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From human immunodeficiency virus non-nucleoside reverse transcriptase inhibitors to potent and selective antitrypanosomal compounds

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ABSTRACT

The presence of a structural recognition motif for the nucleoside P2 transporter in a library of pyrimidine and triazine non-nucleoside HIV-1 reverse transcriptase inhibitors, prompted for the evaluation of antitrypanosomal activity. It was demonstrated that the structure–activity relationship for anti-HIV and antitrypanosomal activity was different. Optimization in the diaryl triazine series led to 6-(mesityloxy)-N_2-phenyl-1,3,5-triazine-2,4-diamine (**69**), a compound with potent in vitro and moderate in vivo antitrypanosomal activity.

1. Introduction

After malaria and lymphatic filariasis, human African trypanosomiasis (HAT, sleeping sickness) is estimated to have one of the highest disease burdens of the neglected parasitic diseases (NPD).[1] HAT is endemic in 36 sub-Saharan countries and approximately 70 million people are estimated to be at risk, with at least 10,000 mortalities annually.[2,3] However, gross underreporting due to difficulties in diagnosis and remoteness of some affected areas makes estimation of accurate rates of morbidity and mortality difficult. African trypanosomiasis is transmitted by the tsetse fly and caused by *Trypanosoma brucei gambiense*, causing endemic or chronic disease in Central and West Africa and *Trypanosoma brucei rhodesiense*, causing the more acute disease in East and Southern Africa. The animal form of African trypanosomiasis is called Nagana and caused by *Trypanosoma brucei brucei*.[4] Together with HAT, Nagana is a major cause of rural underdevelopment with significant economic damage to cattle farming. Chemotherapy currently remains the only treatment option. However the available drugs that were developed decades ago show limited efficacy, are not affordable, cause severe adverse effects and are threatened by increasing drug resistance.[4,5] Currently used drugs are pentamidine, suramin, melarsoprol, eflornithine and nifurtimox. HAT is classified as a neglected disease since investments are negligible compared to its impact on human and veterinary health. In the last decades, little progress has been made in addressing this lack of effective treatments and the initiative to tackle this pressing medical need has largely moved to academic research in collaboration with public–private partnerships, such as the Drugs for Neglected Diseases initiative (DNDi).

It has been well established that phenotypic screening is a successful approach for the discovery of first-in-class drugs, which is especially valid for drug discovery in infectious diseases, since the 'whole micro-organism' is the best possible target.[6,7] Hence, the screening/evaluation of existing libraries of drug-like molecules for novel anti-infectives is the most logical and straightforward approach. In the framework of a drug discovery programme for new anti-HIV microbicides,[8–11] we developed a library of novel

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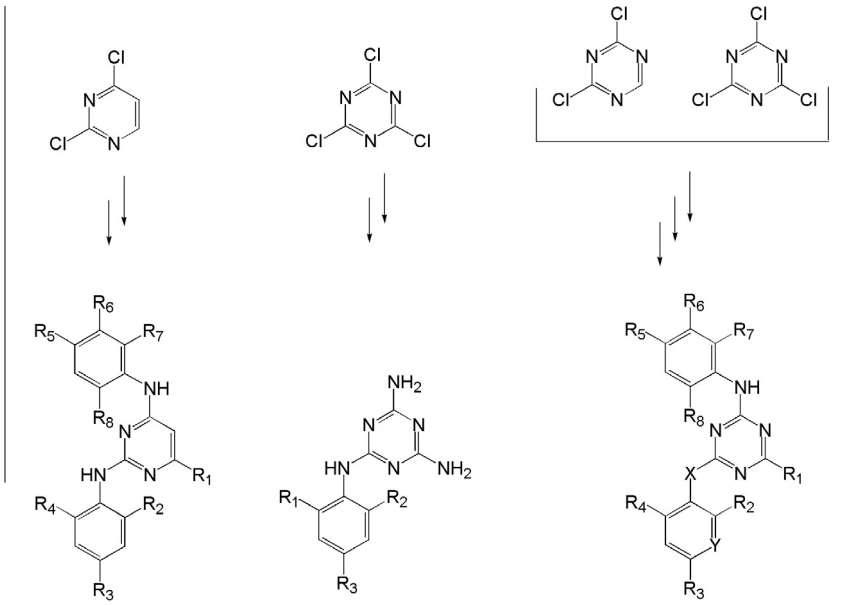
pyrimidines and triazines[11] related to TMC120[12] (**1**) as non-nucleoside reverse transcriptase inhibitors (NNRTIs). The antiprotozoal activity of NNRTIs has never been described, but the triazine core is a substrate for the unique nucleoside P2 transporter in *T. brucei* and is present in melarsoprol.[13] The triazine core has been used in drug delivery to trypanosomes for compounds such as polyamine analogues and nitroheterocycles.[14–19] However, since there are no reports of diarylsubstituted pyrimidines and triazines as antitrypanosomal agents, we broadly screened and optimized our compounds against *T. b. brucei* and *T. b. rhodesiense* (Fig. 1).

