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Identification of the benzodiazepines as a new class of antileishmanial agent

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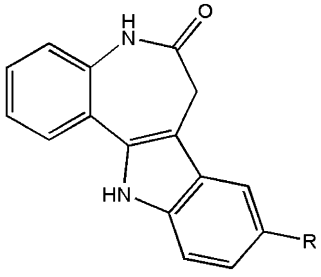
Abstract—The continual increase in drug resistance; the lack of new chemotherapeutic agents; the toxicity of existing agents and the increasing morbidity with HIV co-infection mean the search for new antileishmanial agents has never been more urgent. We have identified the benzodiazepines as a structural class for antileishmanial hit optimisation, and demonstrated that their in vitro activity is comparable with the clinically used drug, sodium stibogluconate, and that the compounds are not toxic to macrophages.

Leishmaniasis, which threatens approximately 350 million people in 88 countries, was the cause of death of 59,000 people in 2002, and of the 1.5–2 million new cases reported, one-third were the life-threatening form of leishmaniasis.[1] The continual increase in drug resistance, the lack of new chemotherapeutic agents and the toxicity of existing agents means that new drugs are required. A recent study by Meijer and coworkers[2] demonstrated that paullones **1**, previously identified as antitumour agents through their inhibition of cyclin-dependent kinases, completely inhibit the growth of *Leishmania mexicana* promastigotes in vitro. Whilst paullones offer a promising starting point for chemical investigation,[2] we considered their non-selectivity and multi-step, low yielding synthesis[3] a disadvantage for development of structurally related compounds with increased antiparasitic efficacy. Consequently, we prepared a series of synthetically amenable benzodiazepines and pyrrolobenzodiazepines structurally related to the paullone nucleus to probe for activity. Our aim was to chemically modify the basic benzodiazepine skeleton and optimise for antileishmanial activity in a macrophage amastigote infection model,[4] rather than activity against the promastigote stage, so that we could test for antiparasitic activity against the host-relevant form, potential cytotoxicity and ability to enter the macrophage in the same assay.[5,6]

We report here the short, efficient synthetic route and the results of the biological testing for antileishmanial activity. Whilst modifications were made to probe the effects of structural changes, low cost economics and synthetic efficiency were also important factors in selecting compounds for synthesis.



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To establish whether tricyclic or tetracyclic systems similar to paullones exhibited antileishmanial activity, compounds **3**–**5** were prepared in a one-step reaction[7] by condensation of isatoic anhydride **2** and the appropriate amino acid derivative in DMSO at 100 °C (Scheme 1).

