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Antileishmanial actions of tricyclic neuroleptics appear to lack structural specificity

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Abstract—There is continuing interest in the antimicrobial effects of tricyclic neuroleptics and antidepressants, particularly against trypanosomatid protozoa, but few studies have attempted to dissociate antiparasitic actions from effects on the central nervous system. In this report, we have measured the antileishmanial potency of a range of structural analogues in this drug group, and conclude that no one structural determinant is critical for activity. Instead, potency may be predicted from an overall determination of the lipophilic and steric components of the structure by a simple combination of two descriptive parameters, valence connectivity and an interactive term.

Key words: Leishmania; phenothiazine; antidepressant; antimicrobial; QSAR; connectivity

The activity of tricyclic neuroleptics against *Leishmania* and *Trypanosoma* parasites has been reported in previous studies [1–5]. These did not dissociate the potent central effects of the compounds from their antiparasitic activities, although various specific interactions were proposed. Pearson *et al.* [1] reported the activity of 10 phenothiazines against *L. donovani* both *in vitro* and *in vivo*. Zilberstein and Dwyer [2] demonstrated that related tricyclic antidepressants based on a dibenzazepine nucleus were effective against culture promastigotes of *L. donovani*, provided that ring 3 substituents were present. These authors proposed that tricyclic compounds interfered with a membrane transport system unique to trypanosomatids, an ATPase proton pump. Seebeck and Gehr [3, 4] found that phenothiazines were also active against *Trypanosoma brucei in vitro*, and provided evidence for a rapid, irreversible effect on subpellicular microtubule assembly. They also stressed the correlation between antiparasitic and neuroleptic activity, since the metabolite chlorpromazine sulphoxide was inactive. Most recently, Benson *et al.* [5] designed a computer graphics technique based on the three dimensional structure of recombinant trypanothione reductase, a unique component of trypanosomatid redox defence mechanisms. They demonstrated the ability of a phenothiazine, a dibenzazepine and a dibenzocycloheptadiene compound to act as competitive inhibitors of this enzyme, and described a molecular conformation which allowed the tricyclic region to overlie the hydrophobic regions of the active site while the polar sidechain interacted through hydrogen bonding.

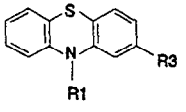
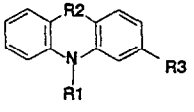
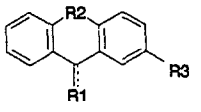
Conversely, Hammond *et al.* [6], considered the tricyclics to be members of a larger group of cationic, amphiphilic drugs with an activity against *T. cruzi* trypomastigotes in stored blood at 4° which was independent of parasite metabolic activity. Seeman *et al.* [7] summarized evidence for the existence of two separate classes of tricyclic drug action; specific effects occurring at nanomolar concentrations and exhibiting distinct structural requirements (e.g. stereospecificity), and nonspecific membrane stabilization occurring at micromolar concentrations. The

Table 1. Antileishmanial activity of tricyclic compounds *in vitro*

Compound	*L. amazonensis* Max. Cells Clear (se)	ED_{50} (μM)	(Range)	*L. donovani* ED_{50}
Amitriptyline	100 (0)	12.1	(8.9–15.3)	14.0
Clomipramine	97 (2)	4.3	(2.3–6.3)	8.0
Chlorpromazine	93 (2)	6.9	(6.3–7.5)	9.1
Desipramine	99 (1)	8.5	(7.8–9.1)	16.0
Dibenzepin	18 (18)	69*	(48.0–90.0)*	Inactive
Doxepin	97 (2)	40.0	(29.6–50.2)	35.0
Fluphenazine	77 (5)	5.4	(3.9–7.1)	9.3
Imipramine	99 (10)	14.3	(8.6–20.3)	16.2
Nortriptyline	93 (3)	5.5	(3.1–8.2)	10.0
Opipramol	64 (4)	20.0	(16.7–23.5)	28.7
Protriptyline	92 (3)	6.3	(4.0–8.6)	9.6
Thioridazine	97 (2)	4.4	(3.9–4.9)	ND
Trifluperazine	97 (2)	3.7	(3.7–3.9)	5.3
Triflupromazine	83 (8)	6.7	(5.4–8.0)	7.6
Trimipramine	82 (3)	9.4	(6.2–12.9)	14.1

All compounds were >90% effective at their maximum tolerated dose except opipramol (64 ± 3%) and dibenzepin (18 ± 18%), * extrapolated values are used.

