NOTE

In vitro antitrypanosomal activity of five low-MW antibiotics

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As part of our screening program to find new antitrypanosomal compounds, we evaluate isolates from soil-dwelling microorganisms as well as compounds from existing antibiotic libraries. We have previously published findings of various microbial metabolites exhibiting potent antitrypanosomal properties.^{1–7} We have recently investigated five compounds of low MW from Kyowa Hakko Kirin Co., Ltd, with MWs ranging from 238 to 511 Da. The compounds, all known to possess antibiotic properties, are EI-1507-1, EI-1941-1, EI-1941-2, leinamycin (DC 107) and UCS1025A. All showed antitrypanosomal activity *in vitro*. Here, we report the antitrypanosomal profiles of these antibiotics (Figure 1), in comparison with two clinically used antitrypanosomal drugs, eflornithine and suramin. We also present some insights with regard to structure–activity relationships.

Test compounds were obtained from the antibiotic library of the Medicinal Chemistry Research Laboratories, Kyowa Hakko Kirin Co., Ltd (Tokyo, Japan). *In vitro* antitrypanosomal activity against *Trypanosoma brucei brucei* strain GUTat 3.1 and cytotoxicity against human diploid embryonic cell line MRC-5 were measured, as described previously.¹

Table 1 shows the *in vitro* antitrypanosomal activities of the compounds tested, as well as the two leading antitrypanosomals. EI-1941-1 showed the highest trypanocidal activity, with an IC₅₀ value of 14 nM. This compound was 79–891-fold more potent than eflornithine and suramin. Leinamycin was slightly less active than EI-1941-1, showing an IC₅₀ value of 17 nM. EI-1507-1, EI-1941-2 and UCS1025A were 42–145-fold less active than EI-1941-1, showing IC₅₀ values of 593–2029 nM. Although the antitrypanosomal activities of these compounds were similar to that of suramin, those activities were 6–21-fold more effective than that of eflornithine.

The *in vitro* cytotoxicity of all compounds tested is also presented in Table 1. Leinamycin showed the highest cytotoxicity against MRC-5

cells, with an IC₅₀ value of 313 nM. EI-1507-1, EI-1941-1 and EI-1941-2 were found to be only slightly cytotoxic, demonstrating IC₅₀ values of 9.6–12.7 μM, whereas UCS1025A had an IC₅₀ value of >25 μM, exhibiting very low cytotoxicity but still far more than eflornithine and suramin.

For a more relevant comparison of the suitability of these compounds for possible development as human medicaments, we devised a Selectivity Index (SI), determined by (cytotoxicity [IC₅₀ for the MRC-5 cells]/antitrypanosomal activity [IC₅₀ for the GUTat 3.1

Figure 1 Structures of antitrypanosomal antibiotics.

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Table 1 *In vitro* antitrypanosomal activity against *Trypanosoma brucei* GUTat 3.1 and cytotoxicity in MRC-5 cells of some low-MW antibiotics and two commonly used antitrypanosomal drugs

Compound	<i>IC</i> ₅₀ (nM)		Selectivity index MRC-5/GUTat 3.1
	Antitrypanosomal activity GUTat 3.1	Cytotoxicity MRC-5	
EI-1507-1	593	12 684	21
EI-1941-1	14	9833	702
EI-1941-2	2029	9580	5
Leinamycin	17	313	18
UCS1025A	864	>25 014	>29
Eflornithine	12 473	>549 451	>44
Suramin	1106	>69 979	>63

strain]), as presented in Table 1. Among the tested compounds, EI-1941-1 showed the highest SI, with a ratio of 702. EI-1507-1, leinamycin and UCS1025A showed a moderate SI, with ratios of around 18–>29, less than those of both eflornithine and suramin. EI-1941-2 showed a low SI, with ratio of 5.

The discovery of the antitrypanosomal activities of EI-1507-1, EI-1941-1, EI-1941-2, leinamycin and UCS1025A are novel, and this Note represents the first report of such properties.

Among the compounds, EI-1941-1 showed the most potent and selective antitrypanosomal property. Comparison with EI-1941-2 provides some very significant information about structure–activity relationships. EI-1941-1 possesses a hydroxyl group at the C-3 position, and the compound is 140-fold more trypanocidal than EI-1941-2 (which possesses a carbonyl group at the C-3 position). However, cytotoxicity in human cells of EI-1941-1 was similar to EI-1941-2. These data suggest that the hydroxyl group at the C-3 position in EI-1941-1 is responsible for significantly increasing antitrypanosomal activity. Further studies are required to fully evaluate the structure–*in vitro* antitrypanosomal activity of EI-1941-1 and related compounds.

EI-1507-1 is a benz[*a*]anthracene antibiotic containing an epoxide ring. EI-1941-1 and EI-1941-2 are hexaketide antibiotics that contain an epoxide ring. These compounds are reported to inhibit interleukin-1 β -converting enzyme,^{8–10} although the mode of action is not clearly understood. However, a related cycloepoxydon is reported to inhibit TPA-induced NF- κ B activity and AP-1 mediated secreted alkaline phosphatase expression.¹¹ Leinamycin is an 18-membered lactam macrocyclic antibiotic, containing a disulfide moiety and thiazole ring, and it is known to have antitumor and antibacterial properties.¹² Its mode of action is via inhibition of DNA synthesis through

thiol-mediated DNA alkylation in bacterial cells.¹³ UCS1025A is a pentacyclic polyketide antibiotic, known to exhibit antitumor and antibacterial activity¹⁴ via inhibition of telomerase.¹⁵ These compounds may have similar modes of action against *Trypanosomes*.

The above results reveal that the low-MW antibiotics tested may be promising ‘drug-like’ lead compounds for possible development into novel antitrypanosomal drug. Further studies of the trypanocidal, cytotoxic and other bioactive properties of these antibiotics are under way.

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