Keywords: Antileishmanial agents; Amastigote; Benzodiazepine; Pyrrolobenzodiazepine; Paullone.
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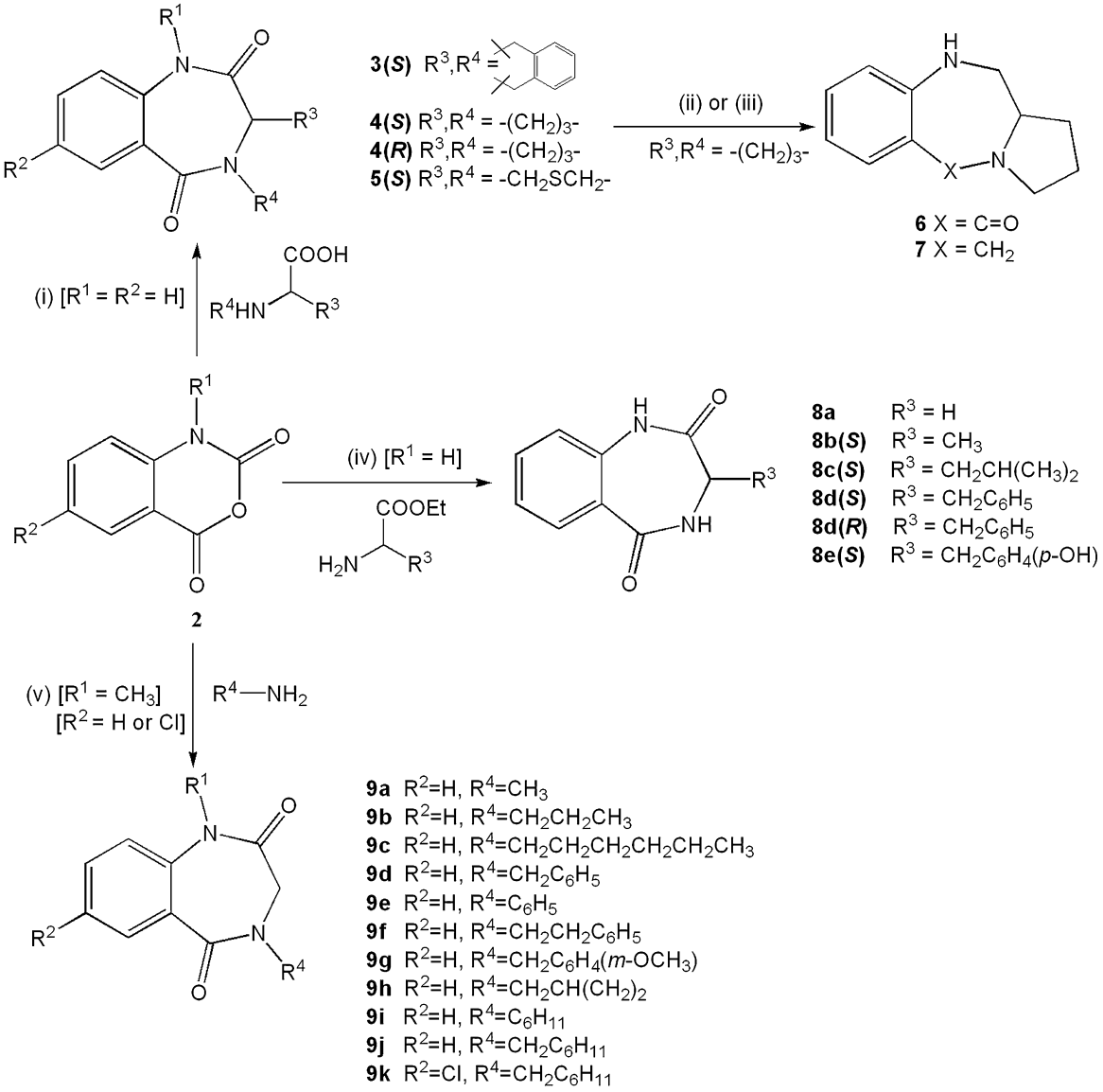

Scheme 1. Simple and affordable route to the pyrollobenzodiazepines and the benzodiazepine diones. Reagents and conditions: (i) 1—DMSO, 100 °C (2) H_2O, 0 °C; (ii) $NaBH_4$, TFA, glyme, reflux; (iii) $LiAlH_4$, THF, reflux; (iv) 1—pyridine, reflux; 2—H_2O, 0 °C; (v) 1—CH_2Cl_2, KPhos, pH 7.0, $BrCOCH_2Br$, 5 °C, DBU.

The effects of chirality on activity were investigated with both enantiomers of proline. The compounds were isolated by precipitation of the cooled reaction mixture using iced water. To establish whether the hydrogen-bond acceptors of the amide carbonyls were necessary for activity, the secondary amide of dione **4** was selectively reduced to the corresponding secondary amine using sodium borohydride and trifluoroacetic acid in glyme to give **6** in a quantitative yield. Both amides in dione **3** were reduced with lithium aluminium hydride to give the diamine **7**.[8] Reductions were performed on both the *R*- and *S*-isomers of **4** to produce enantiomerically pure compounds. In order to establish whether a tricyclic system was an activity requirement, proline was replaced with a series of amino acid ester hydrochlorides,[9] which in refluxing pyridine, produced benzodiazepine-2,5-diones **8** with different hydrophobic substituents (or no substituent in the case of glycine) at C_3, whilst leaving N_4 free as the secondary amide. Finally, to investigate the role of substituents at N_4, a high yielding, 'one-pot' reaction between *N*-methyl isatoic anhydride, an appropriate primary amine and bromoacetyl bromide in the presence of DBU was used to produce a series of N_4-substituted benzodiazepine-2,5-diones **9**.[10]

The antileishmanial activity of all compounds against a clinically derived strain of *Leishmania donovani* (antimony-sensitive 200016)[4] is shown in Table 1. In addition, the activity of the clinically used drug sodium stibogluconate is also shown for comparison.

These data indicate that whilst the tetracycle ***3(S)*** is inactive, the tricyclic pyrrolobenzodiazepine-2,5-diones are more effective antileishmanial agents than sodium stibogluconate at the concentrations tested, with no evidence of toxicity against the host macrophage cells. Activity appears to be independent of chirality in the tri-