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5-CYANO-2-THIOURACILS AND THEIR DERIVATIVES: A NEW CLASS OF LEISHMANICIDES+

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Abstract: Several 5-Cyano-6-substituted-2-thiouracils **(1a-e)** and their monocyclic **(2,3, 4,6)** and bicyclic **(5)** derivatives have been prepared and evaluated for *in vivo* leishmanicidal activity against *L. donovani* in hamster.

Introduction: Chemotherapy of leishmania is limited to handful of drugs with dire side effects but patients are bound to compromise with the existing drugs. The first regimen of drugs in clinical use are sodium stibogluconate[1,2] (pentostam) and glucantime[3,4]. These are highly toxic to the host and their efficacy depends upon the quantitative presence of Sb^{5+} oxidation state in the complex[5]. Pentamidine is a drug of choice in resistant cases of sodium stibogluconate while amphotericin B is useful in unresponsive and relapse cases of visceral leishmaniasis[6,7].

The alarming situation of chemotherapy of leishmania aroused considerable interest to design and synthesize molecules which kill the parasites and also boost the impaired immune system of the host, based on the pattern recognition approach. Levamisole and its metabolites possessing S-C=N structural unit display leishmanicidal as well as immunostimulant properties[8,9]. It was presumed that simulation of recognised structural unit in flexible or rigid forms may exhibit antileishmanial activity of high order. In order to testify our presumption 5-cyano-2-thiouracils **(1a-e)** and their derivatives **2-6** were designed and synthesized incorporating recognised structural unit in the ring skeleton. Among compounds of various prototypes, only compounds **1a,e, 2c,d,e,g** displayed maximum 80-85% of inhibition, almost parallel to sodium stibogluconate (86%), a standard drug, while **1b, 2f,h, 4b, 5a** demonstrated activity in the range of 73-78%. Like other antileishmanial drugs, all the active compounds did not show any dose-response relationship. Activity of these compounds increases upto certain dose level and thereafter decreases. At 10 mg/kg dose only compounds **1b, 1e, 2e** and **2g** retained the leishmanicidal activity while **1a, 2c** and **2d** lost their efficacy. Two of them **1e** and **2e** were found effective even at 50 mg/kg dose and thereafter become toxic. Rest of the compounds were either inactive or moderately active.

Synthesis: Various 5-cyano-6-substituted-2-thiouracils **(1a-e)** were prepared[10,11] by the base catalysed condensation-cyclization of aldehyde, ethyl cyanoacetate and thiourea. The 2-alkyl/aralkyl-thio-5-cyano-3,4-dihydro-6-substitutedpyrimidin-4-ones **(2)** were obtained by selective monoalkylation of **1** with alkyl halide at < 5°C. Further, alkylation of **2** or dialkylation of **1** with two moles of alkyl halide yielded **6.** Reaction of **2** with $POCl_3$ provided corresponding chloro compound **3** which on reaction with amine provided **4.** Interaction of **1** with dihaloalkane yielded **5** (Scheme 1).

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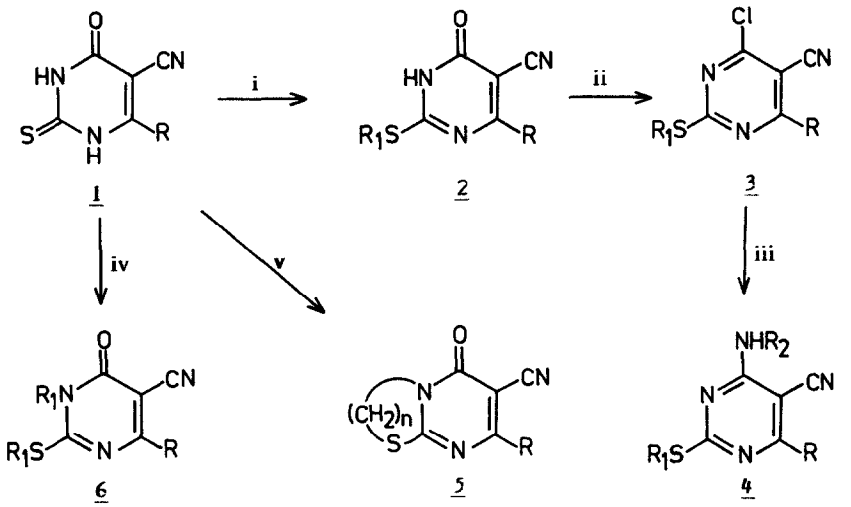
Biological activity: The *in vivo* leishmanicidal activity against amastigotes of *L. donovani* was determined[12] in golden hamsters (Mesocricetus auretus) inoculated with $1x10^7$ amastigotes/hamster intracardially. The established infection with 11-50 amastigotes per 100 spleen cells nuclei were selected for the study. The infected hamsters were divided into three groups of three each. One group was left untreated while second group received sodium stibogluconate (2.5 mg/kg x 5 days, i.p.) and the third received test chemicals (2.5 mg/kg x 5 days, i.p.). On day 28, animals were sacrificed and spleens of untreated and treated animals were disected and number of amastigotes/ 100 spleen cells nuclei were counted. The percent inhibition of the amastigotes was calculated by using formula described in the literature[12,13]. Sodium stibogluconate, a standard drug at above mentioned dose demonstrated only 65% of inhibition.

Scheme-1



Reagents/Conditions: i) $K_2CO_3/R_1X/DMF/<5°C$, ii) $POCl_3/C_6H_5N(CH_3)_2/100°C$, iii) $R_2NH_2/C_2H_5OH/90°C$, iv) $K_2CO_3/2R_1X/DMF/35°C$, v) $(CH_2)_nBr_2/K_2CO_3/DMF/80°C$.

