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Activity of Novel Tryptophan Analogs against Mammalian and Trypanosomal Monoamine Oxidases

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Monoamine oxidases were assayed in live *Trypanosoma brucei brucei* and in trypanosomal homogenate using the oxygen electrode method. Serotonin and tryptamine were used as standard monomine oxidase A and B substrates, respectively. The ability of live trypanosomes to metabolize tryptamine and serotonin was also monitored by the more sensitive high performance liquid chromatography method. No measurable enzyme activity could be detected in either live trypanosomes or trypanosomal cell homogenates. These results obtained suggest that *T. b. brucei* do not possess monoamine oxidase activity. Thus trypanocidal tryptophan analogs that were previously thought to act through inhibition of monoamine metabolizing enzymes may be acting by a diferent mechanism. The activity of these tryptophan analogs against mammalian MAO was tested to establish their potential toxicity in man. Two compounds, 5-(1-benzenesulphonylindol-2-ylidene)-5-methoxy-3-ethyl-thiazolidene-2-thione and 5-(1-benzenesulphonylindol-2-ylidene)-3-methylthiazolidine-2-thione had significant activity against mammalian monoamine oxidase. The enzyme kinetics for the latter was also derived.

Key Words: *Trypanosoma brucei brucei*, monoamine oxidase, tryptophan metabolites.

INTRODUCTION

Trypanosomes causing African trypanosomiasis are known to have a high capacity to catabolize aromatic amino acids [1,2]. Tryptophan, for instance is broken down to the indole pyruvate, indole lactate and indole-3-ethanol via the aminotransferase pathway [3,4]. Indole-3-ethanol is neuroactive and is thought to induce the CNS pathology observed in the later stages of trypanosomiasis [5-7].

Mammalian enzymes on the other hand catabolize trytophan using monoamine oxidase (MAO) to tryptamine, 5-hydroxytryptamine (serotoning, 5-HT) and melatonin among other products [7]. However, it has not been shown whether the trypanosomes produce these products and are able to metabolize them further [1-4]. It is noteworthy that tryptamine, 5-HT and their metabolites are neuroactive and could contribute to the pathogenesis of trypanosomiasis [8].

Certain amino acid analogs possess trypanocidal properties. Dithroromethyl orhithine (DFMO) is marketed for treatment of *Typanosoma rhodesiense* infection [9]. The trypanocidal potential of synthetic tryptophan analogs has been reported previously [10]. In the present study, we set out to determine whether the activity of nine tryptophan analogs (figure 1) can be attributed to inhibition of parasite MAO.

EXPERIMENTAL

Buffers

HGRP buffer for maintenance of *T.b. brucei* during the experiments consisted of N-hydroxyethylpiperazine - N'-2–ethanesulphonic acid sodium, HEPES (25 mM), Na_2HPO_4 (30 mM), $Ca(NO_3)_2$ (0.42 mM), KCl (5.4mM), $MgSO_4$ (0.41 mM), NaCl (100 mM), glucose (56 mM), pH 7.4.

