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Original article

Synthesis of 2-sulfanyl-6-methyl-1,4-dihydropyrimidines as a new class of antifilarial agents

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Abstract

A series of 2-sulfanyl-6-methyl-1,4-dihydropyrimidines (**8**–**21**) were synthesized in good yields by alkylation of 5-methyl-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid ethyl esters (**2**–**7**) with different alkyl or aralkyl halides in the presence of a combination of anhydrous K_2CO_3 and catalytic amount of tetrabutyl ammonium bromide. The title compounds were evaluated for their antifilarial activity against adult parasites of human lymphatic filarial parasite *Brugia malayi* (sub-periodic strain) in vitro and in vivo at various concentrations. One of the compounds (**18**) showed promising antifilarial activity.

Keywords: Dihydropyrimidines; 1,4-Dihydropyrimidines; Antifilarial; *Brugia malayi*; Tetrabutyl ammonium bromide

1. Introduction

Current global estimates suggest that around 80 countries are endemic for lymphatic filariasis (LF) [1,2]. Of the three parasites causing LF, *Wuchereria bancrofti* accounts for over 90% of the global burden. *Brugia malayi* is limited to Asia and *Brugia timori* to a few islands in Indonesia. It has been estimated that 1.1 billion people living in areas endemic for this disease are exposed to the risk of infection, and that there are about 120 million cases with either disease or infection (microfilaria carriers) [3]. An estimated population of 22 million is known to be the host for circulating microfilaria and 16 million people suffer from filarial manifestations like elephantiasis of limbs, genitals and hydrocele. Low priority was given to this disease, although it is responsible for significant morbidity and consequently the World Health Assembly has adopted a resolution on the global elimination of lymphatic filariasis as a public health problem [4–6].

The available control strategies have significant limitations as current drugs are principally microfilaricidal and require annual repeated treatment for a number of years, thus there is still a need for the development of a macrofilaricidal agent or drug combination for the curative treatment or sustained suppression of the microfilariae [7–10]. Drug resistance to ivermectin appears to be another issue of concern, especially in areas where diethylcarbamazine (DEC) cannot be given. Besides, research is required on the progression and reversibility of disease manifestations.

A number of molecules from heterocycles are known as good antifilarials [11]. Benzimidazoles [12] and triazines [13,14] have recently been shown to possess very good antifilarial activity. In spite of tremendous medicinal chemistry in dihydropyrimidines [15] and their derivatives only scanty reports exist for their antifilarial activity. In recent years interesting dihydropyrimidines are being looked as an important class of molecules since many of them are clinical candidates

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for different diseases [16–18]. S-DABO (Fig. 1) and many other analogues have recently been shown to possess anti-HIV activities [19,20]. We have recently shown that dihydropyrimidinones show interesting antiparasitic activities [21] via inhibition of crucial enzymes of parasite biochemistry. Herein we have synthesized 2-sulfanyl-6-methyl-1,4-dihydropyrimidines and evaluated them for the first time for their antifilarial activity both in vitro and in vivo. The method of synthesis is simple and efficient and does not involve any special apparatus or reagents.

2. Results and discussion

The starting 1,2,3,4-tetrahydropyrimidine-2-thione derivatives were prepared in excellent yields by our earlier reported protocol [22]. It involves a modified Biginelli three-component reaction of aromatic aldehydes, ethyl acetoacetate and thiourea in diethylene glycol at 120 °C. The yields and characterization of these tetrahydropyrimidine thiones were already described. In the proposed reaction ethyl acetoacetate and thiourea were kept constant while aromatic aldehydes were the variant.

The target molecules, 2-sulfanyl-6-methyl-1,4-dihydropyrimidines, were synthesized from the respective tetrahydropyrimidine-2-thiones by reaction with an alkyl or aralkyl halides in anhydrous acetone in the presence of anhydrous K_2CO_3 and catalytic amount of tetrabutyl ammonium bromide (Scheme 1 and Table 1). It is important to mention here that this class was earlier synthesized by Atwal et al. [16] as possible calcium channel blockers, however, our method of synthesis is far better with respect to environmental consideration, time and yields of the final product. Use of tetrabutyl ammonium bromide as phase transfer catalyst not only improves the yield of the sulfanyl derivatives but the reaction time is also considerably reduced. The structures of all the products were established on the basis of their spectral data and analyses. In IR spectra of the compounds the carbonyl frequency of the ester moiety was observed at around 1650 cm^{-1} along with other usual characteristic frequencies. All the compounds displayed $[M + H]^+$ corresponding to their molecular formulae. In ^1H NMR of the compounds three protons of 6-methyl substituent appeared as singlets at around δ 2.45 along with other protons at their usual chemical shifts. Out of the two possibilities of 1,4- and 3,4-dihydroisomers (**A** and **B**) (Fig. 2) only the former prevailed as the characteristic signal H-4 was observed at around δ 6.50 as singlet in all the products. Further, the structure of one of such prototype (compound **12**) is established by X-ray crystallographic data (Fig. 3). Interestingly out of the two enantiomers of dihydropyridine **12** only one enantiomer crystallizes out and the other remains in solution. Such a crystallization of single enantiomer from a racemic mixture has been reported earlier by different workers involving a process known as ''chiral amnesia'' [23–25].

