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SYNTHESES AND ANTIFILARIAL PROFILE OF 5-AMINO AND 5,8-DIAMINO-ISOQUINOLINE DERIVATIVES : A NEW CLASS OF ANTIFILARIAL AGENTS[1]

Sanjay K. Srivastava[a], P.M.S. Chauhan*[a], S.K. Agarwal[a], A.P. Bhaduri[a], S.N. Singh[b], Nigar Fatma[b], R.K. Chatterjee[b], Chhanda Bose[c] & V.M.L. Srivastava[c]

Divisions of Medicinal Chemistry[a], Parasitology[b] and Biochemistry[c], Central Drug Research Institute, Lucknow-226 001 (India).

Abstract: The syntheses of 5-amino (**4-12,15**) and 5,8-diamino(**16-17**) isoquinoline derivatives, their antifilarial activity and their effect on metabolic activities of filariids are delineated. Some of the screened compounds have shown promising filaricidal response against *Acanthocheilonema viteae* in rodents. Copyright © 1996 Elsevier Science Ltd

Introduction: Filariasis is a major health problem in most of tropical and subtropical zones of the world. More than 900 million people are living in endemic areas and over 120 million people are infected with lymphatic filariasis. In India, approximately 31.2 million people are known to harbour circulating microfilariae (mf) and another 19 million to suffer from filarial manifestations caused by lymph dwelling filariids, *Wuchereria bancrofti* and *Brugia malayi*[2].

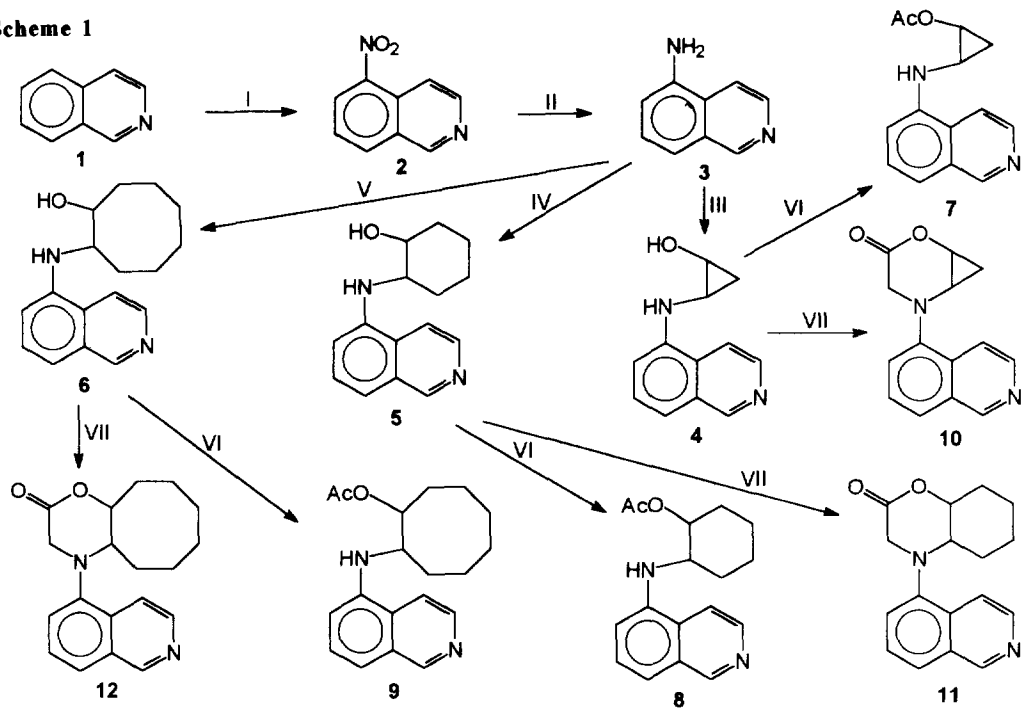
No satisfactory drug is available for the treatment of this disease except diethylcarbamazine (DEC) which kills mf but has no effect on most of the adult filarial species and causes side effects[3]. No drug yet has been found to be effective against the adult worms[4,5] The search for new molecular structures associated with macrofilaricidal activity as lead molecules is, therefore, needed.