Table 2. Structures of tricyclic antidepressants and neuroleptics

R1	R2	R3	Phenothlazine 	Dibenzazepine 	Dibenzocyclo-heptadiene 
$CH_2(CH_2)_2NHCH_3$	C_2H_4	H		Desipramine	Nortriptyline
	C_2H_4	H			Protriptyline
$CH_2(CH_2)_2N(CH_3)_2$	C_2H_4	H		Imipramine	Amitriptyline
		Cl	Chloropromazine	Clomipramine	
		CF_3	Trifluperazine		
	CH_2O	H			Doxepin
CH_3	$O(CH_2)_3N(CH_3)_2$	H		Dibenzepin	
$CH_2.CH.CH_2.N.(CH_3)_2CH_3$	C_2H_4	H		Trimipramine	
$(CH_2)_3.N$ $N(CH_2)_3OH$	C_2H_4	H		Opipramol	
		CF_3	Fluphenazine		
$(CH_2)_3.N$ $N\ CH_3$		CF_3	Trifluperazine		
$(CH_2)_2HC—N\ CH_3$		SCH_3	Thioridazine		

ability of neuroleptics to protect erythrocytes from haemolysis [7] and their lysosomotropic behaviour [8] are examples of non-specific effects mediated through cationic or amphiphilic character. Since a wide range of structural analogues exist (Table 1), we have been able to examine in more detail the structural requirements for antileishmanial activity *in vitro*.

Materials and Methods

Test compounds were kindly donated by the following manufacturers: Ciba Geigy Pharmaceuticals; Lilly Research Centre Ltd; May and Baker Ltd; Merck, Sharp and Dohme; Pfizer Ltd; Sandoz Products Ltd; or purchased from Sigma Chemical Co. (Poole, U.K.) All were supplied as a salt and were fully soluble in the incubation media at the concentrations tested. Antileishmanial potency was determined *in vitro* using methods described in detail elsewhere [9, 10]. Briefly, compounds were tested against amastigote initiated infections of CD1 mouse peritoneal macrophage primary monolayer cultures by *L. donovani* (MHOM/ET/67/HU3) and *L. amazonensis* (MPRO/BR/72/M1845) at 37° and 33°, respectively. The test period was 7 days, and compounds were used in 2-fold dilution series covering the full range of activity. The culture medium (RPMI 1640 plus 10% foetal calf serum) was changed twice during this period and the cultures maintained in an atmosphere of 5% CO_2/95% air. The percentage of cells remaining infected at the end of this period was determined from Giemsa stained preparations, and ED_{50} values calculated by log probit analysis. Data are the means of two separate experiments in which all compounds were tested in parallel. Each dilution of drug was tested in four replicate cultures in each experiment. Valence connectivity indices were calculated from the planar structure diagram of each compound according to the methods of Kier and Hall [11]. Interactive terms were adapted from the method of Mellors and McGowan [12], to distinguish between secondary and tertiary amines.

Results and Discussion

The tricyclic antidepressants and neuroleptics form an interesting group of closely related compounds which are potent antileishmanial agents *in vitro* (Table 1), and the present study confirms that the dibenzocycloheptadiene group display an equivalent potency range to the phenothiazines and dibenzazepines.

No single component of molecular structure appears to be critical in determining the activity of the compounds studied, however (Table 2). Thus, a comparison of the activities of chlorpromazine, amitriptyline and imipramine suggests that the phenothiazine nucleus confers greater activity than the dibenzocycloheptadiene ring structure, which in turn gives greater activity than the dibenzazepine type. However, the dibenzeazepine, desipramine was more active than either amitriptyline or chlorpromazine. Similarly, although compounds with straight sidechains are more active than their branched or cyclic counterparts (e.g. desipramine > imipramine; protriptyline > opipramol), trifluperazine, a piperazine derivative, was the most active compound tested. The third variable, substitution in position 3, appeared to improve activity, but was by no means essential for high potency (e.g. nortriptyline).

The drugs tested in the present study had ED_{50} values in the range 3.0 to 5.0 μM for antiparasitic activity, whilst their therapeutic levels in plasma for neuroleptic activity are typically 0.1–50 nM. The combination of a steric parameter (molecular connectivity, $^1X^v$) [10] with an interactive term (E_B) [11] is sufficient to describe quantitatively the variation in activity in these compounds, and the relationship is linear to a cut off point of 9.0–9.5 (Fig. 1). Although the phenothiazines and antidepressants display similar antileishmanial activity, their central effects differ markedly. In addition, antileishmanial potency