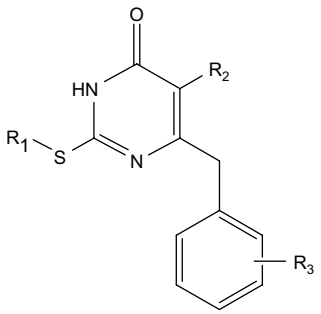
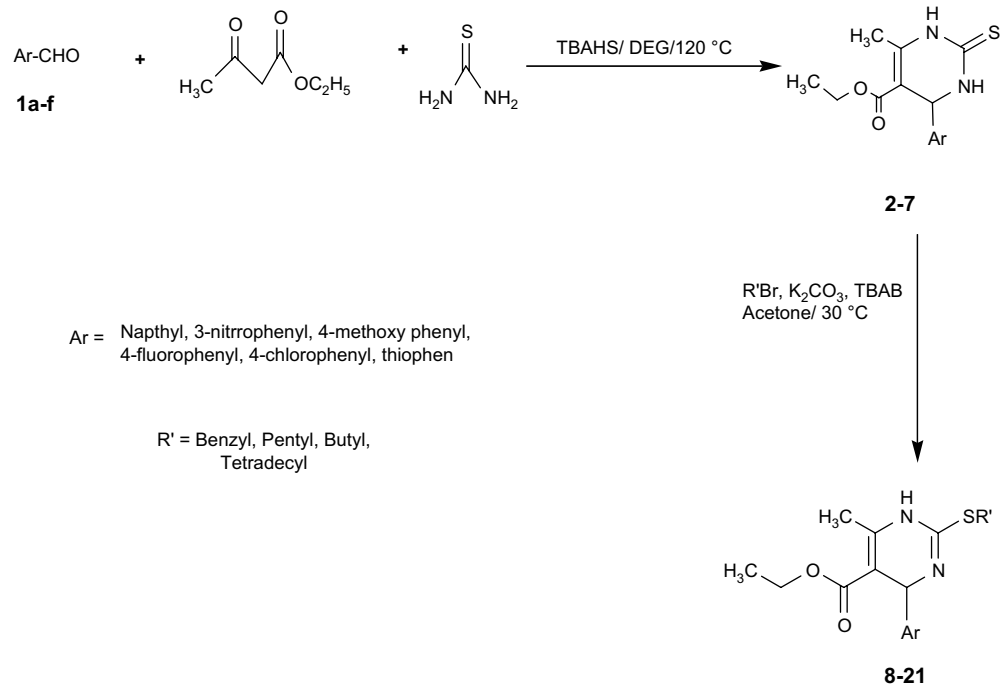


Fig. 1. S-DABO.

Compounds **8–20** synthesized were evaluated in vitro for their macrofilaricidal activity against *B. malayi* according to the method of Murthy and Chatterjee [26]. Micro- and macrofilaricidal activities were evaluated following the methods described by Lammler and Wolf [27], Chatterjee et al. [28] and Gaur et al. [29].

As evident from Table 2 compounds **8**, **10–12**, **14**, **15**, **18** and **19** resulted in complete loss of motility of adult worms of *B. malayi* at 100 μM concentrations and they have shown 15.4–68.61% inhibition in MTT reduction assays while compound **19** did not show any inhibition in MTT reduction assay. Of these, three compounds (**10**, **15** and **18**) affected both motility (irreversible loss) and MTT reduction (~50% inhibition or more) and compounds **8**, **11**, **12**, **14** and **19** either affected motility with <50% MTT reduction or only motility. Further, the compounds showing MTT reduction to the level of around 50% were screened at lower concentration also. Compound **10** at 50 μM concentration resulted in complete loss of motility of filarial worms with no inhibition in MTT reduction assay, while at 25 μM concentration it showed only sluggish motility of filarial worm and displayed approximately 30% inhibition in MTT reduction assay. Compound **15** on the other hand displayed complete loss of motility in filarial worms at 50 μM concentration with 19% inhibition in MTT reduction assay. Compounds **17** and **19** were not effective at lower concentrations. The best compound of the series was found to be compound **18** which resulted in complete loss of motility at two lower concentrations (50 and 25 μM) screened and good inhibition (around 70%) in MTT reduction assay. DEC upto 200 μM was ineffective. However, at higher dose (400 μM), DEC exerted irreversible paralysis of the worms i.e. the worms failed to gain normal motility even after transferring the treated worms in fresh medium without any drug at 37 °C for 30 min. The control vehicle did not display any activity against the adult worms of *B. malayi*. Although no definite conclusion on SAR can be drawn with this sizable number of compounds, it is clear that 4-aryl-1,4-dihydropyridines bearing less bulky electron-withdrawing substituent at the 4th position of phenyl ring (4-F–phenyl) compound **18** is the best compound of the series.