This search led to the design and syntheses of compounds described in schemes 1 & 2. The design is based on the observations that 4-aminoquinolines exhibited antifilarial activity *in vivo*[6] and phenoxycyclohexane derivatives inhibited the enzyme phosphoenolpyruvate-carboxylase in non-competative manner and killed the parasites *in vitro*[7]. The details of the syntheses, antifilarial potential *in vivo* and effect on certain metabolic activities of adult *A. viteae in vitro* are reported here.

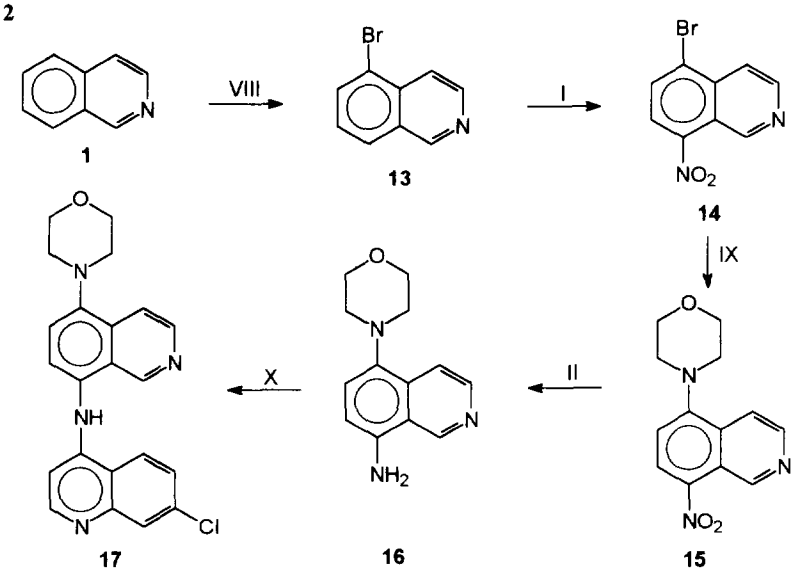
Chemistry: 5-Aminoisoquinoline[8] (**3**) was prepared by the nitration followed by the catalytic hydrogenation of isoquinoline (**1**). The reaction of **3** with cycloalkene oxides afforded the corresponding aminoalcohols (**4-6**) which were eloborated, as in scheme 1, in two ways:

In the first, the alcoholic group was converted into the respective acetyl derivatives (**7-9**) with acetic anhydride and which were characterized by spectroscopic evidences. In the IR spectrum, acetates (**7-9**) showed their characteristic peak at the range ν 1750-1730 cm^{-1} along with disap-

Scheme 1



Scheme 2



Reagents / Condition: (I) KNO_3 , H_2SO_4, 10% aq. Na_2CO_3, 25°C; (II) H_2, Pd-C, MeOH; (III) Cyclopropylene oxide, acetone; (IV) Cyclohexene oxide, acetone; (V) Cyclooctene oxide, acetone; (VI) Acetic anhydride, pyridine, 10% aq. NaOH, 0°C; (VII) Chloroacetyl chloride, THF, 25°C; (VIII) $AlCl_3$, Br_2, Et_2O; (IX) Morpholine, ethanol, reflux; (X) 4.7-Dichloroquinoline, ethanol, reflux.

pearance of the OH peak. Furthermore, EIMS showed a prominent peak of an ion corresponding to the loss of acetic acid (M^+-CH_3COOH) in all acetate derivatives (**7-9**).

In the second, these (**4-6**) were cyclized into hetrocyclic derivatives (**10-12**) with chloroacetyl chloride and these structures were confirmed by various techniques. In the IR spectrum, we got a characteristic peak at the range ν 1750-1730 cm^{-1} of these compounds (**10-12**) due to lactone (O=C-O) and therefore, it supports the linkage of carbonyl function with oxygen atom. Moreover, EIMS showed a strong peak at m/z 156 (M^+-CO_2-cycloalkene) along with a prominent peak of an ion corresponding to the loss of carbon dioxide (M^+-CO_2) indicating the presence of lactone in the all cyclized compounds (**10-12**).

