H	H	thio-anthranil†	21	2×10^{-4}
36	N	S	CH	CH_3	H	H	H	thio-anthranil†	21	3×10^{-5}
37	N	S	CH	H	Cl	H	H	thio-anthranil†	21	6×10^{-6}
38	N	S	CH	H	H	Cl	H	thio-anthranil†	21	3×10^{-5}
39	N	S	CH	NO_2	H	H	H	thio-anthranil†	21	3×10^{-5}
40	N	O	N	H	H	H	H	benzofurazan‡	22	4×10^{-5}
41	N	O	N	NO_2	H	H	H	benzofurazan‡	23	2×10^{-5}
42	N	O	N	NH_2	H	H	H	benzofurazan‡	24	3×10^{-5}
43	N	O	N	NO_2	H	H	$N(CH_3)_2$	benzofurazan‡	25	3×10^{-5}
44	N	O	N	NO_2	H	H	NHPh	benzofurazan‡	26	3×10^{-6}
45	N	O	N	NO_2	H	H	N CH_3/Ph	benzofurazan‡	26	4×10^{-6}
46	N	O	N	NO_2	H	H	$NHCH_2Ph$	benzofurazan‡	26	2×10^{-5}
47	N	O	N	Br	OH	H	H	benzofurazan‡	27	1×10^{-4}
48	N	O	N^+—O^-	H	H	H	H	benzofuroxan§	22	8×10^{-6}
49	N	O	N	C_4H_4		H	H	naptho-furazan‡	28	6×10^{-7}
50	N	O	N^+—O^-	C_4H_4		H	H	naptho-furoxan‡	28	2×10^{-6}
51	N	O	N	N_2O_2		H	H	furoxano-benzofurazan‡§	29	1×10^{-6}
52	N	O	N^+—O^-	N_2O_2		H	H	furoxano-benzofuroxan‡§	29	4×10^{-7}
53	N	S	N	H	H	H	H	benzo-thiadiazole	17	1×10^{-4}
54	N	S	N	NO_2	H	H	H	benzo-thiadiazole	17	1×10^{-5}
55	N	S	N	H	Cl	H	H	benzo-thiadiazole	30	4×10^{-5}

* Anthranil = benzisoxazole.

† Thioanthranil = benzoisothiazole; kindly supplied by Dr. M. Davis, La Trobe University, Melbourne.

‡ Benzofurazan = benzo-2,1,5-oxadiazole.

§ Benzofuroxan = benzo-2,1,5-oxadiazole-1-oxide.

Reaction velocity was linear under these conditions for at least 30 min so that reaction rates measured after incubation periods of 15 min reflected true initial velocities. K_m and V_{max} values were computed from the results by the method of Wilkinson[7] and inhibition constants were determined from these values by the method of Friedenwald and Maengwyn-Davies.[8]

RESULTS AND DISCUSSION

The *in vitro* molar inhibition constants (K_i) for the heterocycles described are recorded in Tables 1 and 2.

The effects of inhibitors on Michaelis constants and maximum velocity values of the enzyme system showed that inhibition was in all cases of the mixed competitive and non-competitive types.

In the oxazole series the introduction of methyl substituents lowered the inhibition constant by 5–10 times, i.e., increased activity. A similar trend was observed for the isoxazole series, although the activity of these compounds was overall less than the oxazoles. The phenyl and *p*-toluene sulphonyl hydrazones of the 5-acetyloxazoles (cpds 3, 7 and 8) were moderately potent inhibitors of this enzyme system, a property probably due to the presence of the hydrazone function in these molecules.

The thiazoles and isothiazoles were less active than their oxygen analogues. The activating effect of methyl groups observed for oxazoles and isoxazoles was absent in the sulphur systems. The introduction of one or more aryl groups (cpds 15 and 16) in the 4- and 5- positions of the thiazole nucleus greatly enhanced activity, suggesting the presence of hydrophobic bonding areas adjacent to the site of action on the enzyme surface. A nitro group in the isothiazole 4- position (cpd 22) also raised activity relative to the parent. The introduction of a ring bound nitrogen atom into the isoxazole 3- position to produce the 1,2,5-oxadiazoles (cpd 23) in itself did little to increase enzyme inhibition. However the addition of methyl groups in the oxadiazole 3- and 4- positions (cpd 24) raised the activity of the oxadiazole parent to an extent greater than was observed for the oxazoles and isoxazoles.

The addition of hydrophobic groupings to oxazoles, isoxazoles, thiazoles and oxadiazoles greatly enhanced *in vitro* MAO inhibition (Table 1). Therefore, the molecules derived by fusion of the heterocycle with a benzene ring were considered likely to provide more effective inhibitors of this enzyme. Table 2 records the results of such a study. The unsubstituted benzoxazole molecule (cpd 25) was more active than the simple monocyclic relative; however, the introduction of methyl groups into the 2- and 6- positions (cpd 26) was detrimental to activity. The fusion of the isoxazole ring with a benzene ring to produce the anthranils revealed an even greater increase in activity, 3-methyl anthranil (cpd 28) having approximately one thousand times the activity of its monocyclic relative. The addition of a nitro group onto the anthranil system (cpd 29) caused, in contrast to its monocyclic analogue, a decrease in activity.

The benzothiazoles generally revealed a lower level of activity than the benzoxazoles. The highest inhibition resulted from the presence of a mercapto group in the 2- position of benzothiazole (cpd 34). The thioanthranils, on the other hand, showed a moderate activity, 6-chloro thioanthranil (cpd 37) having a molar inhibition constant of 6×10^{-6}. The substitution of a methyl group in the 7- position of thioanthranil (cpd 36) also increased activity relative to the unsubstituted parent (cpd 35).

The most interesting system studied was the benzo 2,1,5-oxadiazoles. Progressive enhancement of enzyme inhibition was observed with (a) benzene fusion; (b) *N*-oxide formation; and (c) addition of a second oxadiazole ring to the aromatic nucleus.

Thus the fusion of a single benzene ring to the oxadiazole nucleus to give compound 40 raised activity 100 times. Further annulation to the naphthofurazan (cpd 49) increased parent heterocycle activity some 10,000 times.

The *N*-oxide (cpd 48) of benzo-2,1,5-oxadiazole raised activity by a factor of approximately 10, while the combination of an additional oxadiazole ring and two oxides (cpd 52) produced the most potent compound of the series, furoxanobenzofuroxan (4,5-oxadiazolobenzoxadiazole dioxide) with a molar K_i of 4×10^{-7}.

The presence of substituents on the benzene ring of benzoxadiazoles had negligible effect on biological activity, generally. Thus, benzoxadiazole (cpd 40), 4-nitro benzoxadiazole (cpd 41) and 4-amino benzoxadiazole (cpd 42) possessed comparable activities. This suggests that the extent of binding of these molecules with the enzyme is largely predetermined by the benzheterocycle itself. On the other hand, the presence of additional aryl groups on the nitrogen function as in 4-anilino-7-nitrobenzoxadiazole (cpd 44) and 4-methylanilino-7-nitrobenzoxadiazole (cpd 45) is advantageous to biological activity.

The benzo-2,1,5-thiadiazoles (cpds 53, 54 and 55) were less active than the correspondingly substituted benzo-2,1,5-oxadiazole. The finding possibly reflects the lower electronegativity of sulphur relative to oxygen which would undoubtedly influence the binding of these molecules to the enzyme surface.

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