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THE TRYPANOCIDAL ACTIVITY OF DITHIOBIURET DERIVATIVES

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During the routine screening of various types of compounds for chemotherapeutic activity, trypanocidal activity was noted in several dithiobiuret derivatives. As a consequence, a more detailed study of compounds of this type was carried out.

The chemistry of the compounds mentioned in this paper will be reported elsewhere by Fairfull and Peak.

MATERIALS AND METHODS

Most of the compounds had a very low solubility in water, and only for those marked with an asterisk in the Tables was this greater than 1 in 500. The drugs were therefore used in aqueous suspension, with Dispersol LN as a wetting and dispersing agent. They were given subcutaneously to mice showing a moderate degree of parasitaemia following inoculation with diluted infected mouse blood. Many of the compounds were tested against several trypanosome species—*Trypanosoma rhodesiense, T. gambiense, T. brucei, T. congolense,* and *T. cruzi*—but none showed appreciable trypanocidal activity against any species other than *T. congolense.* In the Tables, therefore, the results given are for activity against the F.N. strain of *T. congolense* only. The largest dose given was usually a high fraction of the acute subcutaneous LD50, but the maximum dose used was 500 mg./kg. Doses were given on one occasion to one series of mice, and repeated daily for five days in a second series of animals. In general the drugs were more active when the same total amount was given in repeated doses than in a single dose. The CD50 has been calculated on the basis of removal of parasites from the peripheral blood for one month.

RESULTS

1-Alkyldithiobiurets, etc. (Table I).—Dithiobiuret itself, RD2491, is known to be a compound of fairly high toxicity. Nevertheless, repeated doses can cure mice infected with *T. congolense.* 1-Methyl substitution (RD1658) increased activity, while the 1-ethyl compound was less toxic than, and approximately as active as, unsubstituted dithiobiuret. However, lengthening the alkyl chain increased toxicity and decreased activity.

TABLE I

THE TRYPANOCIDAL ACTIVITY OF 1-ALKYLDITHIOBIURETS, ETC.

R—NH.C(=S).NH.C(=S).NH$_2$

Reference No.	R =	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD2491	H—*		Delayed death at 100 mg./kg. s.c.×1. CD50 *ca.* 40 mg./kg. s.c.×5
RD1658	Methyl	140	Delayed death at 80 mg./kg. s.c.×1. CD50 *ca.* 20 mg./kg. s.c.×5
RD1659	Ethyl	*ca.* 1,000	CD50 *ca.* 300 mg./kg. s.c.×1. *ca.* 40 mg./kg. s.c.×5
RD1668	*n*-Butyl	*ca.* 200	Delayed death at 100 mg./kg. s.c.×1. CD50 *ca.* 80 mg./kg. s.c.×5

1-Aryldithiobiurets.—Many of the compounds listed in Table II had considerable activity when repeated doses were given, and the toxicity generally was low. The soluble compounds (marked with an asterisk) were usually more toxic than the practically insoluble derivatives, so that the apparent low toxicity of, for example, RD1501 might result from poor absorption. Nevertheless, even compounds of low solubility, such as RD1501, had quite marked trypanocidal activity. Of the members of the homologous series of 1-*p*-alkylphenyldithiobiurets, the methyl compound, RD1548, was the most potent. In the alkoxyphenyl- series the *para-* substituted compound RD1145 was more effective than the *meta-* or the *ortho-* analogues when five doses were given; but, rather curiously, it was the least effective of the three when only a single dose was administered. The ethoxy- and *n*-butoxy-derivatives were less potent than the methoxy-compound. Of other *p*-phenyl substituents tried, only the *p*-chloro-compound had retained appreciable activity.

None of the naphthyl-, pyridyl-, *iso*quinolyl-, or quinolyl-derivatives tried had more than slight activity.