2. Results and discussion

The target compounds were synthesized from 2,4-dichloropyrimidines, 2,4-dichlorotriazines and 2,4,6-trichlorotriazines by nucleophilic aromatic substitution (S_NAr) reactions (Schemes 1–5 in Supplementary content).

The activity of compounds **1–5**, **6–10** and **11–70** toward *T. b. brucei* and *T. b. rhodesiense* and their cytotoxicity on a human cell line (MRC5) is presented in comparison with anti-HIV-1 activity (Tables 1–5). The compounds were also tested against *Trypanosoma cruzi*, *Leishmania infantum* and *Plasmodium falciparum* (Tables 1–3 in Supplementary content). As a control, we used standard anti-parasitic compounds as well as known NNRTIs. The antiparasitic potential of all compounds was analyzed by applying the WHO/TDR screening activity criteria specified for each parasite.[20] All the disubstituted pyrimidines including TMC120 (**1**) as well as the monosubstituted triazines **6–10** were inactive (Table 1). In contrast, among the diaryltriazines several compounds exhibited submicromolar activity against both *T. b. brucei* and *T. b. rhodesiense* (Tables 2–5), with **69** being equipotent to suramin and melarsoprol.

Based on the activities of the 60 diaryltriazines, we were able to deduce a structure–activity relationship (SAR) (Fig. 2). At R_1, the presence of NH_2 (e.g., **29**) is clearly favoured, whereas hydrogen (e.g., **14**), chloro- (e.g., **16**) and some alkylamino- (**37–44**) substituents are less active and/or cytotoxic. Other small substituents at this position such as cyano- (e.g., **24**) and methoxy- (e.g., **26**) are not tolerated. The presence of methyl groups at R_2, R_3 and R_4 originating from the SAR of the anti-HIV-1 NNRTIs (TMC120, **1**) resulted in potent antitrypanosomal compounds. Generally, these methyl groups can be replaced with Br or Cl, especially at R_2 and R_4, but removal of one of the methyls is less well tolerated. The *para*-cyano group on the other aryl group (R_5), which is known to be crucial for anti-HIV-1 activity can be removed and offers a way to dissociate antitrypanosomal and anti-HIV-1 activity. For instance, in the series **60** (*para*-cyano), **70** (*meta*-cyano) and **69** (no cyano group), the antiviral activity decreases whereas the antitrypanosomal activity increases. Replacement of one aryl group with a pyridyl group (**45–48**) was not tolerated. Changing the arylamino substituent (X = NH) to an aryloxy substituent (X = O, Table 5) was more favourable in almost all cases (e.g., **60** vs **28**). X = NMe did not seem to alter the activity much (**36** vs **28**). Concludingly, **16**, **29**, **63** and **69** are the four most potent antitrypanosomal compounds (IC_{50} = <0.25 μM) from the library of disubstituted triazines **11–70**.



R_1 = H, Cl, OMe, CN, NH_2, NHR_9, NR_9R_{10}, Piperidinyl, N-Me piperazinyl
R_2 = H, Me, OMe, Br, Cl
R_3 = H, Me, Br, Cl, CN, CHO
R_4 = H, Me, OMe, Br
R_5 = R_6 = H, CN
R_7 = R_8 = H, Me
R_9 = Me, iPr, cyclopropyl, 3-Methoxypropyl, phenyl
R_{10} = Me
X = NH, NMe, O
Y = CH, N

Figure 1. Structures of target compounds.