Only three compounds **10**, **15** and **18** showing potent in vitro antifilarial activity were screened in vivo against *B. malayi* in *Mastomys coucha* model to see the effect of the compounds on parasitological parameters and the results are depicted in Table 3. As evident from Table 3 a significant effect on adult worms (50%; $P < 0.001$) was shown by compound **18** at 100 mg/kg. In terms of embryostatic activity, it exerted significant ($P < 0.01$) efficacy by sterilizing about 68% of the



Scheme 1. Synthesis of 6-methyl-2-sulfanyl-1,4-dihydropyrimidines.

surviving female worms compared to 20% observed in the untreated controls. The efficacy of the compound was found to be lower at 50 mg/kg as compared to 100 mg/kg. In normal *M. coucha* at this dose the animals showed no mortality or adverse effect on general and gross health of the animals during the treatment and thereafter upto 15 days post-treatment. Compound **15** at 100 mg/kg marginally (20%) exerted macrofilaricidal effect. On the other hand, compounds **10** and **15** at 100 mg/kg affected the female reproductive potentials to the tune of only 35 and 26%, respectively. None of the compounds affected circulating mf. The control drug DEC at a dose of 25 mg/kg (i.p.) caused more than 65% ($P < 0.001$) reduction in microfilaraemia and around 30% female sterilizing action. Untreated control animals showed progressive rise in microfilaraemia till termination of the experiment. About 20% of live female worms recovered from these animals were sterile.

Table 1
4-Aryl-6-methyl-2-sulfanyl-1,4-dihydropyrimidines (**8**–**21**) synthesized

Entry	Compound	Ar	R′	Time	Yield (%)
1	**8**	1-Naphthyl	Benzyl	3	70
2	**9**	1-Naphthyl	*n*-Pentyl	6	72
3	**10**	1-Naphthyl	*n*-Butyl	5	80
4	**11**	1-Naphthyl	*n*-Tetradecyl	6	65
5	**12**	3-Nitrophenyl	Benzyl	3	60
6	**13**	3-Nitrophenyl	*n*-Pentyl	5	75
7	**14**	3-Nitrophenyl	*n*-Butyl	4	70
8	**15**	4-Methoxyphenyl	Benzyl	3	65
9	**16**	4-Methoxyphenyl	*n*-Pentyl	3.5	78
10	**17**	4-Methoxyphenyl	*n*-Tetradecyl	5	80
11	**18**	4-Fluorophenyl	*n*-Pentyl	6	70
12	**19**	4-Fluorophenyl	*n*-Tetradecyl	7	85
13	**20**	4-Chlorophenyl	*n*-Pentyl	4	70
14	**21**	Thiophenyl	Benzyl	3	65

As compound **18** with 4-flurophenyl and *n*-pentyl substituents was found to be the most potent antifilarial in the above two experiments we were prompted to see its effect on the adulticidal activity against filarial worms in *B. malayi*-jird model. It is evident from the results (Table 4) the standard antifilarial drug DEC (12.5 mg/kg, the effective dose of the drug against animal filariid, *Litomosoides carinii*) did not show any noticeable microfilaricidal activity and about 10% adulticidal activity was observed only as compared to untreated controls. However, the synthetic compound **18** displayed about 46% adulticidal ($P < 0.01$) activity and 34% ($P < 0.05$) of the sterilized female worms were recovered. Untreated control animals showed no effect on mf of peritoneal cavity of any of the animals. About 16% of live female worms recovered from these animals was sterile. The results indicate that compound **18** has potential for further optimization and development of novel class of antifilarial agents.

In conclusion we have developed an efficient synthesis of a series of 4-aryl-2-sulfanyl-6-methyl-1,4-dihydropyrimidines

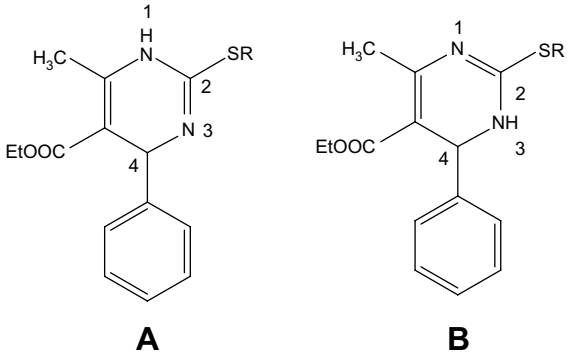


Fig. 2. Two possible isomers of dihydropyrimidines.