Bromination of isoquinoline (**1**) followed by the nitration afforded 5-bromo-8-nitroisoquinoline[9] (**14**) which was treated with morpholine yielded **15**. Catalytic hydrogenation of **15** furnished the respective aminoderivative **16** which on condensation with 4,7-dichloroquinoline provided 5,8-diaminoisoquinoline (**17**) (Scheme 2).

All compounds were characterized by spectroscopic analysis[10].

Material and Methods for Biological Evaluation

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

2. Metabolic Studies and preparation of parasite extract: Adult female worms of *A. viteae* were isolated from subcutaneous tissues of *Mastomys coucha* harbouring 70-90 days old infection.

Six to eight motile worms (15-20 mg) were incubated with 1 μM concentration of the test compounds taken in DMSO. After 24 hr, the medium was analyzed for glucose and lactate. The worms after homogenization and centrifugation were determined for ATP and tubulin content. Detailed methodology has been described elsewhere[12,13]

Results and Discussion

All the synthesized compounds (**4-12**, **15-17**) were examined *in vivo* against *A. viteae* but only two **15** and **17** exhibiting 81% and 76% adulticidal alongwith 60% microfilaricidal action respectively, were found active (Table 1).

On the other hand, 5-(2′-hydroxycyclopropane)aminoisoquinoline (**4**) produced only 31% sterilization of female worms whereas its cyclised congener **10** exhibited 34% adulticidal activity which was complete lost after acetylation (**7**). In addition to it, on incorporation of other cycloalkane systems like cyclohexane and cyclooctane at position-5 in isoquinoline, either these

compounds have become toxic (**11**,**12**) or inactive (**5**,**6**,**8**,**9**) at the same dose level against *A. viteae* infection.

Table 1: Antifilarial *in vivo* activity of isoquinoline analogs against *A. viteae*.

Compound	Activity 50 mg/kg x 5 days (*i.p.*)		
	mf	maf	(sterl. of ♀)
4	0	0	(31)
10	0	34	(0)
15	0	81	(0)
17	60	76	(0)
DEC citrate	90*	0	(0)

0=inactive; DEC=Diethylacarbamazine; *at 350 mg/kgx5 days (*i.p.*).

With a quest to find out biochemical basis of antifilarial activity, the above said active compounds (**4**,**10**,**15** and **17**) were investigated for their impact at 1 μM concentrationn on some important parameters. This concentration was chosen on the basis of information available in literature. The plasma concentration of DEC[14] and thiabendazole[15] following single oral dose of 10 mg/kg was found to range between 0.5 to 20.0 μM. Furthermore, some of the active benzimidazole caused 50% inhibition of tublin binding[16] within a narrow range of 1.8 to 6.3 μM.

The results on biochemical parameters indicate that the uptake of glucose by *A. viteae* was although blocked by all the four active compounds (**4**,**10**,**15** & **17**) but the degree of inhibition was not very high (Table 2). Likewise, the increase in lactate production was also of poor order. The maximum effect on both the parameters which ranged between 20-30% only, was exerted by **4**, **15** and **17**. ATP content, on the other hand, was markedly lowered (~73%) by the latter two antifilarials. **10** also caused a decrease by 49%. The binding of [^{3}H]-colchicine with the filarial proteins, taken as an index of tubulin content, was significantly inhibited by only two compounds namely **10** and **15**.

A comparative analysis of data clearly revealed that only two of the four antifilarial compounds namely **10** and **15** markedly affected all the four parameters studied while **17** lowered ATP and tubulin contents significantly. Glucose uptake and tubulin and ATP levels, the three closely related parameters, are essential for survival and functional integrity of the parasite. Lesser uptake of glucose may result in lower production of ATP through glycolysis and would adversely affect the level and functionality of tubulin. Conversely, the malfunctioning of tubulin would reduce cytosolic movement of solutes and may hinder proper transport of nutrients. The reduced uptake of glucose may thus be the result rather than the cause of a disorder in tubulin integrity. Hence, at this stage of study it is difficult to draw any firm conclusion regarding exact site of action of any of the above antifilarial compounds. Nevertheless, a close similarity between adulticidal action and lowering of ATP level by compounds **15** and **17** suggest that these compounds act possibily by interfering with energy metabolism of the *A. viteae*.

Table 2: Impact of antifilarial compounds on certain biochemical parameters of *Acanthocheilonema viteae in vitro*.

