NOTE

Promising lead compounds for novel antiprotozoals

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Malaria and human African trypanosomiasis (HAT), also known as sleeping sickness, are devastating diseases caused by infection with the protozoan parasites of the *Plasmodia* and *Trypanosoma* genera, respectively. The diseases infect millions of people. HAT, caused by infection with *Trypanosoma brucei*, is a complex disease and represents a major cause of morbidity and mortality in sub-Saharan Africa, where it is endemic and an estimated 70 million people are at risk of contracting it. Several epidemics of HAT occurred during the last century, but a combination of vector control, disease surveillance and early drug treatment of those infected caused the disease to almost disappear by the mid-1960s. Over the following 2–3 decades the disease re-emerged, but recent control efforts have again reduced its incidence, annual cases totaling an estimated 50 000–70 000. HAT has two distinct forms (gambiense and rhodesiense) depending on the parasite involved, each form having two specific stages. HAT is fatal if left untreated, with treatment depending on the stage of the disease. Drugs for the early-stage (pentamidine and suramin) are well tolerated, easier to administer and more effective. Drugs used for the late-stage (melarsoprol, and eflornithine which is ineffective for the acute rhodesiense form) have to cross the blood–brain barrier. They are toxic (particularly melarsoprol), often causing severe and sometimes fatal side effects, and have to be administered under costly medical supervision. Consequently, the swift development and introduction of safer, more effective, easy-to-use and affordable drugs will be instrumental in helping to achieve recent initiatives aimed at eliminating the disease as a public health problem.

Unlike HAT, which is restricted to Africa, malaria is widespread globally, with an estimated 3.5 billion people at risk of contracting the disease, 1.3 billion of whom are at high risk. Massive recent global initiatives to combat the disease, integrating vector control, prevention and treatment, have resulted in significant progress. Nevertheless, some 243 million cases occurred in 2008, resulting in an estimated 863 000 deaths, almost 90% of which were in Africa.¹ Many antimalarial drugs of varying effectiveness have been developed over the past 100 years, but drug-resistant strains of *Plasmodium* parasites have

evolved rapidly and evidence of parasite resistance to the currently most effective antimalarial, artemisinin, was discovered in 2009, with such reports increasing markedly since then (<http://www.dndi.org/> and <http://www.who.int/>).

Therefore, to sustain progress in controlling both these major diseases and to help the current global initiatives achieve the goal of eliminating them, there is an urgent need for new antitrypanosomal and antimalarial drugs that are more effective, safer and easier to administer. More especially, to avoid the problems faced as a consequence of expanding drug resistance, there is a pressing need for compounds that have novel structures and modes of action.

During the course of our screening program, to discover new antiprotozoal (antitrypanosomal and antimalarial) chemicals, we have evaluated isolates from soil microorganisms, as well as compounds from the antibiotic libraries of the Kitasato Institute for Life Sciences and Bioscience Associates. We have previously reported on various microbial metabolites exhibiting potent antiprotozoal properties,² antitrypanosomal properties^{3,4} and potent antimalarial properties.^{5–7} We have recently evaluated a further 10 antibiotics *in vitro*. We report here the antitrypanosomal and antimalarial profiles of 10 antibiotics (Figure 1), in comparison with those of clinically used antitrypanosomal drugs (pentamidine, suramin and eflornithine), and two commonly used antimalarial drugs (artemisinin and chloroquine).

Test compounds were obtained from the antibiotic libraries of the Kitasato Institute for Life Sciences and Bioscience Associates (Tokyo, Japan). *In vitro* antiprotozoal activities were investigated using the *T. brucei brucei* strain GUTat 3 model, as well as *Plasmodium falciparum* strains K1 (drug-resistant) and FCR3 (drug-susceptible).^{3,5} Cytotoxicity against human diploid embryonic cell line MRC-5 was measured as described previously.⁵

Table 1 shows the *in vitro* antiprotozoal activities of 10 antibiotics and standard antiprotozoal drugs tested. 7-Deoxypactamycin (cranomycin) showed the highest antitrypanosomal activity, with an IC₅₀ value of 0.5 ng ml⁻¹. The compound was 30-fold more potent than

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Figure 1 Structures of antiprotozoal antibiotics.

Table 1 *In vitro* antiprotozoal activity against *Trypanosoma brucei brucei* GUTat 3.1 and *Plasmodium falciparum* (K1 and FCR3 strains) plus cytotoxicity in MRC-5 cells of 10 microbial metabolites and some commonly used antiprotozoal drugs

