Antitrypanosomal Activity of Novel Tryptophan Analogs

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A range of 16 novel tryptophan analogs designed as enzyme-activated inhibitors of FAD and Pyridoxal Phosphate - dependent enzymes containing acetylenic, alkenyl and/or dithiocarbamate moieties were tested for *in vitro* activity against *Trypanosoma brucei brucei.* Nine of these compounds, l-(indole-2-yl)-2-piperazinomethyl ethyne, 1-(1-benzenesulphonylindol-2-yl)-2-piperazinomethyl ethyne, 1-(1-benzenesulphonylidol2-yl)-2-(4-methylpiperazinomethyl) ethyne, 5-(methoxyindol-2-yl)-2-piperidino methylethyne, 1-(1-benzenesulphonylindol-3-yl)-2-(4-methylpiperazinomethyl) ethyne, 1(1-benzenesulphonyl-5-methoxyindol-3-yl)-2-(4-methylpiperazinomethyl) ethyne, bis [1-thiocarbonyl-4-(3-(1 -benzene-sulpholindol-2-yl} prop-2-ynyl) piperazine] disulphate, bis[1 -thiocarbonyl-4-(3-{indole-2-yl} prop-2-ynyl) piperazine] disulphide and bis(1 -thiocarbonyl-4-(3-{5-methoxyindol-2-yl)prop-2-ynyl) piperazine) disulphide were found to have a significant inhibitory activity at micromolar concentrations.

The structure-biological activity relationships for the novel compounds is discussed with reference to the most active compound, 1-(1-benzenesulphonylindol-2-yl)-2-(4-methylpiperazino methyl) ethyne. Possible chemical modifications on the most active "lead" compounds in order to modulate the antitrypanosomal activity and explore the possible modes of action are presented.

Key Words: *Trypanosoma brucei brucei;* Tryptophan analogs; Antitrypanosomal activity

INTRODUCTION

Human African trypanosomiasis remains a significant public health problem in many parts of Sub-Saharan Africa. In 1993, the World Health Organization estimated the number of new cases to be 25,000 throughout Africa, with 50 million people at risk of infection [1]. Trypanosomiasis in domestic animals is also a major problem in many parts of rural Africa, with approximately 25 million cattle exposed to the risk of infection. This scourge denies many Africans a major source of food and income. [2,3]. The drugs currently used for the treatment of human and animal trypanosomiasis, such as suramin, pentamide, the arsenicals and phenanthridines (for animals), were introduced early this century. They are either too toxic, ineffective against some stages and forms of the disease or resistance has developed due to the persistent use of the same drugs over many years [4]. The newly introduced drug, difluoromethylornithine (DFMO) is effective only against Gambian trypanosomiasis and requires a very large dosage necessitating frequent daily administrations of the drug by injection [5]. There is therefore an urgent need for new drugs for the treatment of trypanosomiasis. The trypanosomal tryptophan metabolizing pathways appear to differ from mammalian pathways with production of large quantities of a sleep mediator, tryptophol [6].

While tryptophol has been shown to have a pronounced central nervous system activity in mammals, its role in the pathology of the disease, especially the advanced stages of the disease, is not yet clear [7]. There is no doubt that tryptophan metabolism is a major activity of trypanosomes and this is seriously disturbed in infected mammals, including man [6-11]. Although the importance of the tryptophan metabolic pathway in trypanosomes is not very clear [8], we postulated that inhibition of this pathway would either inhibit the growth of trypanosomes directly or reduce the neuropathological effects of the disease by decreasing the amounts of tryptophol and other potential toxic indoleamine metabolites formed. This would also reverse the fast depletion of tryptophan and its essential metabolites from the host [6].

As part of a programme to develop novel trypanocidal drugs we have designed and synthesized novel tryptophan analogs with a potential to inhibit the high rate of tryptophan metabolism in the mammalian host infected with trypanosomes [12]. These compounds, containing an acetylenic and/or a dithiocarbamate group, were designed to interfere with the parasite catabolism of host tryptophan [13]. This was expected to be achieved through the enzyme-activated inhibition of the putative pyridoxal and FAD - dependent enzymes in the

postulated tryptophan metabolic pathways in trypanosomes, that is, monoamine oxidases, decarboxylases, transaminases, alcohol and aldehyde reductases [6]. In this study, sixteen novel tryptophan -acetylenic and/or dithiocarbanate compounds ~ were tested, *in vitro,* for their antitrypanosomal activity against *Trypanosoma brucei brucei.*

