Brief Articles

Potent 1,3-Disubstituted-9*H*-pyrido[3,4-*b*]indoles as New Lead Compounds in Antifilarial Chemotherapy[†,‡]

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Substituted 9*H*-pyrido[3,4-*b*]indoles (*β*-carbolines), identified in our laboratory as potential pharmacophores for designing macrofilaricidal agents, have been explored further for identifying the pharmacophore responsible for the high order of adulticidal activity. This has led to syntheses and macrofilaricidal evaluations of a number of 1-aryl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate derivatives (**3**−**7**). The macrofilaricidal activity was initially evaluated in vivo against *Acanthoeilonema viteae.* Among all the synthesized compounds, only 12 compounds, namely **3a, 3c, 3d, 3f, 4c, 4d, 4f**, **5a**, **6f, 6h, 6i**, and **7h**, have exhibited either >90% micro- or macrofilaricidal activity or sterlization of female worms. These compounds have also been screened against *Litomosoides carinii,* and of these only **3f** and **5a** have also been found to be active. Finally these two compounds have been evaluated against *Brugia malayi.* The structure−activity relationship (SAR) associated with position 1 and 3 substituents in *β*-carbolines has been discussed. It has been observed that the presence of a carbomethoxy at position 3 and an aryl substituent at position 1 in *β*-carbolines effectively enhances antifilarial activity particularly against *A. viteae.* Among the various compounds screened, methyl 1-(4-methylphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**4c**) has shown the highest adulticidal activity and methyl 1-(4-chlorophenyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**3a**) has shown the highest microfilaricidal action against *A. viteae* at 50 mg/kg × 5 days (ip). Another derivative of this compound, namely 1-(4-chlorophenyl)-3-(hydroxymethyl)-9*H*-pyrido[3,4-*b*]indole (**5a**), exhibited the highest activity against *L. carinii* at 30 mg/kg × 5 days (ip) and against *B. malayi* at 50 mg/kg × 5 days (ip) or at 200 mg/kg × 5 days (po).

Introduction

The successful treatment of filariasis, a disease of many tropical and subtropical areas, is not possible because of the nonavailability of macrofilaricidal drugs.[1−3] The age old drug diethylcarbamazine (DEC) continues to be the mainstay of clinical practice despite its well-known deficiencies.[4,5] Ivermectin, a semisynthetic macrocyclic lactone antibiotic, may take impact as a microfilaricide for onchocerciasis, but it did not irreversibly damage the adult filarial worms.[6] Although organic arsenical compounds have long been known as good macrofilaricides,[7] their potential toxicity to the host has prevented their development as useful antifilarial drugs. Besides these antifilarials, a number of phenoxycyclohexane derivatives,[8] 2,4,6-substituted-triazines,[9] 5-amino- and 5,8-diaminoisoquinolines,[10] aplysinoposin derivatives,[11] and 1,1′-dicyano-2-substituted-ethylenes[12] were identified as potential filaricides, but most of the compounds exhibited very poor adulticidal response. The benzimidazole group of anthelmintics exhibits high order of activity against intestinal helminths but has not found application for the treatment of tissue-dwelling helminths.[13,14] Therefore the need arose to identify structural prototypes associated with macrofilaricidal activity.

In earlier communications,[15−24] the macrofilaricidal activities of 1-substituted- and 1,5-, 1,6-, 1,7-, and 1,8-disubstituted-9*H*-pyrido[3,4-*b*]indoles and representatives of pyrido[3,4-*b*]imidazo[1,2-*c*]quinazolo[4,5-*e*]- and -[4,5-*g*]indoles were reported. These research activities did not reveal the optimal structural requirements to evoke a very high order of macrofilaricidal activity. In continuation of this work, it was considered essential to evaluate 1,3-disubstituted-9*H*-pyrido[3,4-*b*]indoles because of reasons stated later.