TABLE II

THE TRYPANOCIDAL ACTIVITY OF 1-ARYL-DITHIOBIURETS

R—NH.C.NH.C.NH_2
‖ ‖
S S

Reference No.	R=	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD1501	Phenyl	4,500	CD50 *ca.* 150 mg./kg. s.c.×1; *ca.* 35 mg./kg. s.c.×5
RD1548	*p*-Tolyl	>5,000	CD50 *ca.* 180 mg./kg. s.c.×1; *ca.* 25 mg./kg. s.c.×5
RD2138	*p*-*n*-Propylphenyl	>5,000	Delayed death at 200 mg./kg. s.c.×1; CD50 *ca.* 70 mg./kg. s.c.×5
RD2286	*p*-*n*-Butylphenyl	>3,200	CD50 *ca.* 400 mg./kg. s.c.×1; *ca.* 200 mg./kg. s.c.×5
RD1519	*o*-Methoxyphenyl	*ca.* 3,200	CD50 *ca.* 180 mg./kg. s.c.×1; *ca.* 80 mg./kg. s.c.×5
RD1549	*m*-Methoxyphenyl	>5,000	CD50 *ca.* 200 mg./kg. s.c.×1; *ca.* 50 mg./kg. s.c.×5
RD1145	*p*-Methoxyphenyl	*ca.*500 i.p.	Delayed death at 200 mg./kg. s.c.×1; CD50 *ca.* 40 mg./kg. s.c.×5
RD1524	*p*-Ethoxyphenyl	>5,000	Delayed death at 500 mg./kg. s.c.×1; CD50 *ca.* 115 mg./kg. s.c.×5
RD1547	*p*-*n*-Butoxyphenyl	>5,000	Delayed death at 500 mg./kg. s.c.×1; CD50 *ca.* 150 mg./kg. s.c.×5
RD1518	*p*-Chlorophenyl	2,000	CD50 *ca.* 150 mg./kg. s.c.×1; *ca.* 45 mg./kg. s.c.×5
RD1546	*p*-Ethylsulphonylphenyl	>5,000	Delayed death at 500 mg./kg. s.c.×1; CD50 *ca.* 350 mg./kg. s.c.×5
RD1657	*p*-Carboxyphenyl	*ca.* 1,000	Delayed death at 400 mg./kg. s.c.×1; or 300 mg./kg. s.c.×5
RD1656	*p*-Carbethoxyphenyl	*ca.* 4,000	Delayed death at 500 mg./kg. s.c.×1; or 500 mg./kg. s.c.×5
RD1609	*p*-Dimethylaminophenyl	>5,000	De'ayed death at 500 mg./kg. s.c.×1; or 500 mg./kg. s.c.×5
RD1898	*p*-Dimethylaminophenyl (methochloride) *	*ca.* 400	Inactive at 100 mg./kg. s.c.×1; or 100 mg./kg. s.c.×5
RD2103	*p*-Acetamidophenyl	>5,000	Inactive at 400 mg./kg. s.c.×1; CD50 *ca.* 250 mg./kg. s.c. ×5
RD1573	β-Naphthyl	200	Inactive at 100 mg./kg. s.c.×1; or 50 mg./kg. s.c.×5
RD1666	α-Naphthyl	>5,000	Delayed death at 500 mg./kg. s.c.×1; CD50 *ca.* 300 mg./kg. s.c.×5
RD1844	2-Pyridyl	*ca.* 4,000	Delayed death at 400 mg./kg. s.c.×1; or 400 mg./kg. s.c.×5
RD1951	3-Pyridyl	*ca.* 800	Inactive at 400 mg./kg. s.c.×1; delayed death at 400 mg./kg. s.c.×5
RD2039	3-Pyridyl (methochloride)*	280	Inactive at 50 mg./kg. s.c.×1; or 50 mg./kg. s.c.×5
RD2040	4-*Iso*quinolyl	>5,000	Delayed death at 400 mg./kg. s.c.×1; or 400 mg./kg. s.c.×5
RD2137	4-*Iso*quinolyl (methochloride) *	*ca.* 2,000	Inactive at 400 mg./kg. s.c.×1; or 400 mg./kg. s.c.×5
RD2284	5-Quinolyl	*ca.* 1,000	Delayed death at 500 mg./kg. s.c.×1; CD50 *ca.* 100 mg./kg. s.c.×5
RD1985	6-Quinolyl	*ca.* 2,000	Inactive at 500 mg./kg. s.c.×1; delayed death at 500 mg./kg. s.c.×5
RD1986	6-Quinolyl (methochloride) *	>5,000	Inactive at 500 mg./kg. s.c.×1; delayed death at 250 mg./kg. s.c.×5 (toxic at 500 mg./kg. s.c.×5)