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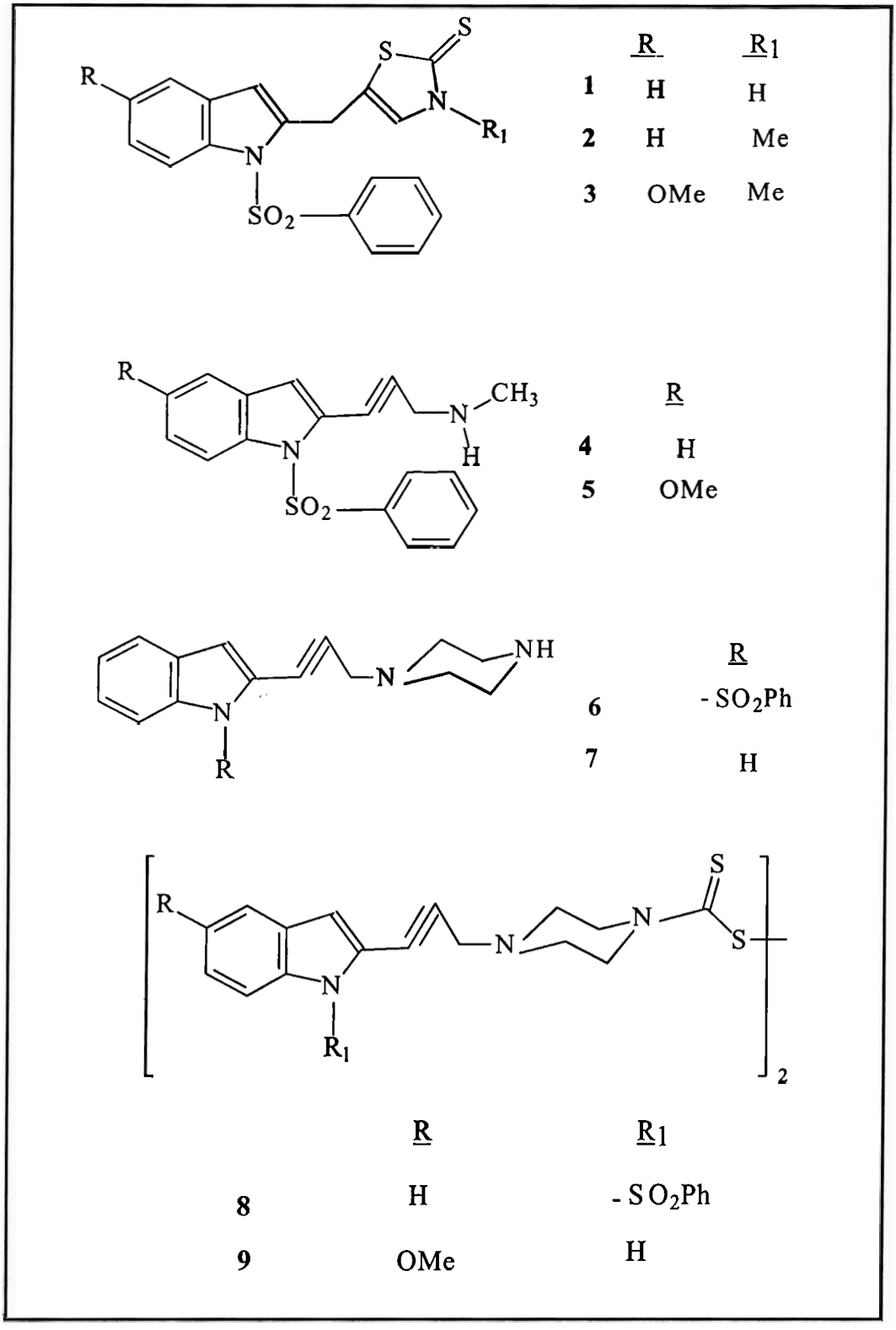

	R	R1
1	H	H
2	H	Me
3	OMe	Me

	R
4	H
5	OMe

	R
6	- SO_2Ph
7	H

	R	R1
8	H	- SO_2Ph
9	OMe	H

Figure 1: Structures of the tryptophan analogs under study

Homogenizing buffer was used during parasite and liver tissue homogenization with the aid of acid washed beads. It consisted of sucrose (250 mM) and TRIS (10 mM for parasite and 3.4 mM for liver homogenates respectively).

Liver tissue

Two Witstar rats (male and female), were sacrificed and their livers removed and washed in ice-cold homogenizing buffer to remove excess blood before use.

Oxygen consumption by *T.b. brucei*

Trypanosoma brucei brucei trypomastigotes (S4 27/118) were obtained from infected rat blood [11] and maintained in HGRP buffer. The rate of oxygen consumption was measured for 2 minutes using a Clarke-type oxygen electrode in a stirred solution kept at 37 °C [12]. This was carried both with and without the MAO substrates, tryptamine and 5-HT. During the experiment oxygen utilization via the glycerol phosphate was arrested by use of salicylhydroxamic acid. The presence of oxygen was confirmed by addition of dithionate.

MAO activity of *T. b. brucei* homogenates

Purified trypanosomes were homogenized in homogenizing buffer and acid washed beads. The homogenate was treated to centrifugation and protein content determination by the Biorad protein assay technique [13]. The procedure was designed to incorporate nuclei and large organelles in the final homogenate. Oxygen consumption was measured in the presence of 5-HT and tryptamine.

