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## FUNCTIONALIZED AZOLES AND TRIAZOLO[1,5-a]PYRIMIDINES AS LATENT

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**Abstract:** Triazolo[1,5-a]pyrimidine (3-6), benzoxazole (7a,b) and benzimidazole (7c) derivatives have been synthesized and evaluated for their *in vitro* leishmanicidal activity against *L. donovani* promastigotes.© 1997 Elsevier Science Ltd.

Introduction: Triazolo[1,5-a]pyrimidines being an isostere of purine display diverse pharmacological activity as inhibitors of xanthine  $oxidase^1$ , nucleosidephosphotransferase<sup>2,3</sup> and phosphodiesterase<sup>4</sup> but none had so far demonstrated their latent leishmanicidal activity. Based on structure activity analyses of different class of heterocycles it has been rationalised that the presence of N-C-N and N-C-N structural units either in flexible or rigid form in their molecular make up is basic requirement to demonstrate leishmanicidal activity while specific functionality at specific position potentiate it. It was therefore, considered to synthesize molecules simulating the recognised structural units for evaluating their leishmanicidal efficacy. Since azoles and azines are well documented for their antiparasitic properties<sup>5-8</sup>, synthesis of triazolo[1,5-a]pyrimidines (3-6) with diverse functionalities was undertaken to demonstrate their potential as leishmanicide. Some benzoxazoles or benzimidazole derivatives were also synthesized for establishing structure activity relationship and to generate new leads.

**Synthesis** : The intermediate 3-amino-5-arylsulphonamido-4H-1,2,4-triazole (2) prepared<sup>9</sup> from the reaction of S,S-dimethyl-N-arylsulfonylcarbodithioimidates (1) and aminoguanidine was used as precursor for the construction of fused heterocycles. 2-Arylsulphonamido-1,2,4-triazolo[1,5-a]pyrimidine derivatives (3,4,6) and 2-arylsulphonamido-5,6cycloalkyl-1,2,4-triazolo[1,5-a]pyrimidines (5a-c) were synthesized by acid catalysed condensation-cyclization of 2 with acyclic or cyclic, 1,3-dicarbonyl compounds or either of the ethoxymethylene derivatives of ethyl cyanoacetate/diethylmalonate/malononitrile and ketene dithio-

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acetal separately. Reaction of 1 with 2-aminophenol and 1,2-phenylenediamine led to the formation of oxazole (**7a-b**) and benzimidazole (**7c**) derivatives respectively (Scheme 1). All the synthesized compounds were characterized by elemental and spectroscopic analyses.

## Scheme 1



**Reagents & Conditions:** (i)  $NH_2-C = (NH)-NHNH_2/(CH_2OH)_2/KOH/170^{\circ}C$ ; (ii)  $\beta$ -diketone or  $\beta$ -ketoester/acetic acid/160°C; (iii)  $C_2H_5OCH = C(R_1R_2)$  [ $R_1 = R_2 = CN$ ;  $R_1 = CN$ ,  $R_2 = COOEt$ ;  $R_1 = R_2 = COOEt$ ]/acetic acid/160°C; (iv) cyclopentyl or cyclohexyl-1,3-ketoester/acetic acid/160°C; (v) (CN)\_2C = C(SMe)\_2/200°C; (vi) 2-aminophenol/ 2-phenylene diamine /DMF/ NaOH, reflux.

**Biological Activity:** The *in vitro* leishmanicidal activity of the synthesized compounds was determined by measuring the <sup>3</sup>H thymidine incorporation<sup>10</sup> in promastigotes of *L. donovani*.

Promastigotes of L. donovani being maintained in vitro were harvested in the log phase and resuspended in fresh Dulbecco's Modified Eagle's Medium (DMEM) so as to obtain 1-2x10<sup>6</sup> promastigotes/ 200  $\mu$ l of the medium. 1-2 x 10<sup>6</sup> promastigotes in 200  $\mu$ l of the growth medium per well were dispensed into each well of 96 wells microtitretissue culture plate. The test compound was added to the final concentration of 200 AM or as specified and cultures were allowed to grow at 26<sup>0</sup>C. After 72 hours, culture was pulsed with <sup>3</sup>H thymidine (0.2  $\mu$ Ci/well) and allowed to grow further at 26<sup>o</sup>C for atleast 18 hours. After 18-24 hours the cells were harvested on glass fibre filters (Whatman) and transferred to scintillation vials and after addition of scintillation cocktail, radioactivity was measured using liquid scintillation counter (LKB, 1209 Rackbeta). The parallel controls were also run without using drug at all. The compound effect was measured in terms of 8 inhibition of growth usina dissociations/disintegration per minute (DPM) counts. Each assay was run atleast in tetraplicates. The results are presented in Table 1.

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Compound No.	Inhibition (%) (at 200 µM conc.)	IC <sub>50</sub> values (µM)
$\begin{array}{c} 2\\ 3a\\ 3b\\ 3c\\ 3d\\ 3e\\ 4a\\ 4b\\ 4c\\ 5b\\ 6c\\ 7a\\ 7b\\ 7c\\ Pentamidine (100 A\\ (Standard drug) \end{array}$	97 100 100 3 98 5 0 0 0 0 0 15 17 74 92.6 80 100	2 1.5 2 - - - - - - - - - - - - - - - - - -

Table 1: In vitro leishmanicidal activity of azoles (2,7 a-c) and triazolo[1,5-a]pyrimidines (3-6)

Structure-activity relationship of screened compounds was established from the leishmanicidal activity of synthesized compounds. Among all the 13 screened compounds only prototypes 2,3 and 7 displayed *in vitro* leishmanicidal activity while others were either inactive or poorly active. The order of activity of highly potent compound was 3a>2=3b=3d>7a>7c>7b (based on IC<sub>50</sub> values). Of all the compounds **3a** (100%), **3b** (100%), **3d** (98%) and **2** were almost equipotent and activity wise equivalent to pentamidine, a standard drug used at 200  $\mu$ M concentration. The overall activity profile of compounds **2,7b,7c** and **7a** demonstrated 97%, 92.5%, 80% and 74% of growth inhibition at same concentration though there is a great difference in their IC<sub>50</sub> values.

As evident from the screening data of compounds 2 (97%), 3a (100%), 3b (100%), 3c (3%), 3e (5%), 7a (74%), 7b (92.6%) and 7c (80%) that electron donating substituents R=CH<sub>3</sub>, NH<sub>2</sub>, in 2,3 and 7 displayed high order of activity than the presence of electron withdrawing substituents, R=Cl in 3e and 4a-c except 3d which demonstrated 98% of growth inhibition. Among compounds 7a-c, 7b (92.6%) was found most active having electron donating methylsubstituent in the phenyl ring. A change of benzoxazole (7a,b) to benzimidazole 7c (80%) did not change the activity profile of the compounds.

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