Centrally acting agents known to interact with benzodiazepine and *γ*-aminobutyric acid (GABA) receptors also exhibit anthelmintic activity,[25−28] and since 3-carboxy-*β*-carbolines also exhibit a high order of affinity for the benzodiazepine receptor,[29,30] it was considered desirble to evaluate the macrofilaricidal activities of

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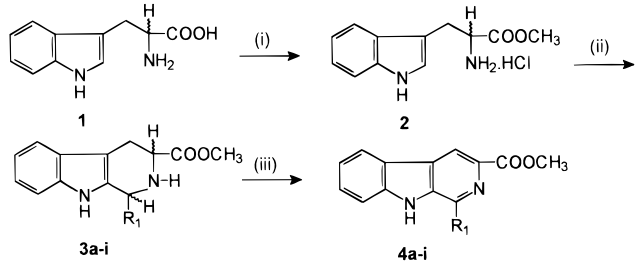
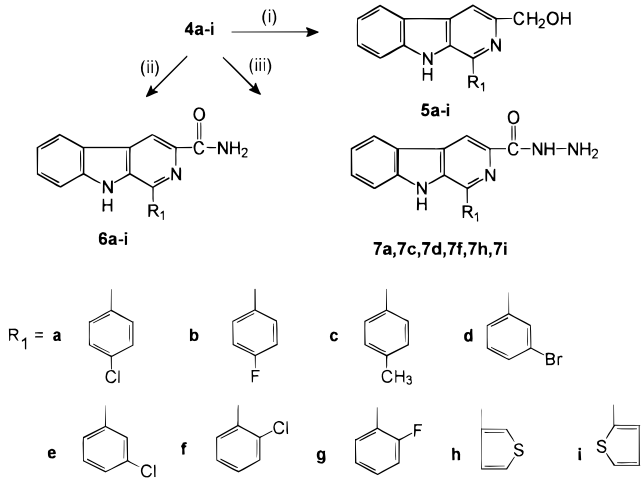
Scheme 1[a]



[a] Reagents: (i) MeOH, $SOCl_2$; (ii) R_1CHO, MeOH, 10% aq Na_2CO_3; (iii) sulfur, xylene, reflux.

Scheme 2[a]



[a] Reagents: (i) $LiAlH_4$, dry THF, reflux, 10% aq NaOH; (ii) aq ammonia, MeOH, 80 °C; (iii) hydrazine hydrate, EtOH, reflux.

esters of 1-substituted-3-carboxy-9*H*-pyrido[3,4-*b*]indoles as macrofilaricidal agents. The details of this study are presented here.

The design of 1,3-disubstituted-9*H*-pyrido[3,4-*b*]indoles (hereafter called *β*-carbolines for the sake of convenience) was based on earlier experience.[15−24] It was observed that a phenyl or thiophene ring at position 1 in *β*-carboline was necessary for evoking weak macrofilaricidal activity. The choice of substituents at position 3 was limited to ester, amide, and hydroxymethyl groups, and for specific structure−activity relationship studies, the corresponding hydrazides were also prepared.

Chemistry

Our synthetic approach was focused on the preparation of the key compounds 1-aryl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indoles **3a**−**i** in order to allow easy elaboration of the functional group attached to position 3. Pictet−Spengler cyclization[31] of L-tryptophan methyl ester hydrochloride (**2**) in the presence of the appropriate aldehyes (R_1CHO) furnished the corresponding methyl l-aryl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylates **3a**−**i**. Dehydrogenation[32] of **3a**−**i** over sulfur in xylene yielded the respective methyl 1-aryl-9*H*-pyrido[3,4-*b*]indole-3-carboxylates **4a**−**i** as described in Scheme 1.

Methyl esters **4a**−**i**, chosen as convenient intermediates, were elaborated in three ways (Scheme 2). In the first, the ester group at position 3 was reduced to its corresponding alcohol (**5a**−**i**) by lithium aluminium hydride ($LiAlH_4$) in dry THF;[33] in the second, the ester was reacted with aqueous ammonia in a steel bomb to provide the respective amides[34] (**6a**−**i**); in the third, compounds **4a**, **4c**, **4d**, **4f**, **4h**, and **4i** were reacted with hydrazine hydrate in ethanol to furnish their corresponding carboxylic acid hydrazides[32] (**7a**, **7c**, **7d**, **7f**, **7h**, and **7i**).