Compd.	Concentration	Glucose uptake	Lactate Production	ATP Content	Tubulin Content
		μmoles/mg worms		p moles/mg worm protein	
Control (DMSO)	0.005%	8.71±0.12	5.72±0.13	145.3±27.1	63.5±6.3
4	1.0 μM	6.67±0.14* (-20.1)	7.42±0.17* (+30.0)	107.9±4.9* (-25.7)	70.2±11.4 (+11.0)
10	-do-	6.09±0.24* (-27.0)	6.42±0.21* (+12.3)	74.2±13.5** (-48.7)	35.4±6.9* (-44.5)
15	-do-	6.15±0.52* (-26.5)	7.19±0.18* (+25.7)	39.1±6.7** (-72.6)	38.5±9.7* (-39.0)
17	-do-	6.26±0.39* (-25.0)	7.30±0.52* (+27.7)	37.9±3.8** (-74.0)	45.1±10.5 (-28.8)

Data are means ±SD of 3 experiments; *$p<0.05$; **$p<0.005$; figures in parenthesis denote % change with respect to control (DMSO).

This would indicate that morpholine at position-5 in isoquinoline plays an important role in eliciting biological response against *A. viteae*. Thus this class of compounds may provide a useful lead to conduct further modifications to generate better macrofilaricidal drug to combat filarial infections.

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10. Spectroscopic data for representative compounds **4** : Yield 68%; m.p. oil; MS m/z 200(M^+); IR(KBr) 780, 1370, 1570, 1640, 3180, 3320, 3440 cm^{-1}; ^1H NMR($CDCl_3$): δ 2.10-2.15(m, 2H), 2.18-2.20(m, 2H), 4.15(s, 1H), 4.30(s, 1H), 6.95-7.00(m, 1H), 7.40-7.42(m, 2H) 7.60(d, J=8 Hz, 1H), 8.55(d, J=8 Hz, 1H), 9.25(s, 1H); **7**: yield 54%; m.p. oil; MS m/z 242(M^+); IR(KBr) 770, 1310, 1640, 1750, 3040, 3100, 3420 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.00-2.20(m, 2H), 3.45(s, 3H), 3.80-4.00(m, 2H), 4.35(s, 1H), 6.24-6.26(m, 1H), 7.28-7.30(m, 2H), 7.66(d, J=8 Hz, 1H), 8.55(d, J=8 Hz, 1H), 9.24(s, 1H); **10**: yield 51%; m.p. oil; MS m/z 240(M^+); IR(KBr) 680, 830, 1400, 1600, 1730, 3200, 3440 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6): δ 2.85-3.00(m, 4H), 4.30(s, 2H), 7.63-7.68(m, 2H), 8.08-8.10(m, 1H), 8.58(dd, J=8 Hz, 2H), 10.22(s, 1H); **15**: yield 80%; m.p. 80°C; MS m/z 259(M^+); IR(KBr) 740, 1080, 1440, 1550, 1580, 2980, 3300 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.00-3.30(m, 4H), 3.80-4.10(m, 4H), 7.10(d, J=8.2 Hz, 1H), 7.85(d, J=8.2 Hz, 1H), 8.25(d, J=8.2 Hz, 1H), 8.55(d, J=8.2 Hz, 1H), 9.80(s, 1H); **16**: yield 69%; m.p. 67°C; MS m/z 229(M^+); IR(KBr) 780, 1100, 1430, 1500, 1650, 2950, 3300 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.60-3.10(m, 4H), 3.70-4.00(m, 4H), 4.70(s, 2H), 6.70(d, J=8.2 Hz, 1H), 7.15(d, J=8.2 Hz, 1H), 7.90(d, J=8.2 Hz, 1H), 8.45(d, J=8.2 Hz, 1H), 9.30(s, 1H); **17**: yield 67%; m.p. oil; MS m/z 391(M^+); IR(KBr) 790, 850, 1460, 1550, 1600, 2960, 3400 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.00-3.20(m, 4H), 3.60-3.80(m, 4H), 4.30(s, 1H), 6.30-6.80(m, 4H), 7.52-7.58(m, 2H), 7.80-8.20(m, 2H), 8.35(s, 1H), 8.70(s, 1H).

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