Determination of MAO metabolites using liquid chromatograhy

Trypanosoma brucei brucei trypomastigotes and rat liver homogenate were separately incubated in HGRP buffer enriched with 5-HT and tryptamine for 2 h. The control solutions incorporated neat buffer, buffer with tryptamine, buffer with 5-HT and liver homogenate alone. The viability of the trypanosomes was checked microscopically half hourly. At the end of the incubation period, protein was precipitated using trichloroacetic acid and centrifuged off. The supernatant was subjected to high performance liquid chromatography (LC) analysis on RP C_{18} silica column (250 x 4.6 mm, 5 μm). The mobile phase consisted of acetate buffer- acetonitrile, pH 4.76, pumped on a gradient for optimum elution. The eluents were monitored at 265 nm.

Liver MAO activity

Rat liver homogenates were incubated in homogenizing buffer enriched with KCN (3.33 mM) containing tryptamine and 5-HT. Oxygen consumption was measured and the enzyme activity recorded. The results were fitted into the Michaelis–Menten (MM) and Lineweaver-Burke (LB) plots to obtain the V_{max} and K_m values [14].

Liver MAO inhibition

Two known MAO inhibitors clorgylline (MAO-A) and L-deprenil (MAO-B) were used for this purpose). The substrates, used were 5HT and 2-phenylethylamine. Initially 0.1 ml of KCN (100 mM), 0.3 ml rat liver homogenate and 0.1 ml of the inhibitor solution were added into the reaction chamber. After 2 min, 0.1 ml of substrate solution was added. The oxygen consumption per gram of protein was determined. The control setup did not incorporate the inhibitor substances. Percentage inhibition was calculated with reference to the controls. This procedure was repeated for 6 inhibitor concentrations ranging 0-200 μM. From a plot of percentage inhibition against concentration, the IC_{50} was determined.

MAO inhibition by test compounds

The compounds were dissolved in dimethylsulfoxide (DMSO) and introduced into rat liver homogenates. The substrates, 5HT and 2-phenylethylamine were then added and the oxygen consumption measured. The percentage inhibition of MAO by the test compounds is shown in table 1. Compound 2 was further investigated for the kinetics of its MAO inhibition. The compound was applied in the concentration range 10-100 μM against 3 concentrations of the two substrates (K_m, $2K_m$ and $3K_m$). The data obtained for MAO-A were analyzed and fitted into a Dixon plot [15]. The IC_{50} for the compound was determined as described above.

RESULTS AND DISCUSSION

Figure 2 shows the oxygen consumption of live parasites and liver homogenates in the presence of MAO substrates. The parasites did not show MAO activity as indicated by the level curve. Furthermore, even *T.b. brucei* homogenates produced the same results. This shows that lack of MAO activity is not due to non-penetration of the substrates in the parasite cells. This observation is further supported by the HPLC assay, whereby no peaks corresponding to MAO metabolites were detected. This being a highly sensitive method (compared to the oxygen electrode technique) trace amounts of substrates could be detected.