Table 1. Antifilarial Activity (%) of 1-Aryl-3-substituted-9*H*-pyrido[3,4-*b*]indoles (**3**−**7**) against *A. viteae* at 50 mg/kg × 5 days (ip)[a]

	antifilarial activity[b]				antifilarial activity[b]		
compd	mif	maf	sterl of ♀	compd	mif	maf	sterl of ♀
3a	94	39	100	**4h**	0	60	0
3b	0	25	0	**4i**	62	45	0
3c	0	90	0	**5a**	76	56	75
3d	90	0	0	**5a**[c]	0	94	0
3e	0	50	0	**5b**	0	75	0
3f	0	93	0	**5c**	0	0	40
3f[c]	91	0	0	**6a**	0	0	60
3h	0	57	0	**6d**	0	69	0
4b	0	56	67	**6e**	84	0	50
4c	0	100	0	**6f**	44	86	100
4d	0	84	100	**6h**	0	89	0
4e	0	81	0	**6i**	93	0	0
4f	0	94	0	**7h**	91	0	0
4g	0	44	0	DEC[d] citrate[e]	90	0	0

[a] Intraperitoneal route. Compounds **3g**, **3i**, **4a**, **5d**−**i**, **6b**, **6c**, **6g**, **7a**, **7c**, **7d**, **7f**, and **7i** are inactive and are not described here. [b] O, inactive; ♀, female worms; mif, microfilariae; maf, macrofilariae. [c] At 200 mg/kg × 5 days (oral). [d] DEC, diethylcarbamazine. [e] At 350 mg/kg × 5 days (ip).

Antifilarial Activity

The micro- and macrofilaricidal activities of the synthesized compounds (**3-7**) were evaluated against *L. carinii* in cotton rats (*Sigmodon hispidus)* and *A. viteae* and *B. Malayi* in *Mastomys coucha* as described earlier.[35,36]

Compounds being insoluble in water were made as fine suspensions within 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.

Results and Discussion

All the synthesized compounds (**3-7**) were evaluated for their antifilarial activity in vivo against *A. viteae* at 50 mg/kg × 5 days by intraperitoneal route (ip) and/or 200 mg/kg × 5 days through oral route (po) in *M. coucha.* The antifilarial activity against *A. viteae* is given in Table 1. Compounds which do not exhibit micro- or macrofilaricidal activity are not described in Table 1. During the course of discussion if the route of administration has not been described, the mode of administration of test compounds should be treated as intraperitoneal.

The other test models used in the present study for evaluation of antifilarial activity were *L. carinii* in cotton rats and *Brugia malayi* in *M. coucha. A. viteae* is metabolically similar to human filarial parasites which are anaerobic in nature, and therefore, *A. viteae* in *Mastomys* was used for evaluation of efficacy for antifilarial activity of all newly synthesized compounds. This model has also been recommended by WHO for the experimental chemotherapy of filariasis.[37] *L. carinii*, a metabolically facultative filarial species maintained in cotton rats was earlier used for efficacy evaluation of

DEC[38] and was subsequently tested in human filarials with success. *B. malayi* is target human filarial parasite, and therefore, the use of experimental model for evaluating efficacy of this parasite is obvious. Besides these reasons, the use of three different models was considered necessary because of the envisaged intractions of the synthesized compounds with GABA receptors. A precise comment on this subject has been made later.

The micro- and macrofilaricidal activities in compounds with various substituents at positions 1 and 3 in β-carbolines were monitored as follows: With a particular substituent at position 1, the effect of substituents such as ester, amide, and hydroxymethyl group at position 3 was monitored. Along with this study the effect of the ester group at position 3 with and without a tetrahydropyridine ring on the antifilarial activity was also monitored. In situations where the activity was significantly high, the effect of hydrazide at position 3 was also monitored. The active compounds (with at least 90% micro- and/or macrofilaricidal activity or sterilization of female worms) were short-listed and were subjected for evaluation against *L. carinii* infection. The best compound from this short-listed one was finally evaluated against *B. malayi* infection. The structure−activity relationship, therefore, relates to activity against *A. viteae* infection only.