Experimental

Each of the sixteen novel test compound [Fig 1] was dissolved in dimethylsulphoxide (DMSO) or ethanol (these solvents were shown by controls to have no activity) to give the appropriate final concentrations of 90, 30, 10 or 3 micromoles per litre in the wells of a 96 well microtitre plate. A 100 microlitre suspension of T.b. *brucei* (S427/118 MiTat 1.5, known to be drug sensitive) containing about $2x10^5$ trypanosomes per ml in the growth medium (HMI+20%ECS) and 100 microlitre of each drug solution were added to each well [14]. Each concentration was set in triplicate. Plates were incubated for 72 hours at 37^0C in a carbon dioxide-enriched incubator. Plates were then examined for the trypanosomes. The wells were further examined on an inverted microscope and the number of parasites scored from 0 (no living cells) to (+++) (number of trypanosomes greater than or equal to control). The minimum inhibitory concentration (MIC) was thus determined. The most active compounds (MIC less than or equal to 30 micromolar) were tested further to determine the ED_{50}. In this case four concentrations (30, 10, 3 and 1 micromolar) were prepared in triplicate. T.b. *brucei* ($2x10^5$) were added and grown in the media as mentioned above. To get the number of T.b. *brucei* per ml, each well was counted on a coulter counter after dilution (isotonic diluent). This was used to calculate the average percent inhibition and hence the ED_{50} for each drug. Wells in which no T.b. *brucei* were living were not counted and 100% inhibition was concluded. Pentamidine was used as a positive control for all tests.

RESULTS AND DISCUSSION

Out of the sixteen novel compounds tested for *in vitro* activity against T.b. *brucei,* nine were found to have a significant activity with a minimum inhibitory concentration (MIC) of less that 30 micromoles per litre [Table 1]. The most active compounds were 1-(indole-2y1)-2-piperazinomethylene (4), 1-(1-benzene-sulphony-lindol-2-yl)-2-piperazinomethylethyne (5), 1-(1 -benzene sulphonylindol-2-yl)-2-(4-methylpiperazino-methyl) ethyne (6), 5-(methoxyindol-2-yl)-2-piperi-dinomethyle thyne (7), 1 -(benzenesulphonylindol- 1 -3-yl)-2-(4-methylpiperazinomethyl) ethyne (8), 1-(1-benzene sulphonyl-5-methoxyindol-3-yl)-2-(4-piperazine methyl) ethyne (9), bis (1 4hiocarbonyl-4-(3-(1-benzene sulpho-nylindol-2-yl) prop-2-ynyl) piperazine) disulphide (10a), bias (1-thiocarbonyl-4-(3-(indole-2-yl) prop-2-ynyl) piperazine) disuiphide (1 la), and bis(lthiocarbonyl-4-(3-(5-methoxy indolindol-2-yl)prop-2-ynyl) piperazine) disulphide (11b). Compound 6 was the most active with a MIC of less than 1.0 micromole per litre and an ED_{50} of 2.88 micromoles per litre completely inhibiting growth at 10 micromoles per litre. Compound (10b) had a weaker activity (MIC> 10 micromoles per litre) and compounds (1 a), (lb), (2a), (2b) and (3) did not inhibit the *in vitro* growth of trypanosomes at 90 micromoles per litre, the maximum concentration used in these studies. The most active compound, (6), was found to be 220 times weaker than the standard drug used in these tests (pentamidine, ED_{50} = 0.013 micromoles per litre). However, the activity of these compounds is promising enough to warrant further studies. Our structure-activity analysis of the present *in vitro* activity data has identified the importance of the following:

(i) A hydrophobic indole ring carrying a benzene sulphonyl substituent at the indole nitrogen (ie position 1), (the unsubstituted compounds are less active, e.g. compound (4), ED_{50} = 8.7, compared to compound (5), ED_{50} = 6.78 micromoles per litre;

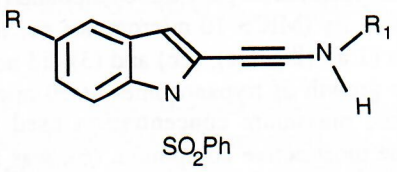
(ii) a 3-carbon bridge at position 2 of the indole ring which includes an ethynyl group (3-substituted indoles are less active, eg compound (8), ED_{50} = 7.5 micrbmoles/1);

(iii) a terminal piperazino group with a 4-methyl substituent (compounds which are unsubstituted at position 4 of the piperazino ring are slightly less active eg compound *(5)* ED_{50} = = 6.78 compared to (6), ED_{50} = 2.88 micromoles per litre). The tertiary amino group of the piperazine ring provides a cationic centre, Pka about 8.5, which at physiological pH, will be about 90% ionized.