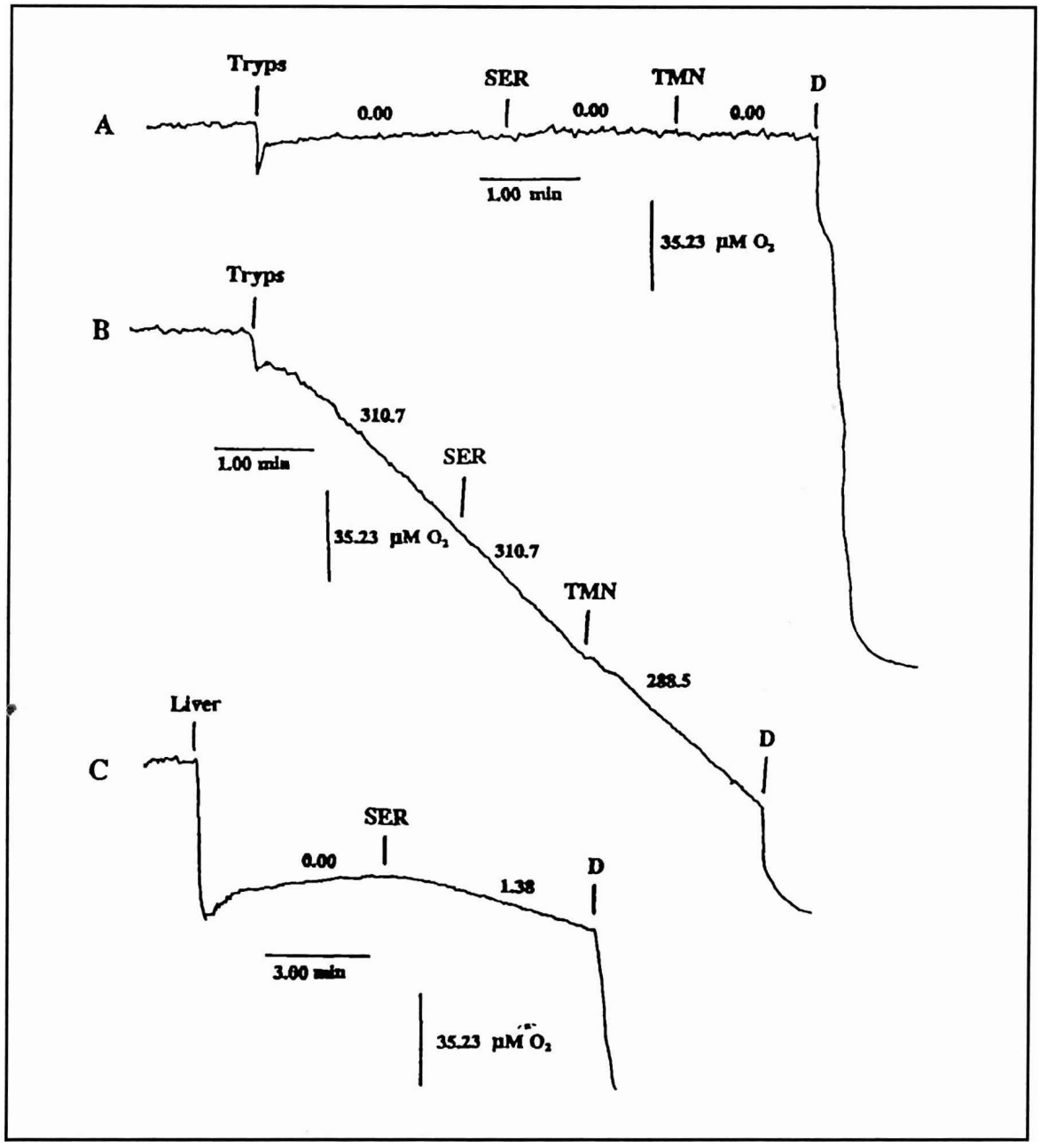

Fig. 2: Oxygen consumption by Trypanosoma brucei brucei; A salicylhydroxamic acid present; B No salicylhydroxamic acid; C Rat liver homogenate in presence of KCN SER: Serotonin: TMN: Tryptamine; D Dithionate. The numbers refer to oxygen consumption (µM/min) by 108 trypanosomes (A, B) and by 1 mg of protein (C).

Liver homogenate on the other hand readily converted tryptamine into the 5-indole acetate, 5-hydroxy indole acetate and 5-hydroxyindole ethanol as confirmed by HPLC analysis (Figure 3).

The antitrypanosomal activity of the compounds tested does not occur through parasite MAO inhibition since the parasites are devoid of MAO activity [10]. Thus *T.b. brucei* represents a unique biochemical system amongst protozoans. Majority of protozoa are reported to metabolize MAO substrates [16-19]. *T.b. gambiense* can convert 5-HT to 5-hydroxylindole ethanol via the transaminase pathway [20]. However, trypanosomes are unable to convert tryptophan to 5-HT [1]. The neuropathology of trypanosomiasis needs to be studied further.

