Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 8, pp. 1087-1090, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00166-2

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

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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[2,3] and phosphodiesterase[4] 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[5-8], 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[9] 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-5,6-cycloalkyl-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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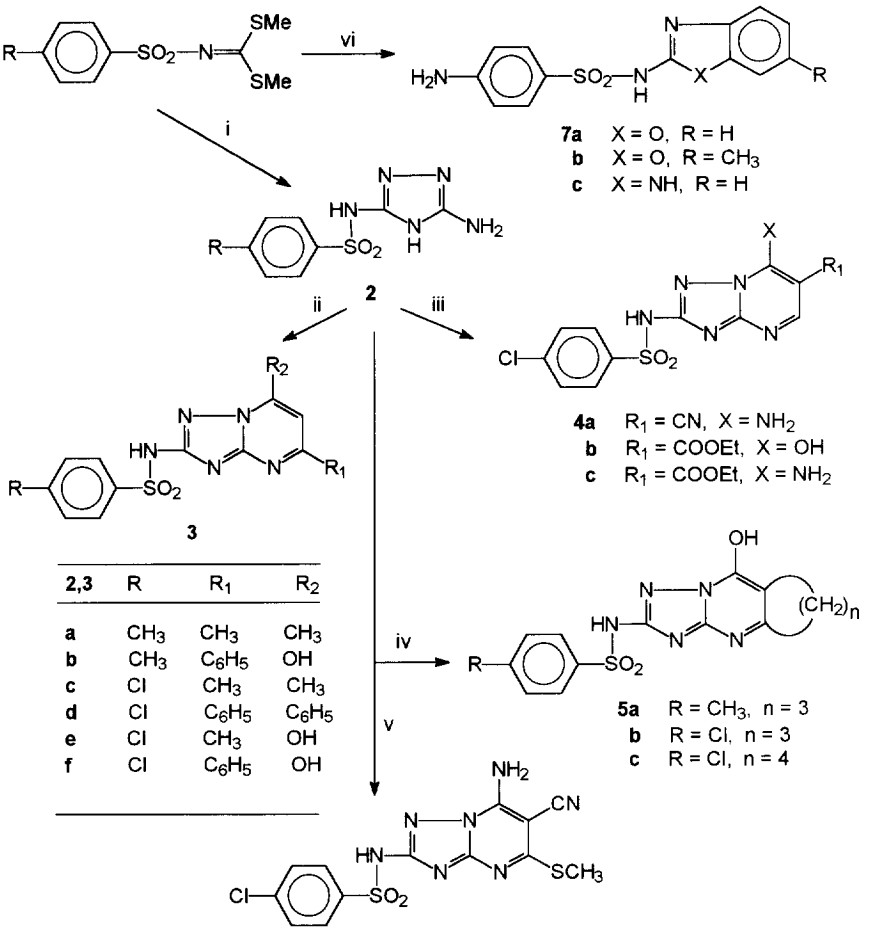
acetal separately. Reaction of **1** with 2-aminophenol and 1,2-phenylenediamine led to the formation of oxazole (**7a-b**) and benzimidazole (**7c**) derivatives respectively (Scheme 1). All the synthesized compounds were characterized by elemental and spectroscopic analyses.

Scheme 1



2,3	R	R_1	R_2
a	CH_3	CH_3	CH_3
b	CH_3	C_6H_5	OH
c	Cl	CH_3	CH_3
d	Cl	C_6H_5	C_6H_5
e	Cl	CH_3	OH
f	Cl	C_6H_5	OH

Reagents & Conditions: (i) NH_2-C=(NH)-$NHNH_2$/$(CH_2OH)_2$/ KOH/170°C; (ii) β-diketone or β-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 ^3H thymidine incorporation[10] 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 1-2x10^6 promastigotes/ 200 µl of the medium. 1-2 x 10^6 promastigotes in 200 µl of the growth medium per well were dispensed into each well of 96 wells microtitre-tissue culture plate. The test compound was added to the final concentration of 200 µM or as specified and cultures were allowed to grow at 26°C. After 72 hours, culture was pulsed with ^3H thymidine (0.2 µCi/well) and allowed to grow further at 26°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 % inhibition of growth using dissociations/disintegration per minute (DPM) counts. Each assay was run atleast in tetraplicates. The results are presented in Table 1.

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

Compound No.	Growth Inhibition (%) (at 200 µM conc.)	IC_{50} values (µM)
2	97	2
3a	100	1.5
3b	100	2
3c	3	-
3d	98	2
3e	5	-
4a	0	-
4b	0	-
4c	0	-
5b	15	-
6	17	-
7a	74	6.4
7b	92.6	15.5
7c	80	10.5
Pentamidine (100 µM) (Standard drug)	100	0.76

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_{50} 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 μM 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_{50} 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_3, NH_2, 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.

Acknowledgement: Pratibha Srivastava and Dr. S.K. Singh are grateful to DOD and CSIR respectively for their financial support.

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(*Received in Japan* 3 *September* 1996; *accepted* 19 *March* 1997)