TABLE III

THE TRYPANOCIDAL ACTIVITY OF :-ARYL-1-ALKYL-DITHIOBIURETS

C_6H_5
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N—C—NH—C.NH_2
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R S S

Reference No.	R	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD1660	Methyl	*ca.* 1,000	CD50 *ca.* 200 mg./kg. s.c.×1; *ca.* 10 mg./kg. s.c.×5
RD1704	Ethyl	1,400	CD50 *ca.* 90 mg./kg. s.c.×1; *ca.* 17 mg./kg. s.c.×5
RD1823	*n*-Propyl	*ca.* 5,000	CD50 *ca.* 100 mg./kg. s.c.×1; *ca.* 25 mg./kg. s.c.×5
RD1705	*n*-Butyl	>4,000	CD50 *ca.* 150 mg./kg. s.c.×1; *ca.* 40 mg./kg. s.c.×5

TABLE IV

THE TRYPANOCIDAL ACTIVITY OF 1,5-DISUBSTITUTED-DITHIOBIURETS

R^1—NH.C.NH.C.NH—R^2
‖ ‖
S S

Reference No.	R^1	R^2	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD1706	Phenyl	Ethyl	*ca.* 5,000	CD50 *ca.* 300 mg./kg. s.c.×1; *ca.* 120 mg./kg. s.c. ×5
RD1707	Phenyl	*n*-Butyl	*ca.* 1,800	Delayed death at 500 mg./kg. s.c. ×1; CD50 *ca.* 250 mg./kg. s.c. ×5
RD1737	Phenyl	Phenyl	>3,800	Inactive at 500 mg./kg. s.c.×1; delayed death at 400 mg./kg. s.c. ×5
RD1750	Phenyl	*p*-Methoxyphenyl	>5,000	Delayed death at 500 mg./kg. s.c. ×1; CD50 *ca.* 300 mg./kg. s.c. ×5
RD1749	*p*-Methoxyphenyl	*p*-Methoxyphenyl	*ca.* 1,500	Delayed death at 400 mg./kg. s.c. ×1; CD50 *ca.* 200 mg./kg. s.c. ×5
RD2276	Phenyl	Diphenylmethyl	>3,200	Inactive at 500 mg./kg. s.c.×1; delayed death at 500 mg./kg. s.c. ×5

1-Alkyl-1-aryl-dithiobiurets (Table III).—These compounds appeared to show considerable promise, and the 1-methyl-1-phenyl-compound (RD1660) was the most effective compound of the whole series tested. Again, however, repeated doses were necessary.

1, 5-Disubstituted-dithiobiurets.—Only slight activity, if any, was shown by this series of compounds (Table IV).

1 : 1 : 5-Trisubstituted-dithiobiurets.—Only two such compounds were tested. Both had appreciable activity, but they were inferior to the 1-alkyl-l-aryl-dithiobiurets (Table V).

Bis(1-dithiobiurets).—In view of the activity of 1-aryldithiobiurets it was thought interesting to try bis(1-dithiobiurets). However, none of the three compounds tested showed any detectable trypanocidal activity (Table VI).

Iso-*dithiobiuret Derivatives* (Table VII).—None of the compounds tested had more than a trace of activity.

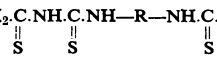
TABLE V

THE TRYPANOCIDAL ACTIVITY OF 1:1:5-TRISUBSTITUTED-DITHIOBIURETS

Reference No.	R	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD2102	Methyl	*ca.* 5,000	CD50 *ca.* 250 mg./kg. s.c.×1; CD50 *ca.* 30 mg./kg. s.c.×5
RD2217	*n*-Propyl	>4,000	CD50 *ca.* 300 mg./kg. s.c.×1; CD50 *ca.* 70 mg./kg. s.c.×5

TABLE VI

THE TRYPANOCIDAL ACTIVITY OF *BIS*(1-DITHIOBIURETS)