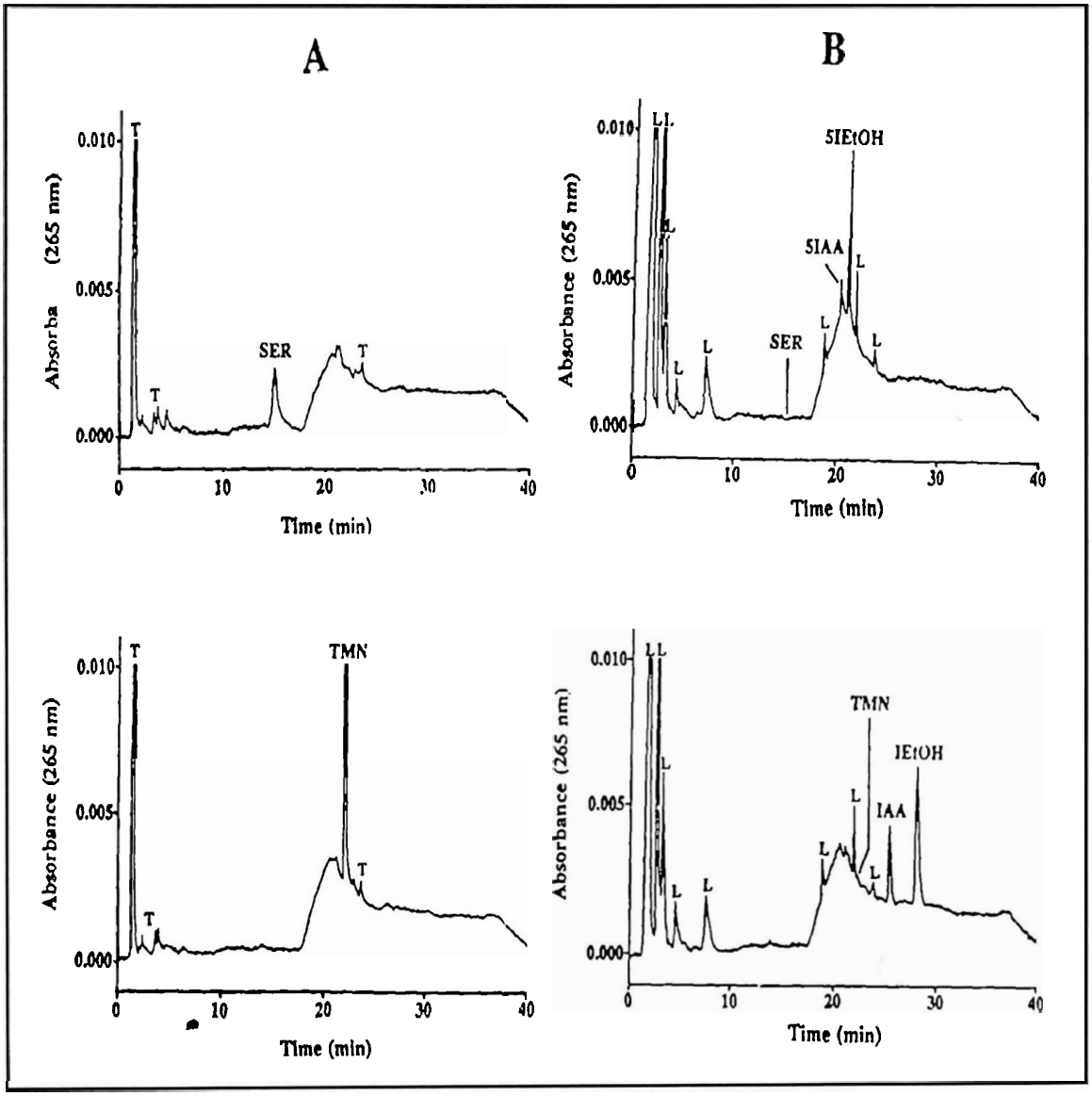

Fig 3: HPLC analysis of monoamine oxidase metabolites in (A) Trypanosoma brucei brucei (5 x 107 ml-1 in HGRP buffer), or (B) rat liver homogenate 1500 x g supernatant (3.60 mg ml-1 protein in HGRP buffer) incubated for 2 hours at 37 ^{0}C SER: serotonin TMN: tryptamine; 5-IetOH; 5-hydroxyindole ethanol; IAA; indole acetate; IetOH; indole ethanol; T, peaks found in trypanosome controls alone; L, peaks found in liver homogenate controls alone.