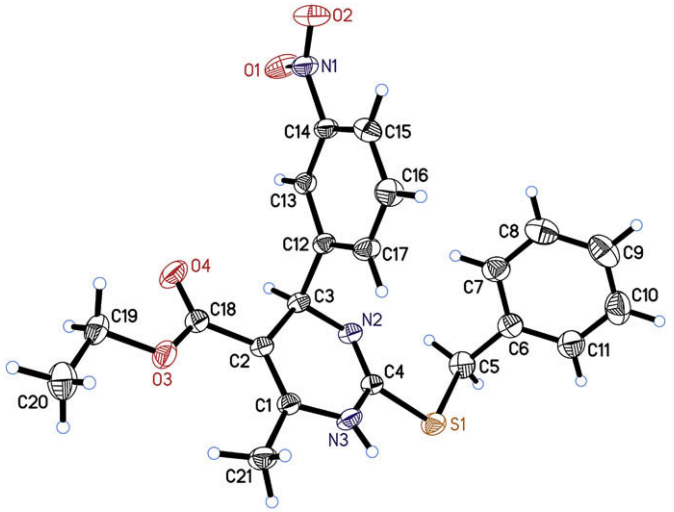


Fig. 3. The ORTEP diagram (at 30% probability) of compound **12** showing the molecular conformation along with atomic numbering scheme.

by simple alkylation of dihydropyrimidine thiones with alkyl halides. One of the compounds (compound **18**) displayed potent activity in vitro and in vivo and it exerted adulticidal activity along with female worms' sterilization. Thus compound **18**, found active with no apparent signs of any toxicity in gross health of treated and normal animals, may serve as a prototype lead for further optimization and development of new antifilarial agents.

Table 2
In vitro macrofilaricidal activity of compounds on *B. malayi*

Compound	Concentration (μM)	Motility[a]	Percent inhibition in MTT reduction over control (means ± SD)
8	100	0	18.95 ± 1.77
9	100	3	NI
10	100	0	49.0 ± 11.50
	50	0	NI
	25	1	30.41 ± 8.7
11	100	0	24.34 ± 3.13
12	100	0	43.14 ± 5.11
13	100	3	15.41 ± 2.82
14	100	0	25.74 ± 3.44
15	100	0	52.11 ± 26.92
	50	0	19.29 ± 9.49
	25	1	NI
16	100	2	22.42 ± 5.84
17	100	2	NI
	50	3	NI
	25	3	NI
18	100	0	68.61 ± 5.13
	50	0	68.46 ± 8.92
	25	0	25.78 ± 17.50
19	100	0	NI
	50	3	NI
20	100	3	NI
DEC (citrate)	400	1	NI
	200	2	NI
	100	3	NI
	50	3	NI
Untreated control	Vehicle	3	NI

[a] Motility is expressed as highly motile (3), low (2), sluggish (1, irreversible) and dead (0); NI = no inhibition.

3. Experimental

3.1. General methods

Melting points were determined on a Buchi 510 apparatus and are uncorrected. Commercially available reagent grade chemicals were used as-received. All reactions were followed by TLC on Merck Kieselgel 60 F_{254}, with detection by UV light and/or spraying 20% $KMnO_4$ aqueous solution. Column chromatography was performed on silica gel (230–400 mesh, Merck). IR spectra were recorded as thin films or neat chloroform solution with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300 in (*D*) chloroform and chemical shift values expressed in parts per million relative to $SiMe_4$ as internal reference, unless otherwise stated; signals are reported as s (singlet), d (doublet), t (triplet), m (multiplet); *J* in hertz. Fast atom bombardment mass spectra (FABMS) were performed by the Mass Spectrometer Jeol SX-102 (FAB). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

3.2. Typical experimental procedure for compounds 8–21

To a magnetically stirred solution of compounds **2–7** (1 mmol) in acetone, K_2CO_3 (1 mmol) was added and the reaction mixture was stirred for 10 min. After that alkyl bromide (1 mmol) and tetrabutyl ammonium bromide (20 mol%) were added and reaction mixture was stirred for desired time at room temperature. After completion, reaction mixture was evaporated and crude mass was extracted by ethyl acetate and water. Organic layer was dried (anhydrous Na_2SO_4) and evaporated under reduced pressure to get the crude mass which was column chromatographed over silica gel (60–120 mesh) using hexane–EtOAc as eluent to give compounds **8–21**.