Compound	$IC_{50}(ng\,ml^{-1})$					
	Antiprotozoal activity		Cytotoxicity		Selectivity index	
	<i>T. b. b. GUTat 3.1</i>	<i>P. f. K1</i> ^a	<i>P. f. FCR3</i> ^b	MRC-5	MRC-5/GUTat 3.1	MRC-5/K1
Acrylamidine	2240	11 740	ND ^c	59 830	27	5
Amidinomycin	420	170	160	320	1	2
Bellenamine	1190	> 12 500	ND ^c	83 080	70	—
BD-12	540	> 12 500	ND ^c	36 580	68	—
BY-81	> 12 500	340	100	> 100 000	—	> 294
Cyclamidomycin	43	1160	1210	1510	35	1
Pactamycin	4.1	7.9	9.4	53	13	7
7-Deoxypactamycin	0.5	0.2	0.2	16	31	70
Neothramycin ^d	130	1090	1180	390	3	0.4
Butylneothramycin A	720	7840	ND ^c	2070	3	0.3
Pentamidine	1.6	ND ^c	ND ^c	5710	3569	—
Eflornithine	2270	ND ^c	ND ^c	> 100 000	> 44	—
Suramin	1580	ND ^c	ND ^c	> 100 000	> 63	—
Artemisinin	ND ^c	6	6	45 170	—	7528
Chloroquine	ND ^c	184	15	18 572	—	101

Abbreviations: *T. b. b. GUTat 3.1*, *Trypanosoma brucei brucei* GUTat 3.1; *P. f. FCR3*, *Plasmodium falciparum* FCR3; *P. f. K1*, *Plasmodium falciparum* K1.

^aDrug-resistant strain.

^bDrug-susceptible strain.

^cNot determined.

^dAn epimeric mixture of neothramycin A and B.

pentamidine and 3100- to 4500-fold more potent than eflornithine and suramin. Pactamycin was eightfold less active than 7-deoxypactamycin, showing an IC_{50} value of 4.1 $ng\,ml^{-1}$. Cyclamidomycin (pyracrimycin A, desdanine) and neothramycin were 86- to 260-fold less effective than 7-deoxypactamycin, with IC_{50} values of 43 and 130 $ng\,ml^{-1}$, respectively. Amidinomycin (myxoviromycin), BD-12 and butylneothramycin A were 840- to 1440-fold less active than

7-deoxypactamycin, having IC_{50} values of 420–720 $ng\,ml^{-1}$. Bellenamine (D-β-lysylmethanediamine) and acrylamidine showed moderate antitrypanosomal activity, with IC_{50} values around 1–2 $\mu g\,ml^{-1}$, comparable with both eflornithine and suramin, whereas BY-81 exhibited virtually no antitrypanosomal activity whatsoever.

With respect to antimalarial properties, 7-deoxypactamycin showed the highest activity against both the drug-resistant K1 and

drug-susceptible FCR3 strains of *P. falciparum*, with an IC_{50} value of 0.2 ng ml^{-1} , almost 30-fold greater than that shown by artemisinin for both strains (Table 1). Pactamycin was 40-fold less active than 7-deoxypactamycin, producing an IC_{50} value of 8 ng ml^{-1} , but its impact was still comparable with artemisinin. Of the other metabolites tested, none bettered the impact of artemisinin and chloroquine on drug-susceptible parasites. However, amidinomycin and BY-81 retained their effectiveness against the drug-resistant strain. Cyclamidomycin and neothramycin produced moderate activity, with IC_{50} values around $1 \mu\text{g ml}^{-1}$. The remaining compounds showed weak or no activity.

The *in vitro* cytotoxicities of the 10 antibiotics and the antiprotozoal drugs are also presented in Table 1. 7-Deoxypactamycin showed the highest cytotoxicity against MRC-5 cells, with an IC_{50} value of 16 ng ml^{-1} . Amidinomycin, pactamycin and neothramycin showed high cytotoxicity, exhibiting IC_{50} values of $53\text{--}390 \text{ ng ml}^{-1}$. Butylneothramycin A and cyclamidomycin were revealed to be slightly cytotoxic, showing IC_{50} values of $1.5\text{--}2.1 \mu\text{g ml}^{-1}$, whereas the remaining four antibiotics had IC_{50} values of $>36 \mu\text{g ml}^{-1}$ and do not seem to be cytotoxic.

To better evaluate the antiprotozoal activities and cytotoxicities of the test compounds, we introduced selectivity indexes (SI; cytotoxicity (IC_{50} for the MRC-5 cells)/antitrypanosomal or antimalarial activity (IC_{50} for the GUTat 3.1 strain or the K1 strain)), as presented in Table 1. Compounds having SI of >100 seem to be excellent compounds from the better activity and the lower cytotoxicity. In the case of the MRC-5 cells/GUTat 3.1 strain, among the tested antibiotics, belenamine and BD-12 showed a moderate SI, with ratios of 68–70, similar to those of eflornithine and suramin. Acrylamidine, cyclamidomycin, pactamycin and 7-deoxypactamycin showed a weaker SI, with ratios of around 13–35. Amidinomycin, neothramycin and butylneothramycin A showed a low SI, with ratios of around 1–6. In the case of the MRC-5 cells/K1 strain, among the new compounds tested, BY-81 showed the highest SI of >294 , which is about threefold more potent than the SI index of chloroquine (for the drug-resistant strain), but about 25-fold less than artemisinin. 7-Deoxypactamycin showed a moderate SI of around 70 for both drug-resistant and drug-susceptible strains (data not shown). Acrylamidine, amidinomycin, cyclamidomycin and pactamycin showed a low SI (of 1–7), with neothramycin and butylneothramycin A exhibiting the lowest SI scores.