Out of the nine tryptophan analogs tested for MAO inhibition, only compounds 2 and 3 showed significant activity against mammalian MAO (Table 1). Compound 2 was a more potent inhibitor of MAO. Both compounds showed higher inhibition of MAO-B relative to MAO-A (Figure 4). Nevertheless, compound 2 was more than x 200 weaker in both MAO-A (IC_{50}, 57 μM) and MAO-B (IC_{50}, 25 μM) inhibition than clorgylline (IC_{50}, 0.09 μM) and deprenil (IC_{50}, 0.125 μM) (Figure 4). Compound 1 differs from compound 2 by a N-Me group. This implies that indole N-methylation decreases MAO activity of 2. The Dixon plot (Figure 5) yielded straight curves for K_m, $2K_m$ and $3K_m$ intersecting the X-axis at the Ki = 60. This clearly shows that compound 2 inhibits MAO-A in a non-competitive way.

Table 1: Inhibition of rat liver MAO by test compounds

	% Inhibition at [I] = 100 μM; [S] = $2K_m$	
Compound	**MAO –A**	**MAO-B**
1	12	16
2	40	82
3	18	52
4	8	-
5	5	6
6	-	-
7	5	3
8	-	4
9	-	3

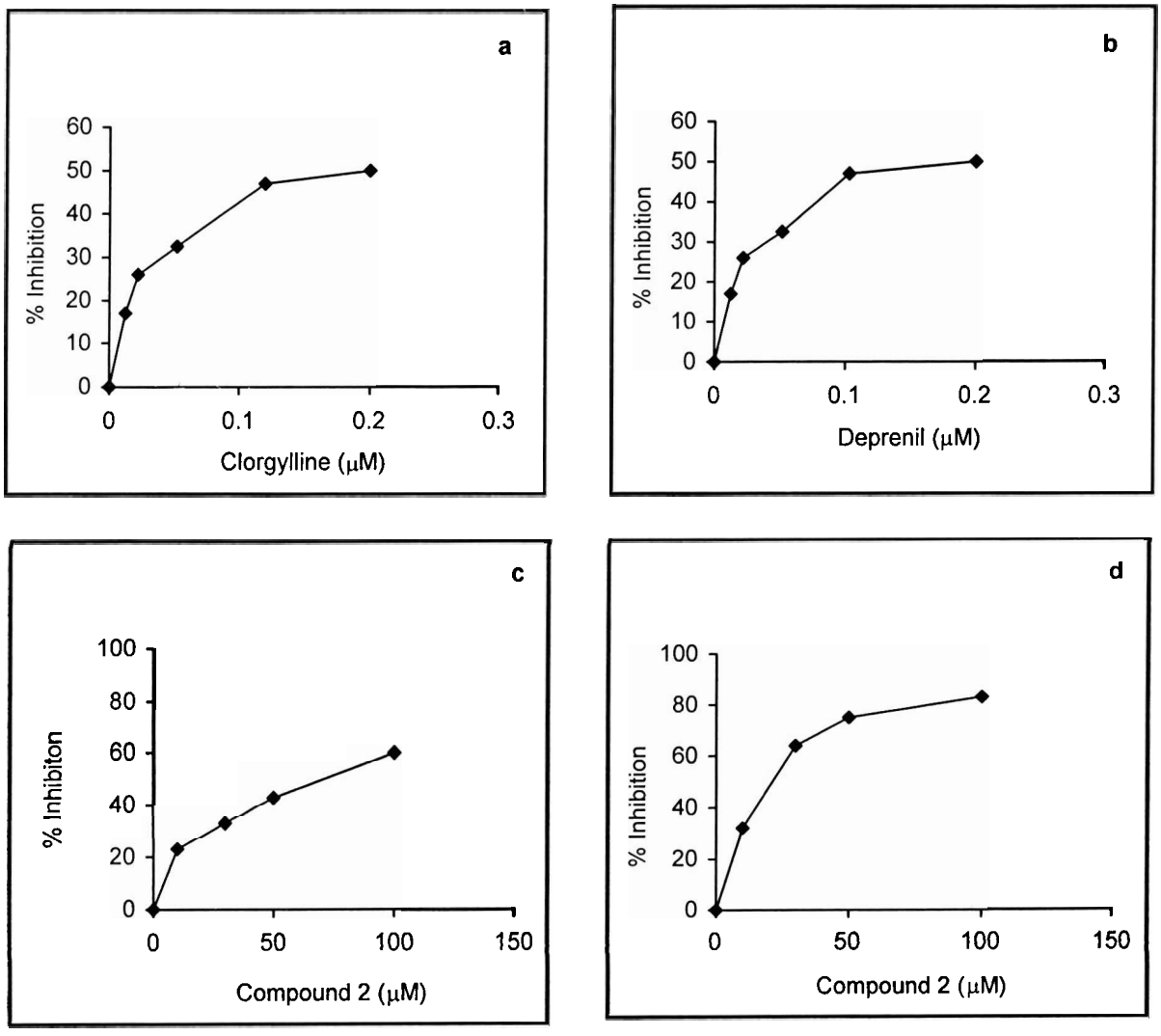

Figure 4: Inhibition (Percentage) of (a) Monoamine oxidase A by Clorgylline (b) Monoamine oxidase B by deprenil (c) Monoamine oxidase A by compound 2 (d) Monoamine oxidase B by compound 2

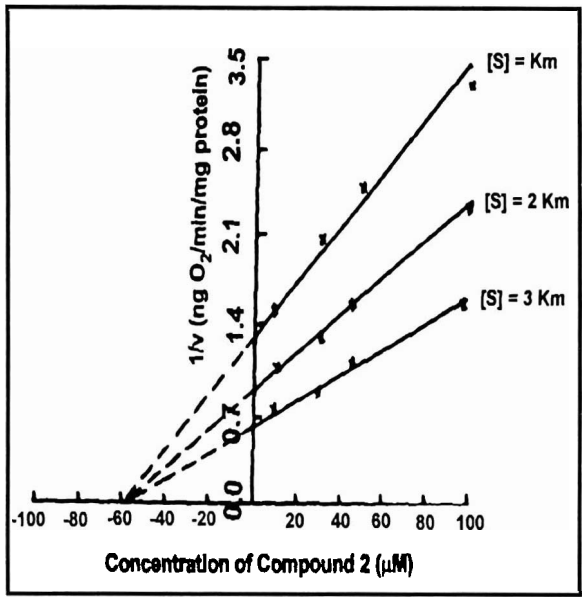

Figure 5: Dixon plot for inhibition of monoamine oxidase A by compound 2