3.2.1. 2-Benzylsulfanyl-6-methyl-4-naphthalen-1-yl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (8)

It was obtained by reaction of **2** (1.1 g, 3.37 mmol), K_2CO_3 (0.47 g, 3.37 mmol) and benzyl bromide (0.4 mL, 3.37 mmol) as a white solid (0.98 g, 70%); m.p. 132–133 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 0.99 (t, 3H, $J = 7.0$ Hz), 2.42 (s, 3H), 3.87–4.16 (m, 4H), 6.57 (s, 1H), 6.83–7.05 (m, 4H), 7.33–7.58 (m, 5H), 7.74–7.89 (m, 2H), 8.74 (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.5, 19.0, 35.6, 56.6, 60.0, 99.6, 124.1, 125.8, 126.3, 127.4, 128.2, 128.8, 129.1, 131.6, 134.5, 138.2, 139.6, 167.0; IR (KBr) cm^{-1}: 3283, 2362, 1650, 1593; MS (FAB): *m/z* 417 $(M + H)^+$.

3.2.2. 6-Methyl-4-naphthalen-1-yl-2-pentylsulfanyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (9)

It was obtained by reaction of **2** (0.8 g, 2.45 mmol), K_2CO_3 (0.38 g, 2.45 mmol) and pentyl bromide (0.31 mL, 2.45 mmol) as a white solid (0.7 g, 72%); m.p. 92–94 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 0.70 (t, 3H, $J = 6.7$ Hz), 0.86–1.05

Table 3
Antifilarial activity of synthetic compounds against *B. malayi* in *M. coucha* (values are means ± SD)

Test compounds (mg/kg, i.p. × 5 days)	No. of animals used	Mf count before treatment	Mf count on day 8 post-initiation of treatment	No. of live worms			Sterilized female worms
				Male	Female	Total[b]	Number (%)
10 (100)	6	38.00 ± 14.18	47.33 ± 20.01	13.00 ± 7.21	15.33 ± 5.69	28.33 ± 12.5 (0.0)	3.00 ± 1.73 (25.77)
15 (100)	6	40.67 ± 2.31	89.67 ± 71. 99	5.67 ± 1.16	10.0 ± 1.00	15.57 ± 2.08 (19.66)	3.33 ± 1.53 (34.58)
18 (100)	9	83.00 ± 45.48	142.67 ± 93.06	3.25 ± 1.89	6.5 ± 1.91	9.75 ± 3.3 (50.0)***	4.0 ± 0.82 (67.71)**
18 (50)	6	45.0 ± 7.07	116.5 ± 94.05	7.0 ± 1.41	6.5 ± 0.71	13.5 ± 0.71 (30.77)	2.5 ± 0.71 (38.1)
DEC[a] (25)	6	59.00 ± 16.09	20.33 ± 9.71[#]	7.33 ± 0.58	13.67 ± 5.51	21.00 ± 5.20 (0.0)	5.67 ± 8.96 (29.7)
Untreated control	6	51.43 ± 29.42	106.71 ± 51.45	7.0 ± 3.58	12.5 ± 3.02	19.5 ± 3.02	2.5 ± 1.38 (20.18)

***$P < 0.001$, **$P < 0.01$ (over untreated control), [#]$P < 0.001$ (over pretreatment level).
[a] Diethylcarbamazine citrate.
[b] % Reduction in worm burden over control.

(m, 7H), 1.19–1.25 (m, 2H), 2.46 (s, 3H), 2.51–2.87 (m, 2H), 4.01 (q, 2H, $J = 7.0$ Hz), 6.51 (s, 1H), 7.23–7.54 (m, 4H), 7.69–7.84 (m, 2H), 8.70 (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.2, 14.5, 19.1, 22.4, 29.7, 31.2, 31.6, 57.0, 60.0, 102.0, 123.8, 125.7, 126.1, 128.1, 128.7, 131.6, 134.5, 139.3, 167.1; IR (KBr) cm^{-1}: 3303, 2362, 1654; MS (FAB): *m/z* 397 $(M + H)^+$.

3.2.3. 2-Butylsulfanyl-6-methyl-4-naphthalen-1-yl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (*10*)

It was obtained by reaction of **2** (1 g, 3.06 mmol), K_2CO_3 (0.42 g, 3.06 mmol) and butyl bromide (0.33 mL, 3.06 mmol) as a white solid (0.93 g, 80%); m.p. 97–98 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.57 (t, 3H, $J = 6.9$ Hz), 0.84–1.25 (m, 7H), 2.46 (s, 3H), 2.64–2.86 (m, 2H), 4.01 (q, 2H, $J = 7.0$ Hz), 6.51 (s, 1H), 7.23–7.84 (m, 7H), 8.74 (d, 1H, $J = 8.1$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.8, 14.5, 19.4, 22.2, 31.3, 32.0, 56.1, 60.0, 123.9, 125.7, 126.1, 128.1, 128.7, 131.7, 134.5, 139.3, 167.1; IR (KBr) cm^{-1}: 3289, 2363, 1653; MS (FAB): *m/z* 383 $(M + H)^+$.

