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1,1'-DICYANO-2-SUBSTITUTED ETHYLENES : A NEW CLASS OF GLUCOSE UPTAKE INHIBITORS IN ANTIFILARIAL CHEMOTHERAPY[1]

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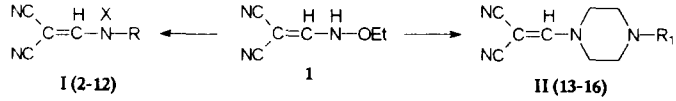
Abstract: Several 1,1'-dicyano-2-substituted ethylenes **(2-16)** were synthesized and evaluated for *in vivo* antifilarial activity. Some of the screened compounds showed significant antifilarial response against *Acanthocheilonema viteae* in rodents. © 1997 Elsevier Science Ltd.

Introduction: Lymphatic filariasis is one of the major public health problem of the tropics affecting more than 120 million people of this world. The disease is caused by lymph dwelling filariids, *Wuchereria bancrofti* and *Brugia malayi*[2].

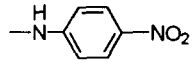
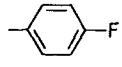
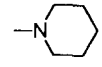
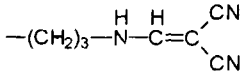
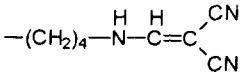
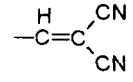
Despite remarkable advances made for developing newer filaricides, no satisfactory drug is yet available for the treatment of this disease. Diethylcarbamazine (DEC) which kills microfilariae but has no effect on most of the adult filarial species, causes side effects[3-5]. These limitations have promoted the search for new biochemical target(s) for designing macrofilaricidal agents. Of the many possibilities specific inhibition of glucose uptake by macrofilariae appears to be an attractive strategy as it is the main energy generating substrate[6,7]. Unfortunately, no specific pharmacophore is known for designing the desired molecule. Nonetheless, several 1,1'-dicyano-2-substituted amino ethylenes are known to possess good antiparasitic activity[8]. This stimulated us to design and synthesize 1,1'-dicyano-2-substituted ethylenes and to evaluate them for antifilarial as well as glucose uptake inhibitory activities.



Reagents / conditions : (I) Amine, EtOH; (II) substituted piperazine, EtOH.
All compounds were characterized by spectroscopic analysis[9].

Chemistry: The synthetic approach involves straightforward reactions of ethoxymethylene malonitrile[10] (1, EMMN). Reaction of EMMN with different primary amines in ethanol furnished the enamines **2-12** and with substituted piperazines yielded the compounds **13-16** (Table 1).

Table 1:

Entry	R = (X = H)	Entry	R = (X = H)
I (2-12)			
2		**10**	$-(CH_2)_7-CH_3$
3		**11**	
4		**12****	
5	$-CH_2-CH_2-OH$	**II (13-16)**	
6*	$-CH_2-CH_2-OH$	**13**	$-CH_3$
7		**14**	
8		**15**	
9		**16**	

*X = CH_2-CH_2OH; **X = Nil

Materials and Methods for Biological Evaluation

Antifilarial activity : The micro and macrofilaricidal activities of the synthesized compounds were evaluated *in vivo* against *A. Viteae* infection in *Mastomys coucha*[11]. Compounds insoluble in water were used as fine suspensions prepared with 1% Tween 80. Two to three animals were used for each dose level study and at least two replicates were used for confirmation of activity.

Metabolic studies and preparation of parasite extract : Adult female worms were isolated from subcutaneous tissues of *M. coucha* bearing 70-90 days old infection.

25-30 mg intact motile worms were incubated with 10 μM concentration of the test compounds taken in ethanol. After 24 hrs the medium was analysed for glucose[12] and lactate[13]. The worms after homogenization and centrifugation were determined for ATP[14] and protein contents[15].

Table 2 : Antifilarial *in vivo* activity of 1,1'-dicyano-2-substituted ethylene analogs against *A. viteae*.

Compound 50 mg/kg x 5 days (i.p.)	Activity (% reduction in parasite load)		
	mf	maf	(Sterl. of ♀)
3	47	0	(0)
4	91	0	(0)
5	82	0	(0)
6	76	0	(0)
15	0	81	(0)
16*	60	60	(30)
DEC citrate	90**	0	(0)
	98***	0	(0)

DEC = Diethylcarbamazine; * = active against *L. carinii* at 30 mg/kg x 5 days (i.p.); ** = at 350 mg/kg x 5 days (i.p.); *** = active against *L. carinii* at 6 mg/kg x 5 days (i.p.); mf = microfilariae; maf = macrofilariae or adult worms.

Results and Discussion

All of the synthesized compounds (**2-12, 13-16**) were examined *in vivo* against *A. viteae* but only six were found active through intraperitoneal route (Table 2). Compound **15** having piperazine moiety, exhibited highest adulticidal activity (81%) while compound **4** possessing *p*-fluorophenyl substituent showed highest microfilaricidal action (91%).