Among the 4-halo substituents at position 1 in β-carboline, the 4-chlorophenyl substituent plays a significant role in eliciting antifilarial response, and particularly, the tetrahydropyridine ring along with the ester function at position 3 was found effective. For example, methyl 1-(4-chlorophenyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**3a**) exhibited highest microfilaricidal (94%) and 39% macrofilaricidal activity along with the sterilization of all the surviving female worms, but after its aromatization, the compound (**4a**) was found to be devoid of any filaricidal activity. The hydroxymethyl derivative (**5a**) of this compound showed a wide range of activity by different routes of administration. For example, it exhibited 76% micro- and 56% adulticidal activity along with sterilzation of 75% of surviving female worms by ip administration, but by oral route it was predominantly macrofilaricidal (94%) without microfilaricidal activity. The amide and hydrazide functions (**6a** and **7a**, respectively) at position 3 in β-carboline of this class of compounds did not exert any significant role. For example, compound **6a** exhibited only sterilization of 60% female worms, whereas **7a** failed to show any antifilarial response. On the other hand incorporation of a 4-fluorophenyl substituent at position 1 in β-carboline, irrespective of the nature of the group present at position 3, led to a low order of antifilarial activity in comparison to compounds with a 4-chlorophenyl substituent at position 1. The tetrahydro compound **3b** showed insignificant macrofilaricidal (25%) activity, whereas its aromatized congener **4b** caused 56% adulticidal activity with 67% sterilization of the surviving female worms. The hydroxymethyl derivative (**5b**) exerted 75% adulticidal activity, but the compound with an amide function at position 3 in β-carboline (**6b**) failed to show any response.

The ester group at position 3 with a 4-methylphenyl substituent at position 1 in 9*H*-pyrido[3,4-*b*]indole played a major role for evoking adulticidal activity against *A. viteae*. The most potent compound, methyl 1-(4-methylphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**4c**), caused the highest macrofilaricidal activity (100%), while its tetrahydro compound (**3c**) showed low but significant adulticidal response (90%). However, the antifialrial activity of the correspondirng hydroxymethyl derivative (**5c**) was limited to sterilization of only 40% of the surviving female worms. Compared to these compounds, variations at position 3 in β-carboline with a 4-halophenyl substituent at position 1 by incorporating amide (**6c**) or hydrazide (**7c**) group made the compounds ineffective against filarial infection.

Unlike 4-substituted-phenyl substituents at position-1 in β-carbolines, compounds with a 3-halophenyl substituent at position 1 exhibited a distinct type of antifilarial action. In this class of compounds, it was interesting to note the effect of the pyridine ring on antifilarial activity. For example, the tetrahydro compound **3d**, possessing 3-bromophenyl at position 1 in β-carboline, exhibited significant microfilaricidal activity (90%) which after aromatization (**4d**) led to enhanced adulticidal activity (84%) with complete loss of microfilaricidal activity and in addition made all the surviving female worms sterile. The adulticidal activity decreased up to 69% or completely disappeared after converting **4d** into its amide (**6d**) and hydrazide (**7d**) derivatives, respectively. The tetrahydro compound **3e**, having a 3-fluorophenyl substituent at position 1 in- β-carboline, exhibited only weak macrofilaricidal activity (50%), but unlike **3d**, adulticidal activity was not only retained but also increased to 81% after its aromatization to **4e**. The 3-chlorophenyl substituent at position 1 in β-carbolines, compounds in which the ester was reduced to the corresponding hydroxymethyl (**5e**) group, led to complete loss of biological response, while an amide function at position 3 (**6e**), unlike **6d**, predominantly evoked microfilaricidal activity (84%) with 50% sterilization of surviving female worms. However, none of the compounds having a 3-halo substituent at position 1 in β-carboline exerted significant adulticidal response (>90%) against *A. viteae*.

Among the compounds with a 2-halophenyl substituent at position 1 in β-carboline, the 2-chlorophenyl substituent at position 1 and an ester function at position 3 elicit interesting adulticidal response against *A. viteae* and were better than a 3-halophenyl substituent at position 1. In this group of compounds the role of the pyridine ring in β-carboline was not very significant. For example, methyl 1-(2-chlorophenyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**3f**) showed significant adulticidal activity (93%) through ip route, while by oral route, it was microfilaricidal (91%) with complete loss of adulticidal activity. Aromatization of **3f** led to the compound **4f**, which exhibited 94% adulticidal activity and was equipotent to its parent compound **3f**. Its amide derivative **6f** showed 86% macro- and 44% microfilaricidal activities with 100% sterilization of surviving female worms. The hydroxymethyl compound **5f** and the hydrazide **7f** were inactive as antifilarial agents. Compounds with a 2-fluorophenyl substituent at position 1 were inactive except aromatized drivative **4g** which showed 44% adulticidal activity.