3.2.4. 6-Methyl-4-naphthalen-1-yl-2-tetradecylsulfanyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (*11*)

It was obtained by reaction of **2** (1.3 g, 3.98 mmol), K_2CO_3 (0.55 g, 3.98 mmol) and tetradecyl bromide (1 mL, 3.98 mmol) as a white solid (1.35 g, 65%); m.p. 86–87 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85–1.26 (m, 30H), 2.46 (s, 3H), 2.52–2.98 (m, 2H), 3.99 (q, 2H, $J = 7.0$ Hz), 6.16 (s, 1H), 6.51 (s, 1H), 7.23–7.55 (m, 4H), 7.69–7.84 (m, 2H), 8.70 (d, 1H, $J = 8.2$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.5, 19.0, 23.1, 29.1, 29.4, 29.8, 30.1, 31.8, 32.3, 56.6, 60.1, 99.5, 123.5, 125.7, 125.9, 126.0, 127.0, 128.0, 128.6, 129.5, 131.9, 134.5, 139.2, 146.0, 149.4, 167.3; IR (KBr) cm^{-1}: 3302, 2363, 1650, 1597; MS (FAB): *m/z* 523 $(M + H)^+$.

3.2.5. 2-Benzylsulfanyl-6-methyl-4-(3-nitro-phenyl)-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (*12*)

It was obtained by reaction of **3** (1.0 g, 3.11 mmol), K_2CO_3 (0.43 g, 3.11 mmol) and benzyl bromide (0.37 mL, 3.11 mmol) as a greenish solid (0.76 g, 60%); m.p. 122–123 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.21 (t, 3H, $J = 7.1$ Hz), 2.31 (s, 3H), 4.06–4.16 (m, 3H), 4.28 (d, 1H, $J = 13.6$ Hz), 5.82 (s, 1H), 6.37 (s, 1H), 7.14–7.22 (m, 5H), 7.41 (t, 1H, $J = 7.8$ Hz), 7.59 (d, 1H, $J = 7.6$ Hz), 8.06–8.11 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.6, 19.1, 35.5, 59.8, 60.5, 100.3, 122.4, 122.5, 127.7, 128.8, 129.0, 129.2, 129.5, 133.9, 137.5, 137.5, 145.6, 147.2, 148.7, 150.4, 166.7; IR (KBr) cm^{-1}: 3326, 2364, 1678, 1653; MS (FAB): *m/z* 411 $(M + H)^+$.

3.2.6. 6-Methyl-4-(3-nitro-phenyl)-2-pentylsulfanyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (*13*)

It was obtained by reaction of **3** (1.0 g, 3.11 mmol), K_2CO_3 (0.43 g, 3.11 mmol) and pentyl bromide (0.39 mL, 3.11 mmol) as a white solid (0.91 g, 75%); m.p. 119–120 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.82 (t, 3H, $J = 7.1$ Hz), 1.16–1.32 (m, 7H), 1.53–1.63 (m, 2H), 2.34 (s, 3H), 2.89–3.04 (m, 2H), 4.11 (q, 2H, $J = 7.0$ Hz), 5.80 (s, 1H), 6.35 (s, 1H), 7.45 (t, 1H, $J = 7.8$ Hz), 7.65 (d, 1H, $J = 7.8$ Hz), 8.05–8.16 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.2, 14.6, 19.2, 22.5, 29.4, 31.1, 31.4, 59.5, 60.5, 100.1, 122.4, 129.5, 133.8,

Table 4
Antifilarial activity of compound **18** (100 mg/kg, i.p. × 5 days) and DEC against transplanted adult worms of *B. malayi* in *Meriones unguiculatus* (values are means ± SD)

Antifilarial agent (mg/kg, i.p. × 5 days)	No. of animals	Effect on microfilariae in peritoneal cavity	No. of live worms			Sterilized female worms
			Male	Female	Total[b]	Number (%)
18 (100)	5	No effect	2.40 ± 0.55	4.40 ± 2.97	6.80 ± 3.11 (46.03)**	2.40 ± 2.07 (34.31)*
DEC[a] (12.5)	5	No effect	4 ± 0.71	7.60 ± 1.14	11.60 ± 1.82	0.80 ± 0.84 (10.53)
Untreated control	5	No effect	4.00 ± 1.00	8.60 ± 0.89	12.60 ± 1.67	1.40 ± 1.14 (16.17)

**$P < 0.01$, *$P < 0.05$ (over untreated control).
[a] Diethylcarbamazine citrate.
[b] % Reduction in worm burden over control.

