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TETRAAZAACENAPHTHENE, TETRAAZAPHENALENE AND 1,3,4-THIADIAZOLE DERIVATIVES AS POTENTIAL LEISHMANICIDES+

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Abstract: Synthesis and leishmanicidal activity of 1,3-dithiolanes (**2a,b**), 1,3-dithiane (**2c**), tetraazaacenaphthene (**4a-h**) and tetraazaphenalene (**4i,j**), imidazolidin-2-ylidene (**5a,6a,b**) and hexahydropyrimidin-2-ylidene (**5b**) derivatives are described. Some of the screened compounds have demonstrated significant leishmanicidal activity in *in vitro* and *in vivo* evaluations.

Introduction: Pyrimidine and imidazole rings, being an integral part of nucleic acid bases and various bioactive substances are involved in diverse pharmacological functions in living organisms. It was contemplated that pharmacological profiles of 1,3-dithiolanes and imidazolidine derivatives being isosteres of imidazole will analogously demonstrate high degree of leishmanicidal activity in presence of specific substituent at specific position which plays a crucial role in potentiating the biodynamic property of the molecule. The importance of pyrimidines as leishmanicides[1-5] aroused considerable interest in developing its rigid analogs as tetraazaacenaphthene (**4a-h**) and tetraazaphenalene (**4i,j**) derivatives which could not only demonstrate leishmanicidal activity but also effectively modulate natural defence of the host and restore the impaired immune systems.

Based on pattern recognition approach and past observations it was rationalised that N-C(=N)-N OR S-C(=N)-N structural unit is the minimum requirement for displaying leishmanicidal as well as immuno-stimulant properties[6,7]. Thus, it was presumed that simulation of either of the structural units in flexible and rigid forms or repetition of such moieties in fused heterocyclic systems may potentiate the antileishmanial activity. The antiparasitic property of azoles such as 1,3,4-thiadiazoles, imidazo[2,1-b]thiadiazoles is well documented[8] and their attachment with other heterocycles often ameliorates or diminishes the bioresponses, depending upon the type of substituent and position of attachment.

Of the various synthesized compounds of prototypes **2,4,5** and **6**, only 17 compounds were screened

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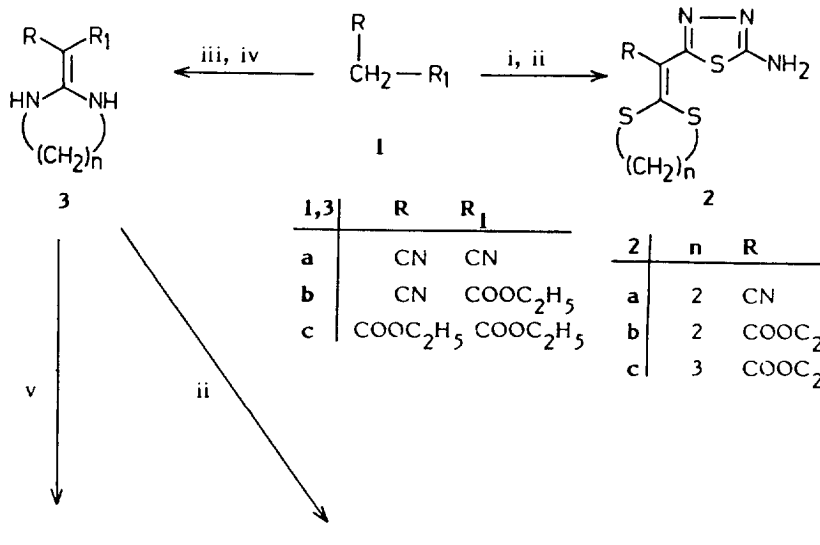
for *in vitro* and 12 compounds for *in vivo* leishmanicidal activity at different concentrations against promastigotes and amastigotes of *L. donovani* respectively. Six compounds **4b-e,g,j** demonstrated high order of *in vitro* leishmanicidal activity at 200 μM concentration against promastigotes of *L. donovani* by measuring ^{3}H-thymidine incorporation. The activity profile of these compounds was almost of the same order and is presented in Table-1. The *in vivo* activity of selected compounds was evaluated against amastigotes of *L. donovani* at 50 mg/kg dose. Only three compounds displayed more than 70% of inhibition and order of activity was as **4e**(81%) > **4b**(79%) > **4a**(71%). The rest of the compounds demonstrated below 50% of inhibition at the same dose level which was treated as insignificant.

Synthesis: Synthesis of (5-amino-1,3,4-thiadiazol-2-yl) (1,3-dithiolan-2-ylidene)acetonitrile (**2a**), ethyl (5-amino-1,3,4-thiadiazol-2-yl) (1,3-dithiolan-2-ylidene)acetate (**2b**) and ethyl (5-amino-1,3,4-thiadiazol-2-yl) (1,3-dithian-2-ylidene)acetate (**2c**) was achieved by transforming nitrile function on corresponding 1,3-dithiolanes[9] and 1,3-dithianes[9] to 5-amino-1,3,4-thiadiazol-2-yl) moiety by acid catalysed condensation-cyclization reaction with thiosemicarbazide[10]. Similarly, ethyl (5-amino-1,3,4-thiadiazol-2-yl) (imidazolidin-2-ylidene)acetate (**5a**) and ethyl (5-amino-1,3,4-thiadiazol-2-yl) (hexahydropyrimidin-2-ylidene)acetate (**5b**) were obtained by reaction of **3** with thiosemicarbazide in TFA. The amino function in **5** was exposed to **ω**-bromoacetophenone for the formation of **6a,b**. Thermal cyclization of **3** with alkyl/aryl isocyanate or alkyl/aryl isothiocyanate provided tetraazaacenaphthene (**4a-h**) and tetraazaphenalene (**4i,j**) derivatives. All the synthesized compounds (Scheme-1) were characterized by elemental and spectroscopic analyses.