Table 2. Antifilarial Activity (%) of **3f** and **5a** against *L. carinii* at 30 mg/kg × 5 days (ip)

compd	antifilarial activity		
	mif	maf	sterl of ♀
3f	0	20	88
5a	29	95	0
DEC citrate	90[a]	0	0

[a] At 75 mg/kg × 5 days (ip).

Table 3. Antifilarial Activity (%) of **5a** against *B. malayi*

dose (mg/kg × 5 days)	route	antifilarial activity		
		mif	maf	sterl of ♀
50	ip	0	62	85
250	po	0	56	69
DEC citrate	ip	90[a]	50	0

[a] At 100 mg/kg × 5 days (ip).

An overview of the activity data of compounds with a thienyl substituent at position 1 in β-carboline clearly indicated that unlike the l-halophenyl substituent, a thienyl group played significant role for evoking a microfilaricidal response. In general, it was interesting to note in this class of compounds the insignificant role of either a pyridine ring or an ester function at position 3 in β-carboline for evoking antifialrial activity. The other derivatives such as hydrazide (**7h**) and amide (**6i**) exhibited interesting microfilaricidal responses. The tetrahydro compound having a thien-3-yl substituent at position 1 **(3h)** showed 57% adulticidal activity which remained almost equipotent (60%) after its aromatization (**4h**), but the hydroxymethyl derivative (**5h**) did not evoke any antifilarial response. A major improvement in macrofilaricidal activity (89%) was recorded for the amide **6h**, whereas the hydrazide derivative **7h** was only microfilaricidal (91%) without the adulticidal activity. Among the compounds with a thien-2-yl substituent at position 1 in β-carboline, the tetrahydro compound (**3i**) was inactive, while its aromatic congener **4i** exhibited 45% adulticidal and 62% microfilaricidal activity. The hydroxymethyl derivative (**5i**) of this series of compounds failed to show any biological response, but the amide **6i** exhibited significant microfilaricidal activity (93%). The hydrazide **7i** was devoid of any antifilarial activity.

Those compounds which showed significant filaricidal action (>90% micro- or macrofilaricidal response or sterilization of female worms) were next examined against *L. carinii* in cotton rats at 30 mg/kg × 5 days (ip). On the basis of this consideration, compounds **3a, 3c, 3d, 3f, 4c, 4d, 4f, 5a, 6f, 6h, 6i**, and **7h** were chosen for testing their antifilarial response against *L. carinii*, and the results are summarized in Table 2.

Among all 12 compounds screened, only **3f** and **5a** were found active, and of the two **5a** exhibited a more pronounced effect against *L. carinii* than **3f**. The compound **5a**, having a hydroxymethyl at position 3 and a 4-chlorophenyl at position l in β-carboline, exhibited 95% adulticidal along with 29% microfilaricidal response, and the tetrahydro compound **3f,** possessing a 2-chlorophenyl substituent at position 1, showed insignificant macrofilaricidal activity (20%) but caused sterilization of 88% surviving female worms.

Since 1-(4-chlorophenyl)-3-(hydroxymethyl)-9*H*-pyrido[3,4-*b*]indole (**5a**) and methyl 1-(2-chlorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**3f**) showed antifilarial activity against *L. carinii*, they were, therefore, evaluated for their efficacy against *B. malayi* by intraperitoneal and oral route of administrations (Table 3).

Compound **3f** failed to show any activity at 50 mg/kg × 5 days (ip) against *B. malayi*, whereas **5a** exhibited antifilarial activity by intraperitoneal as well as by oral route. At 50 mg/kg × 5 days (ip), **5a** showed 62% adulticidal activity and 85% of the surviving female worms were found sterile, while at 250 mg/kg × 5 days (po), activities of **5a** somewhat decreased since it exhibited only 56% macrofilaricidal activity and caused 69% sterilization of the surviving female worms.