145.7, 147.3, 148.6, 151.2, 166.8; IR (KBr) cm^{-1}: 3432, 2364, 1699, 1640; MS (FAB): *m/z* 392 $(M + H)^+$.

3.2.7. 2-Butylsulfanyl-6-methyl-4-(3-nitro-phenyl)-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**14**)

It was obtained by reaction of **3** (0.9 g, 2.80 mmol), K_2CO_3 (0.39 g, 2.80 mmol) and butyl bromide (0.31 mL, 2.80 mmol) as a white solid (0.74 g, 70%); m.p. 98–99 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 0.84 (t, 3H, $J = 7.0$ Hz), 1.22 (t, 3H, $J = 7.1$ Hz), 1.27–1.39 (m, 2H), 1.52–1.68 (m, 2H), 2.35 (s, 3H), 2.91–3.08 (m, 2H), 4.07 (q, 2H, $J = 7.1$ Hz), 5.81 (s, 1H), 6.36 (s, 1H), 7.45 (t, 1H, $J = 7.8$ Hz), 7.66 (d, 1H, $J = 7.7$ Hz), 8.07–8.16 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.9, 14.6, 19.1, 22.1, 31.1, 31.8, 59.4, 60.5, 122.4, 129.6, 133.7, 147.2, 148.6, 166.9; IR (KBr) cm^{-1}: 3391, 1700, 1637; MS (FAB): *m/z* 377 $(M + H)^+$.

3.2.8. 2-Benzylsulfanyl-4-(4-methoxy-phenyl)-6-methyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**15**)

It was obtained by reaction of **4** (1.5 g, 4.90 mmol), K_2CO_3 (0.67 g, 4.90 mmol) and benzyl bromide (0.58 mL, 4.90 mmol) as colorless oil (1.26 g, 65%); ^1H NMR (200 MHz, $CDCl_3$): δ 1.19 (t, 3H, $J = 7.0$ Hz), 2.33 (s, 3H), 3.79 (s, 3H), 4.05–4.46 (m, 4H), 5.69 (s, 1H), 6.79–6.32 (m, 9H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.6, 19.6, 35.2, 55.6, 58.6, 60.2, 102.9, 114.1, 127.7, 128.5, 128.9, 129.4, 130.5, 137.5, 137.9, 159.2, 167.3; IR (neat) cm^{-1}: 3362, 2363, 1690, 1598; MS (FAB): *m/z* 397 $(M + H)^+$.

3.2.9. 4-(4-Methoxy-phenyl)-6-methyl-2-pentylsulfanyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**16**)

It was obtained by reaction of **4** (1.3 g, 4.24 mmol), K_2CO_3 (0.59 g, 4.24 mmol) and pentyl bromide (0.53 mL, 4.24 mmol) as an oil (0.82 g, 78%); ^1H NMR (200 MHz, $CDCl_3$): δ 0.85 (t, 3H, $J = 6.7$ Hz), 1.16–1.30 (m, 3H), 1.53–1.60 (m, 2H), 2.26 (s, 3H), 2.88–2.94 (m, 2H), 3.77 (s, 3H), 5.54 (s, 1H), 6.73–6.83 (m, 2H), 7.14–7.21 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 14.7, 20.6, 22.5, 29.5, 31.2, 55.4, 57.8, 60.0, 102.0, 114.0, 128.3, 130.4, 137.6, 159.1, 167.1; IR (KBr) cm^{-1}: 3298, 1680, 1653; MS (FAB): *m/z* 377 $(M + H)^+$.

3.2.10. 4-(4-Methoxy-phenyl)-6-methyl-2-tetradecylsulfanyl-1,4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**17**)

It was obtained by reaction of **4** (1.5 g, 4.90 mmol), K_2CO_3 (0.67 g, 4.90 mmol) and tetradecyl bromide (1.34 mL, 4.90 mmol) as a foam (1.97 g, 80%); ^1H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.6$ Hz), 1.16–1.25 (m, 21H), 1.42–1.60 (m, 2H), 2.30 (s, 3H), 2.69–3.18 (m, 2H), 3.77 (s, 3H), 4.08 (q, 2H, $J = 7.0$ Hz), 5.66 (s, 1H), 6.10 (s, 1H), 6.79 (d, 2H, $J = 8.6$ Hz), 7.22 (d, 2H, $J = 8.7$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.5, 14.7, 23.1, 29.1, 29.5, 29.7, 29.9, 30.1, 31.3, 32.3, 55.4, 56.0, 60.0, 114.0, 114.6, 128.3, 132.3, 137.6, 159.1, 167.1; MS (FAB): *m/z* 503 $(M + H)^+$.