CONCLUSION

The results obtained from the study show that *T. b. brucei* do not metabolize 5-HT and tryptamine implying that the parasites have no measurable MAO activity. This finding suggests that novel tryptophan analogs, which have been found to have antitrypanosomal activity, do not act by interfering with tryptophan metabolism as originally expected. This is supported by the observation that these compounds do not inhibit mammalian MAO. The thiazolidine thione derivatives of indole (with no acetylenic function), 2 and 3, which weakly inhibit MAO in a non-competitive way possess no antitrypanosomal activity.

REFERENCES

[1] H.H. Stibbs and J.R. Seed. Experientia 29 (1973) 1953-1965.

[2] H.H. Stibbs and J.R. Seed. Int. J. Biochem. 6 (1975) 197-203.

[3] J.E. Hall and J.R. Seed. Comp. Biochem. Physiol. 68B (1981) 521-526.

[4] J.E. Hall and J.R. Seed. Comp. Biochem Physiol. 69B (1981) 791-796.

[5] R. Fukimori, A. Minegishi, T. Satoh, H. Kitagawa and S. Yanaura. Psychopharmacology 69, (1980) 243-246.

[6] J.R, Seed, T.M. Seed. and J. Sechelski. Comp. Biochem. Physiol. 60C (1978) 175-185.

[7] D.A. Bender. Amino Acid Metabolism, John Wiley and Sons, London. 1976. pp 155-191.

[8] T.A. Lambo. Acta Psychiatri. Scand. 42 (1966) 474-484.

[9] A. Sjoedesma and P.J. Schechter. Clin. Pharmacol. Ther. 35 (1984) 287-300.