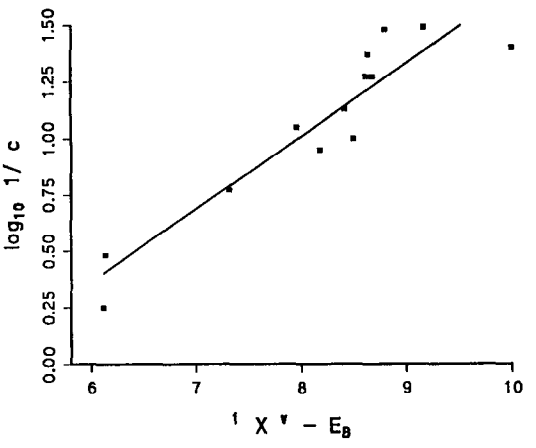


Fig. 1. Relationship between antileishmanial activity and molecular properties. c: molar ED_{50} against *L. amazonensis in vitro*. First order valence connectivity values were calculated according to the method described in Ref. 9. Interactive terms used were: +1.9 oxygen; +1.5 tertiary amine; +0.9 secondary amine; −0.15 halogen. Relationship defined by least squares linear regression analysis: $y = 0.323\,x + 1.575$. $r = 0.93$.

correlates closely with fatal plasma levels obtained from analyses of poisoning cases ($r = 0.74$), but not with therapeutic plasma levels (data not shown). We conclude from this that potency in this system reflects a relatively non-specific physical toxicity involving either membrane stabilizing effects or interaction with more than one receptor having an amphipathic active site. Furthermore, we would conclude that the antiparasitic effects of these compounds are mediated through non-specific interaction, and support their inclusion in the larger group of cationic amphiphilic compounds proposed by Hammond *et al.* [6]. This would seem to be contradicted by the proposed requirement for neuroleptic activity; however the model would not predict significant activity for sulphoxide derivatives due to their hydrophilicity; in fact, chlorpromazine sulphoxide possesses all the structural requirements for neuroleptic activity, but, is unable to penetrate the blood–brain barrier [13]. The activity range available for analysis in our system appears to be limited by absorption and host cell toxicity but at least one compound predicted to be more potent by the model (maprotiline) was shown to be highly effective against *T. cruzi* [6]. Butriptyline and dothiepin would be other candidates for future study. In general, the present system was more sensitive than previously utilized chemotherapy systems, presumably due to the longer dosing regimen (7 days as opposed to a maximum of 3 days). The effective concentrations were also lower than those required to produce 50% inhibition of trypanothione reductase [5]. It is interesting to note that clomipramine was more active in inhibiting this enzyme than the classical calmodulin antagonist, trifluperazine; some specificity may be created through fine structural interaction of these compounds with trypanothione reductase compared with the analogous calcium binding site of calmodulin. Given that they are roughly equipotent antileishmanial agents, however, this further suggests that inhibitory potency against either of these target proteins does not predict antileishmanial activity, at least in this system. Otherwise, there was general agreement in relative potencies with the present data. Imipramine showed no activity in one system [2],

probably due to low sensitivity (approximately 6-fold less on the basis of clomipramine potency). One possible element which is not adequately explained by the present model is the apparent effect on potency of the position of ring substituents. Zilberstein and Dwyer [2] tested two dinitro substituted imipramine derivatives which would be predicted by the model to be equipotent, whereas in fact the 2,8 substituent was at least twice as active as the 4,8 compound. No similar disubstituted compounds were included in the present study, and it would be interesting to explore a range of these derivatives, in order either to refine the predictive model, or produce conclusive evidence for a specific structural requirement.

We believe that the presented data provides a useful baseline of antileishmanial potency for a generally wider range of related tricyclic structures than previously considered, allowing inclusion of the dibenzocycloheptadiene nucleus into structure activity comparisons. Valence connectivity terms are readily calculable and based on planar molecular structure, which precludes subjective speculation about likely conformation at the putative site of action, and this parameter has been shown to be a valid substitute for parachor terms [14]. Combination of this parameter with another simply defined function to account for hydrophilic interaction has provided a predictive model for antiparasitic activity mediated through structurally non-specific interaction, and the high level of correlation of relative potency in an *in vitro* system of tricyclic structures with the model appears to largely confirm the lack of specific structural requirements for antileishmanial potency. It is possible that the tricyclics which deviate from the model (e.g. due to the position of ring substituents) may provide leads to selective inhibitors which lack effects on the central nervous system. Tricyclics are also widely used as model inhibitors in biochemical studies, and have clearly been useful in probing the binding sites of calmodulin and trypanothione reductase. We would conclude, however, that the antileishmanial potency of tricyclics in clinical use is too closely allied to physical toxicity for a single target to be identified.

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