The strong antiprotozoal activity of 7-deoxypactamycin, in comparison with pactamycin, provides a very interesting insight with regard to structure–activity relationships. In contrast to 7-deoxypactamycin, pactamycin possesses a hydroxy group at C-7. This causes the compound to show 8- to 40-fold less antiprotozoal activity and threefold less cytotoxicity than 7-deoxypactamycin. Our data shows that the deoxygenated form at C-7 in pactamycin (giving 7-deoxypactamycin) increases both antiprotozoal activity and the cytotoxicity, suggesting that the hydrogen bonding ability of the 7-hydroxyl group has an important role in both antiprotozoal activity and cytotoxicity. The antiprotozoal activities of neothramycin (an interconvertible mixture of A and B, epimers at C-3, in aqueous solution⁸) in comparison with butylneothramycin A provides further interesting information about structure–activity relationships. In contrast to neothramycin, butylneothramycin A, which was derived from neothramycin with acidic butyl alcohol, possesses an α -butoxy group at C-3, which is responsible for six to sevenfold less antiprotozoal activity and fivefold less cytotoxicity than neothramycin, which has a free hydroxyl group. This suggests that the free 3-hydroxy group in neothramycin has an important role in antiprotozoal activity

and cytotoxicity. The lack of antitrypanosomal activity of BY-81 in comparison with BD-12 and the lack of antimalarial activity of BD-12 in comparison with BY-81 provides very interesting information on structure–activity relationships against trypanosome and malaria parasites. BD-12 has a methyl group at the N-3' position and shows potent selective antitrypanosomal activity compared with BY-81. On the other hand, BY-81 lacks a methyl group at the N-3' position and shows potent antimalarial activity compared with BD-12. This indicates that the presence or absence of a methyl group at the N-3' position in BD-12 and BY-81 has an important role in determining the selective antiprotozoal activity.

With regard to the other compounds examined, acrylamidine is an amidine antibiotic, reported to have antifungal and antitumor activity.⁹ Amidinomycin, another amidine antibiotic, is believed to possess some antibacterial and antiviral activity.^{10,11} Belenamine is an open-chain aldol antibiotic, known to have antiviral and weak antibacterial activity.¹² The mode of action of these three compounds has not been reported. BD-12 and BY-81 are streptothricin-type nucleoside antibiotics, reputed to have antibacterial properties.^{13,14} The mode of action of streptothricin F is known to be through inhibition of protein synthesis, creating miscoding in *Escherichia coli*.¹⁵ Furthermore, the mode of action of the streptothricin-type antibiotics, A-53930A and B, is through inhibition of the N-type Ca^{2+} channel (in chick cerebral cortex synaptosomes).¹⁶ Cyclamidomycin is an acrylamide antibiotic with antibacterial activity.¹⁷ Its mode of action is through inhibition of nucleoside diphosphate kinase and pyruvate kinase (in *E. coli*) and oxidative phosphorylation in rat liver mitochondria.^{18,19} Pactamycin and 7-deoxypactamycin, which were originally isolated as cranomycin, are aminocyclitol antibiotics reportedly showing both antibacterial and antitumor activity.^{20,21} It is known that pactamycin acts through inhibition of the initiation of protein synthesis.²² Neothramycin and butylneothramycin A are pyrrolo(1,4)benzodiazepine antibiotics. Neothramycin has antitumor, weak antibacterial and antifungal activity.^{8,23} It is well known that its mode of action is by inhibition of DNA-dependent RNA and DNA polymerase reactions.²⁴ Of the clinically used antitrypanosomals, the mode of action of pentamidine and suramin remain unknown. Eflornithine works through irreversible inhibition of the biosynthesis of polyamine, which is important for cell growth, differentiation and replication of trypanosomes.²⁵ There is no current consensus on the mode of action of artemisinin, whereas chloroquine is widely believed to act through inhibition of the enzyme that polymerizes and detoxifies ferriprotoporphyrin in the parasite food vacuole.²⁶

The discovery of antitrypanosomal and antimalarial activities in the 10 antibiotics from our compound libraries is the first report of such properties for these metabolites. More significantly, our data show that small structural changes in a parent compound can cause a major difference in antiprotozoal properties.

Our results indicate that, among the antibiotics tested, belenamine and BD-12 show distinct promise as antitrypanosomals, surpassing both suramin and eflornithine with respect to SI values. In addition, BY-81 is as effective as chloroquine against malarial parasites and, moreover, maintains its effectiveness against drug-resistant parasites. Further chemical and biochemical studies are necessary to provide a better, in-depth understanding of the antiprotozoal structure–activity relationship in pactamycins, neothramycins, BY-81 and BD-12-related compounds, so that the compounds may be manipulated to retain or enhance antiprotozoal characteristics while reducing cytotoxicity. Such research is now under way, involving a partnership of multidisciplinary research groups, with a view to swift development and application of promising findings or compounds.

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