A critical structure-activity relationship in the given series revealed that the activity of 2-thiouracils **(1a-e)** depends upon the substituent at position 6 of the pyrimidine ring. As it is evident from the data that electron attracting substituent in the aryl ring at position 6 in **1** diminishes the activity. Substitution at position 2 in **1** by benzyl, 4-chlorobenzyl and allyl groups make significant contribution to the leishmanicidal activity with exception to **2a,b** while presence of methyl group at 2,3 positions in **6a,b** reduces the activity. The order of activity in 2,4-disubstituted products **(3,4)** depends upon the nature of groups at position 4. Presence of electron withdrawing substituent displayed loss of activity while substituents, nucleophilic

in nature ameliorate it. Blocking of 2,3-positions in **1** by methylene groups led to **5a-d** with depletion in order of activity as compared to **1**, while in the same series dihydrothiazolo[3,2-a]pyrimidin-5-ones **(5a,c)** demonstrated better activity than dihydropyrimido[2,1-b]thiazin-6-ones **(5b,d)**. Thus it was concluded that only thiouracils and its mono-benzylated **(2c-f)** and allylated **(2g,h)** derivatives possess high order of leishmanicidal activity.

Table 1: In vivo antileishmanial activity of 2-thiouracils **(1)** and their derivatives **2-6** at 2.5 mg/kg x 5 days in hamster amastigotes of *L. donovani*. Values are the average % inhibition of triplicates afforded by the test compounds after 28 days of drug administration.

	R	R_1	R_2	Inhibition (%)
	Sodium Stibogluconate (10 mg/kg x 5 days)			86**
1a	$3\text{-}CH_3OC_6H_4$	--	--	85**
1b	$4\text{-}CH_3C_6H_4$	--	--	78*
1c	$4\text{-}FC_6H_4$	--	--	63
1d	$2\text{-}ClC_6H_4$	--	--	0
1e	C_2H_5	--	--	80**
2a	$3\text{-}CH_3OC_6H_4$	$C_6H_5CH_2$	--	0
2b	$3\text{-}CH_3C_6H_4$	$C_6H_5CH_2$	--	0
2c	$4\text{-}CH_3C_6H_4$	$C_6H_5CH_2$	--	82**
2d	$3\text{-}CH_3OC_6H_4$	$4\text{-}ClC_6H_4CH_2$	--	85**
2e	3-pyridyl	$4\text{-}ClC_6H_4CH_2$	--	84**
2f	C_2H_5	$4\text{-}ClC_6H_4CH_2$	--	76*
2g	$3\text{-}CH_3OC_6H_4$	$CH_2=CH\text{-}CH_2$	--	80**
2h	$4\text{-}CH_3C_6H_4$	$CH_2=CH\text{-}CH_2$	--	75*
3a	$3\text{-}CH_3OC_6H_4$	CH_3	--	30
3b	$4\text{-}CH_3C_6H_4$	CH_3	--	36
4a	3-pyridyl	$C_6H_5CH_2$	C_6H_5	57
4b	3-pyridyl	$C_6H_5CH_2$	$4\text{-}FC_6H_4$	78*
5a	$3\text{-}CH_3OC_6H_4$	n=2	--	73*
5b	$3\text{-}CH_3OC_6H_4$	n=3	--	63
5c	$4\text{-}CH_3C_6H_4$	n=2	--	65
5d	$4\text{-}CH_3C_6H_4$	n=3	--	62
6a	$3\text{-}CH_3OC_6H_4$	CH_3	--	61
6b	$4\text{-}CH_3C_6H_4$	CH_3	--	50

(**$p < 0.01$; *$p < 0.05$) as compared to standard drug sodium stibogluconate at recommended dose.

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11a. Ram, V.J.; Vanden-Berghe, D.A.; Vleintinck; *J. Heterocycl. Chem.*, **1984**, *21*, 1307.
b. All the compounds **(1-6)** prepared by standard synthetic procedures[11a] gave satisfactory elemental (C,H & N) analyses and characterized spectroscopically as examplified below:
1a: m.p. 244°C; m/z 259 (M^+), 231 (M^+-CO); IR(KBr) 2250 cm^{-1} (CN), 1670 cm^{-1} (CO); ^1H NMR (DMSO-d_6) δ 3.8 (s, 3H), 7.0-7.5 (m, 4H). **2d:** m.p. 172°C; m/z 383 (M^+), 355 (M^+-CO); IR(KBr) 2260 cm^{-1}(CN), 1655 (CO). **3a:** m.p. 140°C; m/z 291 (M^+); IR(KBr) 2255 cm^{-1} (CN); ^1H NMR (Acetone-d_6) δ 2.4 (s, 3H), 3.8 (s, 3H), 6.9-6.55 (m, 4H). **4a:** m.p. 204°C; m/z 395 (M^+); IR(KBr) 2255 cm^{-1} (CN), 3270 cm^{-1} (NH). **5a:** m.p. 170°C; m/z 285 (M^+), 257 (M^+-CO); ^1H NMR (Acetone-d_6) δ 3.7 (t, 2H), 3.8 (s, 3H), 4.55 (t, 2H), 7.05-7.5 (m, 4H). **6a:** m.p. 180°C; m/z 287 (M^+), 272 (M^+-CH_3), 259 (M^+-CO); ^1H NMR (DMSO-d_6) δ 2.26 (s, 3H), 3.43 (s, 3H), 3.8 (s, 3H), 7.05-7.5 (m, 4H).
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13. Formula used for calculating percent inhibition = $\frac{An \times 100}{TI \times IN}$
An = Actual number of parasites in treated animals
TI = Times increased of parasites in control
IN = Initial number of parasites in treated animals.

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