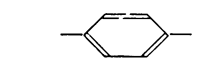
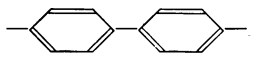
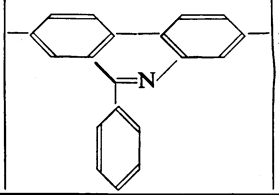
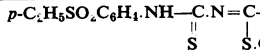
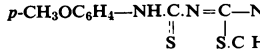
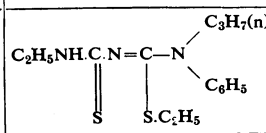
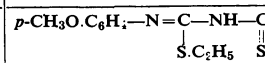
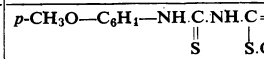
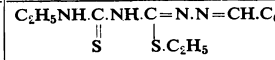
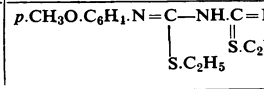
Reference No.	R=	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD2285		>3,200	Inactive at 500 mg./kg. s.c.×1; or 500 mg./kg. s.c.×5
RD2038		>5,000	Inactive at 400 mg./kg. s.c.×1; or 400 mg./kg. s.c.×5
RD2072		>5,000	Inactive at 200 mg./kg. s.c.×1; or 200 mg./kg. s.c.×5

TABLE VII

THE TRYPANOCIDAL ACTIVITY OF *ISO*-DITHIOBIURET DERIVATIVES

Reference No.	Structure	Acute LD50 mg./kg. Subcutaneously	Activity against *T. congolense*
RD1477		>5,000	Inactive at 500 mg./kg. s.c.×1; delayed death at 500 mg./kg. s.c.×5
RD1146		750 (i.p.)	Inactive at 250 mg./kg. s.c.×1; or 250 mg./kg. s.c.×5
RD2109		*ca.* 2,000	Inactive at 400 mg./kg. s.c.×1; or 200 mg./kg. s.c.×5
RD2153			Inactive at 500 mg./kg. s.c.×1; or 500 mg./kg. s.c.×5
RD1249		*ca.* 2,000	Inactive at 500 mg./kg. s.c.×1; or 500 mg./kg. s.c.×5
RD1353		*ca.* 1,500	Inactive at 500 mg./kg. s.c.×1; delayed death at 500 mg./kg. s.c.×5
RD1248		>800	Inactive at 300 mg./kg. s.c.×1; or 300 mg./kg. s.c.×5

Discussion

From the results obtained it is clear that compounds of the dithiobiuret type may possess considerable activity against *Trypanosoma congolense* infections in mice, and that even dithiobiuret itself can cure the infection when five daily doses are given. This need for repeated doses has been noted with practically all the compounds tested, and is rather unexpected. It might be assumed that, if a single massive dose of a compound of low solubility were given subcutaneously, there would be slow absorption and a maintained blood level of the drug. This, apparently, is not so in the dithiobiuret series, and it might be interesting to speculate on the fate of single large doses. In none of the mice killed and examined was there enough encapsulation fully to explain the results, though in some mice residual masses of the drug could be found several days after administration. Most of the compounds had low acute toxicity by the subcutaneous route, and several had a high ratio between acute LD50 and CD50 (five doses); thus RD1660 (Table III) had LD50 approximately 1,000 mg./kg., whereas the CD50 was of the order of 10 mg./kg. given on five successive days. The corresponding figures for RD1823 (Table III) are 5,000 and 25. Such figures compare favourably with all but the most potent of drugs used against *T. congolense*. The need for repeated dosing is, however, an obstacle which might handicap field use of these compounds in cattle. The unavoidable inaccuracies in dosing with suspensions of insoluble drugs would also militate against their use. Nevertheless, it is hoped that some of the more active compounds described will be given a field trial in *T. congolense* infections.

Summary

1. Dithiobiuret and several derivatives have been shown to possess activity against *T. congolense* when given subcutaneously to infected mice.

2. 1-Alkyl-l-aryl dithiobiurets were the most potent derivatives tested.

3. Repeated doses appeared to be necessary for the full therapeutic effect to be shown.

4. The ratio between acute LD50 and CD50 was high for many of the compounds.

5. Because of the need for repeated doses it is concluded that field use of the compounds might be difficult.

I am grateful to Mrs. B. Goodliffe and to Miss H. Riley for much technical assistance, and to Drs. A. E. Fairfull and D. A. Peak, who supplied the compounds tested.