Biological Activity: The *in vitro* leishmanicidal activity of synthesized compounds was determined by measuring the ^{3}H thymidine incorporation[11] 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 Eagles medium so as to obtain 1-2 x 10^6 promastigotes/200 μl of 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 drug or compound was added to the final concentration of 200 μM and cultures were allowed to grow at 26°C. After 72 hours, culture was pulsed with ^{3}H-Thymidine (0.2 μci/well) and allowed further to grow at 26°C for atleast 18 hours. After 18-24 hours the cells were harvested on glass fibre filters using a cell-harvester. The filter disks were transferred into scintillation vials and after addition of scintillation cocktail, radioactivity was measured by

Scheme 1



1,3	R	R_1
a	CN	CN
b	CN	$COOC_2H_5$
c	$COOC_2H_5$	$COOC_2H_5$

2	n	R
a	2	CN
b	2	$COOC_2H_5$
c	3	$COOC_2H_5$

4	n	X	Y	R_2
a	2	NH	S	C_2H_5
b	2	NH	S	C_6H_5
c	2	NH	S	4-FC_6H_4
d	2	O	S	C_2H_5
e	2	O	S	C_6H_5
f	2	O	S	4-FC_6H_4
g	2	O	O	2-$CH_3C_6H_4$
h	2	NH	O	2-$CH_3C_6H_4$
i	3	O	O	C_2H_5
j	3	NH	S	C_2H_5

5

a n = 2

b n = 3

6

a Ar = 4-ClC_6H_4

b Ar = 4-BrC_6H_4

Reagents/Conditions: i) $CS_2/(CH_2)_nBr_2/NaOC_2H_5/0°$, ii) $NH_2C(=S)NHNH_2$/TFA/60-80°, iii) $CS_2/(CH_3)_2SO_4/NaOC_2H_5/20°$, iv) $(CH_2)_n(NH_2)_2/C_2H_5OH/110°$, v) R_2NCY/160-170°, vi) $ArCOCH_2Br/C_2H_5OH$/110°C

using a liquid scintillation counter (LKB, 1209 Rackbeta). The paralle! controls were also run whereby the wells did not receive any drug at all. The drug effect was measured in terms of % inhibition using the DPM counts. Each assay was run in tetraplicates or quadruplicates using pentamidine as standard drug.

The *in vivo* leishmanicidal activity against amastigotes of *L. donovani* was determined[12] in golden hamsters (*Mesocricetus auretus*) inoculated with 1 x 10^7 amastigotes/hamster intracardially. The infection was monitored in spleen after 20 days by biopsy and animals with 11-20 amastigotes/100 spleen cell nuclei were selected for the study. The infected hamsters were divided into three groups. One group was left untreated while second received sodium stibogluconate (10 mg/kg x 5 days, i.p.) and the third received test chemicals (50 mg/kg i.p.) for 5 days. On 7th day post treatment animals were sacrificed and spleens of untreated and treated

Table 1: *In vitro* and *in vivo* antileishmanial activity of 1,3-dithiolan-2-ylidenes(**2**), tetraazaacenaphthenes (**4a-h**), tetraazaphenalene(**4i,j**), imidazolidin-2-ylidene (**5a,6a,b**) and hexahydropyrimidin-2-ylidene(**5b**) derivatives.

Compound No.	*In vitro* screen at 200 μM concentration (%inhibition)	*In vivo* screen at 50 mg/kg dose for 5 days (% inhibition)*
2a	18	-
2b	0	-
2c	0	-
4a	20	71.2
4b	99.3	78.6
4c	99.3	Toxic
4d	76	Toxic
4e	85	81.3
4f	50	0
4g	93	0
4h	7	-
4i	58	0
4j	99.2	50.7
5a	0	-
5b	73	44.7
6a	40	0
6b	31	0
Sodium stibogluconate (10 mg/kgx5days)	-	100
Pentamidine (100 μM conc.)	100	-

*Data are mean of 15 animals

animals were dissected and number of amastigotes/100 spleen cell nuclei were determined in giemsa stained smear. The percent inhibition of the amastigotes was calculated by using formula described in the literature[13]. Sodium stibogluconate was used as standard drug to compare the efficacy of drug.

A critical structure survey of the screened compounds revealed that phenyl substituent at R_2 in **4** potentiates the *in vivo* leishmanicidal activity as evident from the activity profile of **4e**(81.3%) and **4b**(78.6%) while compounds having substituted phenyl groups were either toxic or inactive (**4c,f,g**). In exception only **4a** with ethyl substituent at R_2 demonstrated 71% of inhibition. An enlargement in ring size at bridged nitrogen in **4** and **5** from di- to trimethylene moiety also ameliorated the antileishmanial activity as evident from the *in vitro* screening data of **4j**(99.2%), **4i**(58%), **4a**(20%), **5b**(73%) and **5a**(0%). In general thio analogs demonstrated better activity than corresponding oxo analogs except **4g**(93%).

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13. Formula used for calculating percent inhibition

= (An x 100)/(Ti x In)

An = Actual number of parasites in treated animals

Ti = Times increased of parasites in control

In = Initial number of parasties in treated animals

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