A total analysis of the antifilarial activities of β-carboline derivatives reported earlier[15−24] and of the present study clearly indicate two results: (i) β-carboline framework is a pharmacophore for macrofilaricidal activity and (ii) the nature of the substituents especially at positions 1 and 3 significantly contributes toward the macrofilaricidal efficacy. The present work also reveals that absorption, distribution, and bioclearence of β-carboline derivatives by oral route of administration are substituent-dependent. The results of parallel antifilarial evaluations in vivo against *A. viteae, L. carinii*, and *B. malayi* evoke certain speculations which may provide a basis for future study. Adequate evidence[25,26] exists that β-carboline-3-carboxylic acid derivatives interact with GABA receptors, and it is also known[27] that GABA receptor is a biochemical target site for antifilarial compounds. In light of these observations, it would be reasonable to presume that compounds evaluated in the present study also interact with GABA receptors, which in *A. viteae, L. carinii*, and *B. malayi* are either different or have significant difference in their population.

The next logical step of a future study would be to look into the GABA receptors of different human and experimental filarial worms. The experimental receptor model which will be very near to the human parasites *(Brugia malayi and Wuchereria bancrofti)* would be at great value for developing target sites of quick biological screening, and the results of this study may give valuable inputs for high-throughput screening.

Experimental Section

Chemistry. The compounds were routinely checked for their purity by thin layer chromatography (TLC) on silica gel G, and column chromatography seprations were carried out on Merk silica gel (230-400 mesh). Melting point (mp) were determined in capillary tubes on an Electrothermal melting point Toshiniwal CL-03001 apparatus and are uncorrected. Infrared (IR) spectra were run on a Backman-Acculab-10 spectrophotometer (ν_{max} in cm^{-1}). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker-400-FT instrument, and chemical shifts (δ in ppm) are reported relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at 7.23 ppm, CH_3OH in CD_3OD at 3.4 ppm, and DMSO in DMSO-d_6 at 2.49 ppm) or TMS. Signals are designated as follows; s, singlet; bs, broad signal; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet. EI mass spectra were recorded on a Jeol-JMS-D-300 spectrometer. Chemical analyses were carried out on a Carlo-Erba EA 1108 elemental analyzer. Reagents and solvents were purchased from common commercial suppliers and used as received. Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a Buchi rotary evaporator at low pressure. Yields were of purified product and were not optimized.

Methyl 1-(4-Chlorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (4a). A suspension of **3a** (1.56 g, 4.58 mmol) and sulfur (0.29 g, 9.16 mmol) in xylene (30 mL) was heated at reflux for 8 h and allowed to cool to room temprature, excess sulfur was filtered off, and the filtrate was concentrated in vacuo. The residue was crystallized to afford **4a**, 0.36 g (37%). IR (KBr): 3236, 3010, 2822, 1716, 1600, 1362, 1250 cm^{-1}. MS: *m*/*z* (relative intensity) 338 (M, Cl^{37}, 14.8), 336 (M, Cl^{35}, 39.9), 278 (100), 214 (20). ^1H NMR (400 MHz, $CDCl_3$): δ 8.9 (s, 1H, H-4), 8.8 (bs, 1H, indole NH), 8.25 (d, 1H, ArH, $J = 8$ Hz), 7.9 (d, 2H, ArH, $J = 8$ Hz), 7.64−7.50 (m, 4H, ArH), 7.4 (t, 1H, ArH, $J = 8$ Hz), 4.06 (s, 3H, OCH_3). Similarly, compounds **4b**−**i** were synthesized.

1-(4-Chlorophenyl)-3-(hydroxymethyl)-9*H*-pyrido[3,4-*b*]indole (5a). A solution of **4a** (0.4 g, 1.18 mmol) in dry THF (8 mL) was added dropwise to the stirred solution of $LiAlH_4$ (0.09 g, 2.37 mmol) in dry THF (20 mL) at ambient temperature. The reaction mixture was refluxed for 8 h and was allowed to remain at room temperature. The complex was decomposed by 10% aqueous NaOH solution, solid that separated was filterated and washed with water, and then filterate was concentrated in vacuo. The residue thus obtained was filtered, washed with water, and crystallized to provide **5a**, 0.29 g (80%). IR (KBr): 3180, 3060, 2800, 1630, 1490, 1240, 1010, 720 cm^{-1}. MS: *m*/*z* (relative intensity): 310 (M, Cl^{37}, 4.5), 308 (M, Cl^{35}, 54.9), 306 (100), 278 (47.5). ^1H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 8.46 (bs, 1H, indole NH), 8.12 (d, 1H, ArH, $J = 8$ Hz), 8.1−8.0 (m, 3H, ArH), 7.7−7.49 (m, 4H, ArH), 7.24 (t, 1H, ArH, $J = 8$ Hz), 4.96 (s, 2H, CH_2), 3.7 (s, 1H, OH). Compounds **5b**−**i** were synthesized using a similar procedure.