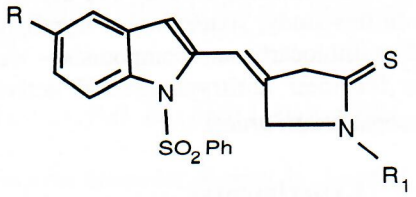
CONCLUSION

This study has shown that some of the acetylenic indole derivatives designed as enzyme-activated inhibitors of putative pyridoxal and FAD-dependent trypanosomal enzymes have a potential for development as antitrypanosomal drugs. The structure-activity studies of the most active compounds in order to increase the antitrypanosomal activity of the "lead" compounds and studies to elucidate the mechanism of action of these compounds forms part of our continuing work.

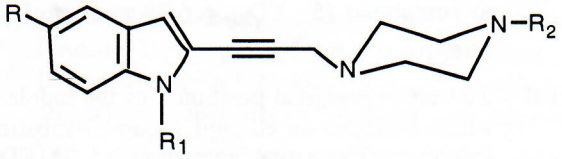
FIG. 1: Target Test Compounds



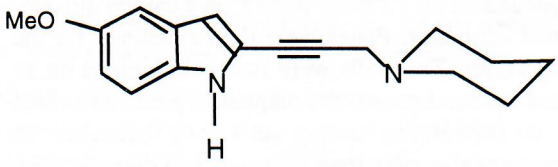
R	R_1	
H	CH_3	(1a)
OMe	CH_3	(1b)



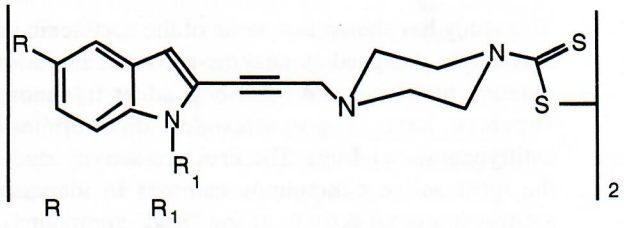
R	R_1	
H	H	(2a)
H	Me	(2b)
OMe	H	(2c)
OMe	Me	(2d)



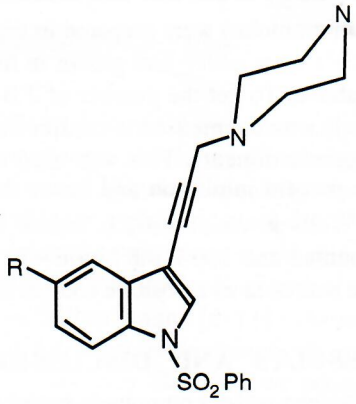
R	R_1	R_2	
H	SO_2 Ph	COOEt	(3)
H	H	H	(4)
H	SO_2 Ph	H	(5)
H	SO_2 Ph	Me	(6)



(7)



R	R_1	
H	SO_2 Ph	(10a)
OMe	SO_2 Ph	(10b)
H	H	(11a)
OMe	H	(11b)



R - H	(8)
OMe	(9)

Table 1: Activity of Target Compounds Against *Trypanosoma brucei brucei* in Vitro

COMPOUND NO.	MIC (Micromol/L)	ED_{50} (Micromol/L)	RANGE (ED_{50})
1a	>90	-	-
lb	>90	-	-
2a	>90	-	-
2b	>90	-	-
2d	>90	-	-
3	>90	-	-
4	10< x>30	8.70	7.75-9.73
5	3 <x> 10	6.78	6.61-6.94
6	0.3 <x> 1	2.88	2.44-3.45
7	3<x>10	15.40	14.94-17.24
8	1< x> 3	7.50	6.50-9.42
9	1<x>3	4.90	4.10-5.22
10a	<3	3.93	3.27-4.82
10b	30<x>90	-	
11a	10<x>30	13.31	12.55-14.15
11b	3<x>10	8.64	7.75-9.60
PENTAMIDINE		0.013	0.01-0.019

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