Introduction of 2-pyridylpiperazine to ethoxymethylene malonitrile (**1**) yielded compound **16** which showed 60% micro- and macrofilaricidal activity along with 30% sterlisation of female worms against *L. carinii* at 30 mg/kg x 5 days (i.p.) while incorporation of other substituents like methyl and phenyl piperazine into compound **1** provided inactive compounds **13** and **14** against *A. viteae* at 50 mg/kg x 5 days (i.p.). Compounds **3** exhibited insignificant 47% microfilaricidal action while its *p*-fluoro analog **4** registered highest (91%) microfilaricidal activity as described earlier against *A. viteae*. Interestingly, α-aminomethylenes having other cyclic amines like cycloheptylamine **2**, 4-aminopyridine **9**, *p*-nitrophenylhydrazine **11**, and 2-piperidinylamine **12**, could not produce effective antifilarial response against *A. viteae*.

On replacement of ethoxy group in **1**, by ethanol amine and diethanol amine afforded the compounds **5** and **6** which yielded better antifilarial response (82% and 76% microfilaricidal action respectively) as compared to other synthesized compounds **7** and **8** having 1,3-diaminopropane and 1,4-diaminobutane substituents, respectively.

The results on biochemical parameters as indicated in Table 3 showed that among six compounds examined only three showed significant effect on energy metabolism of *A. viteae*. Out of these three, compounds **4** and **16** inhibited glucose uptake while **15** accelerated the uptake rate. Lactate production and ATP content, on the other hand, were markedly reduced by all the three antifilarial agents. Compound **4** exerted maximum effect on all the three parameters studied. DEC has no effect on any three parameters of the energy metabolism used in this study. It is not surprising since DEC is a microfilaricidal agent and does not have any effect *in vitro* of *A. viteae*[16].

Table 3: Effect of antifilarial compounds on energy metabolism of *Acanthocheilonema viteae*.

Compound (10 μM)	Glucose Uptake*	Lactate Produced*	ATP Content**	L/G
Control	234±36 -	403±37 -	63.5±6.3 -	1.72
15	327±47 (+39.7[b])	258±51 (-36.0[b])	25.7±4.7 (-59.5[a])	0.79
16	105±26 (-55.1[a])	157±60 (-61.0[a])	37.1±9.6 (-41.6[a])	1.50
3	189±23 (-19.2[c])	443±81 (+9.9[c])	53.5±11.7 (-15.7[c])	2.34
5	164±31 (-29.9[b])	343±59 (-14.9[c])	47.8±13.2 (-24.8[c])	2.09
6	204±24 (-12.8[c])	405±87 (+0.5[c])	49.3±15.6 (-22.3[c])	1.99
4	56±29 (-76.1[a])	222±72 (-44.9[a])	16.9±9.1 (-73.4[a])	3.96
DEC	256±27 (+9.4[c])	461±43 (+14.4[c])	55.6±7.1 (-12.4[c])	1.80

[a]p <0.005; [b]p <0.05; [c]p >0.05; *n mole/mg worm/hr; **p mole/mg protein; data are means ±SD of three experiments. Figures in parenthesis denote % change with respect to control.

Among three compounds which adversely affected energy metabolism of *A. viteae*, compound **15** showed a pattern different than the other two. This compound increased the glucose uptake but decreased the lactate production. It therefore appears that the compound lowers ATP level of the parasite by inhibiting some step in the glycolysis. The drop in L/G ratio to 0.79 supports this view. The other two antifilarials **16** and **4** inhibit both glucose uptake as well as lactate production. Thus the blockade of glucose uptake *per se* appears to account for the lesser production of energy causing a decrease in ATP content of the filariid. In case of **4** glucose uptake is so drastically inhibited that the parasite possibly mobilizes its glycogen reserve to meet the energy requirement. A sharp rise in L/G ratio to 3.96 clearly indicates towards the generation of lactate from some extra source also. Compound **4** which markedly inhibited all the three parameters of energy metabolism (Table 3) remained ineffective *in vivo* (Tablel 2). This may be due to unavailability of the compound in its native form at the required threshold concentration in the host tissue. It may therefore be summarised that compounds **15**, **16** and **4** exert their antifilarial action by inhibiting energy metabolism. The principal site of action of the former two compounds however seems to differ from that of the third.

This would indicate that 1,1'-dicyano-2-substituted ethylenes having piperazine moiety play an important role in eliciting antifilarial response against *A. viteae* infection. Therefore, it may provide a new prototype for further molecular modifications to generate adulticidal drug to combat filarial infection.

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9. Spectroscopic data for representative compound **4** : yield : 72%; m.p. 205°C; MS m/z 187 (M^+); IR (KBr) 3240, 2210, 1510, 1340, 1220, 810, 740 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 10.94(bs, 1H), 7.98(s, 1H), 7.34-7.26(m, 2H), 7.22-7.02(m, 2H). **15** : yield : 70%, m.p. 215°C, MS m/z 238(M^+), IR (KBr) 2986, 2204, 1626, 1444, 1350, 1130, 1007 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 7.80-7.76(m, 2H), 4.08(s, 1H), 3.99-3.90(m, 4H), 3.86-3.78(m, 2H), 3.70(s, 1H).

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