1-(4-Chlorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxamide (6a). A solution of **4a** (0.93 g, 2.75 mmol) in aqueous ammonia solution (8 mL) and methanol (10 mL) was heated at 80 °C under pressure in a steel bomb for 8 h. The reaction mixture was concentrated, and solid thus separated was filtered and on crystallization gave **6a**, 0.33 g (37%). IR (KBr): 3442, 3366, 3220, 1664, 1386, 742 cm^{-1}. MS *m*/*z* (relative intensity): 323 (M, Cl^{37}, 9.5), 321 (M, $C1^{35}$, 25.7), 278 (100), 242 (51.1). ^1H NMR (400 MHz, $CDCl_3$): δ 8.94 (s, 1H, H-4), 8.74 (bs, 1H, indole NH), 8.22 (d, 1H, ArH, $J = 8$ Hz), 8.04 (bs, 1H, NH of NH_2), 7.95 (d, 2H, ArH, $J = 8$ Hz), 7.7−7.5 (m, 4H, ArH), 7.38 (t, 1H, ArH, $J = 8$ Hz), 5.62 (bs, 1H, NH of NH_2). Compounds **6b**−**i** were obtained by using the same method.

1-(4-Chlorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic Acid Hydrazide (7a). Compound **4a** (1 g, 2.97 mmol) and hydrazine hydrate (1.5 mL, 48.2 mmol) were refluxed in ethanol (50 mL) for 4 h. The reaction mixture was concentrated, and solid thus separated was filtered and on crystallization gave **7a**, 0.72 g (72%). IR (KBr): 3930, 3886, 2369, 1623, 1320, 836 cm^{-1}. MS *m*/*z* (relative intensity): 338 (M, Cl^{37}, 10.0), 336 (M, Cl^{35}, 26.9), 277 (29.3), 130 (100). ^1H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 8.83 (s, 1H, H-4), 8.75 (bs, 1H, indole NH), 8.3−8.15 (m, 2H, ArH), 7.8−7.5 (m, 4H, ArH), 7.4−7.2 (m, 2H, ArH), 4.2−3.5 (bs, 3H, NH and NH_2). Similarly, compounds **7c**, **7d**, **7f**, **7h**, and **7i** were synthesized.

Materials and Methods for Biological Evaluation. *1. A. viteae*: *A. viteae* infection was transmitted to 6-week-old male *M. coucha* through the vector *Ornithodorus moubata* by the method as reported in the literature.[39] The micro- and macrofilaricidal activities of the compounds were assessed against *A. viteae* in *M. coucha* at 50 mg/kg intraperitoneally and/or 200 mg/kg orally for 5 consecutive days according to the literature method.[40,41]

2. L. carinii : The infection was transmitted to 6-week-old cotton rats (*S. hispidus*) through the vector *Liponyssus bacoti* by the literature method.[42] Animals showing 250 or more microfilariae/5 mm of blood were chosen for screening. Blood samples of experimental and control animals were examined for microfilariae before starting the treatment and thereafter at weekly intervals till day 42. All the compounds were given 30 mg/kg intraperitoneally for 5 consecutive days. On day 42, all the treated and control animals were sacrified and the conditions of adult male and female worms observed. The micro- and macrofilaricidal actions were assessed as described for *A. viteae*.

3. B. malayi: The 6-week-old male *Mastomys* were infected by inoculum of 50 infective larvae of *B. malayi* recovered from infected mosquitoes *(Aedes aegybti)*.[36] Method of screening of compounds was similar to that of *A. viteae* except blood was examined up to day 92 posttreatment. Animals were sacrificed on day 92 for adult worm recovery.

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