3.2.11. 4-(4-Fluoro-phenyl)-6-methyl-2-pentylsulfanyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**18**)

It was obtained by reaction of **5** (1.5 g, 5.10 mmol), K_2CO_3 (0.70 g, 5.10 mmol) and pentyl bromide (0.63 mL, 5.10 mmol) as an oil (1.3 g, 70%); ^1H NMR (200 MHz, $CDCl_3$): δ 0.94 (t, 3H, $J = 6.6$ Hz), 1.23–1.38 (m, 7H), 1.58–162 (m, 2H), 2.33 (s, 3H), 2.89–2.96 (m, 2H), 4.11 (q, 2H, $J = 7.0$ Hz), 5.72 (s, 1H), 6.96–7.05 (m, 2H), 7.30–7.36 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 14.6, 20.3, 22.5, 29.5, 31.2, 31.3, 58.0, 60.1, 102.3, 115.2, 115.6, 116.1, 128.8, 128.9, 140.9, 159.9, 164.8, 166.9; IR (neat) cm^{-1}: 3293, 2365, 1653; MS (FAB): *m/z* 365 $(M + H)^+$.

3.2.12. 4-(4-Fluoro-phenyl)-6-methyl-2-tetradecylsulfanyl-1,4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**19**)

It was obtained by reaction of **5** (1.5 g, 5.10 mmol), K_2CO_3 (0.70 g, 5.10 mmol) and tetradecyl bromide (1.39 mL, 5.10 mmol) as a light green solid (2.1 g, 85%); m.p. 50–51 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.7$ Hz), 1.16–1.25 (m, 25H), 1.54–1.62 (m, 2H), 2.31 (s, 3H), 2.84–3.07 (m, 2H), 4.07 (q, 2H, $J = 7.0$ Hz), 5.65 (s, 1H), 6.17 (s, 1H), 6.90–6.98 (m, 2H), 7.23–7.29 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.5, 14.6, 19.0, 23.1, 29.1, 29.5, 29.7, 29.8, 30.0, 31.4, 32.3, 59.2, 60.2, 101.2, 115.2, 115.6, 128.8, 128.9, 141.0, 159.9, 164.7, 167.2; IR (neat) cm^{-1}: 3310, 1652, 1601; MS (FAB): *m/z* 491 $(M + H)^+$.

3.2.13. 4-(4-Chloro-phenyl)-6-methyl-2-pentylsulfanyl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**20**)

It was obtained by reaction of **6** (1.6 g, 5.16 mmol), K_2CO_3 (0.71 g, 5.16 mmol) and pentyl bromide (0.64 mL, 5.16 mmol) as an oil (1.37 g, 70%); ^1H NMR (200 MHz, $CDCl_3$): δ 0.86 (t, 3H, $J = 6.6$ Hz), 1.10–1.33 (m, 7H), 1.53–1.60 (m, 2H), 2.32 (s, 3H), 2.89–3.03 (m, 2H), 4.04 (q, 2H, $J = 7.0$ Hz), 5.58 (s, 1H), 7.12–7.33 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.2, 14.6, 19.1, 22.5, 23.0, 29.3, 29.4, 31.1, 31.4, 58.2, 60.5, 101.5, 128.4, 128.7, 129.0, 129.2, 133.2, 143.5, 167.0; IR (neat) cm^{-1}: 3295, 2362, 1652; MS (FAB): *m/z* 381 $(M + H)^+$.

3.2.14. 2-Benzylsulfanyl-6-methyl-4-thiophen-2-yl-1, 4-dihydro-pyrimidine-5-carboxylic acid ethyl ester (**21**)

It was obtained by reaction of **7** (0.8 g, 2.83 mmol), K_2CO_3 (0.39 g, 2.83 mmol) and benzyl bromide (0.34 mL, 2.83 mmol) as a white solid (0.68 g, 65%); m.p. 89–90 °C; ^1H NMR (200 MHz, $CDCl_3$): δ 0.1.25 (t, 3H, $J = 7.0$ Hz), 2.30 (s, 3H), 4.12–4.26 (m, 3H), 4.37 (d, 1H, $J = 13.3$ Hz), 5.97 (s, 1H), 6.87–7.28 (m, 8H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.7, 18.9, 35.8, 55.3, 60.4, 101.5, 123.9, 124.4, 126.8, 127.7, 128.9, 129.4, 137.7, 145.5, 149.2, 151.7, 167.0; IR (KBr) cm^{-1}: 3297, 2358, 1647; MS (ESI): *m/z* 373.1 $(M + H)^+$.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2008.01.038.

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