[10] S. Mung'ong'o, V.K. Mugoyela, A.F. Haule, M. Hooper and S. Croft. East Cent. Afri. J. Pharm. Sci. 2 (1999) 8-11.

[11] S.M. Lanham. Nature 218 (1968) 1273-1274.

[12] A. Shapiro, S.H. Hutner, L. Katz and C.J. Bacchi, J. Protozool. 28 (1981) 3370-3377.

[13] D.F. Weetman and A.J., Sweetman, Anal. Biochem. 41 (1971) 517-521.

[14] Price, N. C., Stephens, L., Fundamentals of Enzymology, Oxford University Press, 2nd Edition.1989. pp 139 –178.

[15] Dixon, M., Biochemical J. 55 (1953) 170.

[16] N.N. Sharma and G.H. Bourne. 17 (1964) 293-301.

[17] N.N. Sharma and G.H. Bourne. Acta Histochem. 27 (1967) 257-266.

[18] K. Janakidevi, V.C. Dewey and G.W. Kidder. J. Biol. Chem. 241 (1966) 2576-2578.

[19] J.M. Feldman, J.M.Roche and J.J. Blum. J. Protozoal. 24 (1977) 459-462.

[20] H.H. Stibbs. J. Parasitol. 70 (1984) 428-432.

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ABBREVIATIONS

Ångström	Å	Gram-molecule	mol	Minimum inhibitory concentration	MIC
Atmosphere	Atm	Hertz	Hz	Molar concentration	M
Atomic weight	at. wt.	High frequency	h.f.	Month	month
Boiling point	b.p.	High pressure liquid chromatography	HPLC	Nanometer	nm
Calorie	cal	Hour(s)	h	Nanomole	nmol
Centimeter	cm	Infrared	i.r.	Normal concentration	N
Central nervous system	CNS	Internal diameter	i.d.	Nuclear magnetic resonance	NMR
Column chromatography	CC	International unit	I.U.	Ohm	Ω
Company	Co.	Joule	J	Outside diameter	o.d.
Corporation	Corp.	Kilocalorie	kcal	Picomole	pmol
Correlation coefficient	r	Kilogram	kg	Probability	P
Coulomb	C	Kilometer	km	Paper chromatography	PC
Counts per minute	cpm	Kilovolt	kV	Proton magnetic resonance	^{1}H-NMR
Counts per second	cps	Kilowatt	kW	Radio-frequency	r.f.
Cubic centimeter	cm^3	Kilowatt-hour	kWh	Relative humidity	r.h.
Cubic inch	in^3	Liter	l	Relative standard deviation	RSD
Cubic meter	m^3	Liquid chromatography	LC	Revolutions per minute	rpm
Cycles per second	$c\ s^{-1}$	Logarithm	log	Root mean square	r.m.s.
Day(s)	day(s)	Logarithm (natural)	ln	Second(s)	s
Degrees		Megaelectron volts	MeV	Square foot	ft^2
Celsius	°C	Melting point	m.p.	Square meter	m^2
Centigrade	°C	Meter	m	Standard deviation	SD
Kelvin	K	Microgram	µg	Standard error	SE
Degrees of freedom	df	Microliter	µl	Standard temperature and pressure	S.T.P.
Direct current	d.c.	Micrometer	µm	Thin-layer chromatography	TLC
Disintegrations per minute	dpm	Micromolar	µM	Ultraviolet	UV
Disintegrations per second	dps	Micromole	µmol	Versus	vs
Dyne	dyn	Millicurie	mCi	Volt	V
Electromagnetic force	e.m.f.	Milliequivalent	mEq	Volt-ampere	VA
Electron spin resonance	ESR	Milligram	mg	Volt-coulomb	VC
Electron volt	eV	Milliliter	ml	Volume	vol.
Erg(s)	erg(s)	Millimeter	mm	Volume by volume	v/v
Feet, foot	ft	Millimolar	mM	Watt	W
Freezing point	f.p.	Millimole	mmol	Watt-hour	Wh
Gas-liquid chromatography	GLC	Millisecond	ms	Weight	Wt
Gauss	G	Milliosmolar	mOsM	Weight by weight	w/w
Gram	g	Minute(s)	min	Weight by volume	w/v