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Original article

Synthesis and antifilarial activity of chalcone–thiazole derivatives against a human lymphatic filarial parasite, *Brugia malayi*☆

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ABSTRACT

Here we report the synthesis of novel chalcone–thiazole compounds and their antifilarial activity. The antifilarial properties of these hybrids were assessed against microfilariae as well as adult worms of *Brugia malayi*. Among all the synthesized compounds, only two compounds, namely **4g** and **4n** were identified to be promising *in vitro*. These active compounds were tested in *B. malayi*-jird (*Meriones unguiculatus*) and *B. malayi*–*Mastomys coucha* models. Compound **4n** showed 100% embryostatic effect and 49% macrofilaricidal in jirds and *M. coucha* models, respectively. This study provides a new structural clue for the development of novel antifilarial lead molecules.

1. Introduction

Lymphatic filariasis is a tropical helminth disease that causes acute and chronic inflammation in patients spanning 72 counties. Recent reports show that 120 million people are infected with around 40 million seriously incapacitated and disfigured by the disease [1,2]. The disease is caused by thread like nematodes *Wucheria bancrofti*, *Brugia malayi* and *Brugia timori*, which are transmitted by anophiline and culicine mosquitoes; 91% of infections are caused by *W. bancrofti* [3,4]. The manifestations of the disease are responsible for the huge loss of man-hours, productivity, and economy of the nation.

The infection is initiated by introduction of third stage infective larvae (L_3) of the parasite into the host by the bites of L_3-bearing mosquitoes. The L_3 migrate to the nearest lymphatics where, after two molts, develop into adult worms, which give birth to microfilariae (mf). The mf enters into the blood circulation from where they are taken up by mosquitoes during blood meal. In the mosquito, the mf develops into L_3. Although all the three life stages were considered to be responsible for development of the disease condition, the mf and macrofilariae (adult worms) have a major impact as they spend a very long time in the host and bring about host's responses that lead to pathology and transmission of the infection [5,6]. For this reason these two stages have been taken as the main targets for antifilarial drug (microfilaricides and macrofilaricides, worm sterilizing agents) development.

In spite of decades of efforts, no single safe antifilarial agent that could effectively eliminate all the parasite stages could be developed. The available drug, diethylcarbamazine (DEC) (Fig. 1) is insufficient because it acts mainly on mf with inadequate effect on adult parasites. Moreover, the treatment is often complicated due to severe side reactions. Another antifilarial drug, ivermectin is a better alternative to DEC, which is less reactogenic, effective in single dose and sterilizing effect on the adult worms but develops resistance to it. Moxidectin (Fig. 1) though looks promising in animal studies is still under development [7,8]. Albendazole is considered to be a macrofilaricide but at a single dose it is devoid of any discernible macrofilaricidal activity and has a transient effect on early embryogenesis [9].

Currently, a combination of DEC plus/albendazole (a benzimidazole derivative) and ivermectin plus/albendazole is being

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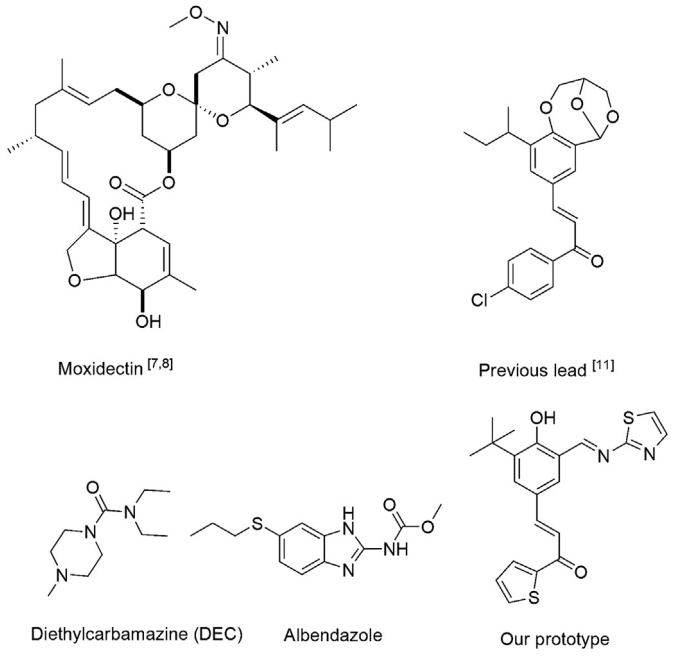


Fig. 1. Chemical structures of some potent antifilarial compounds and our hybrid molecules.

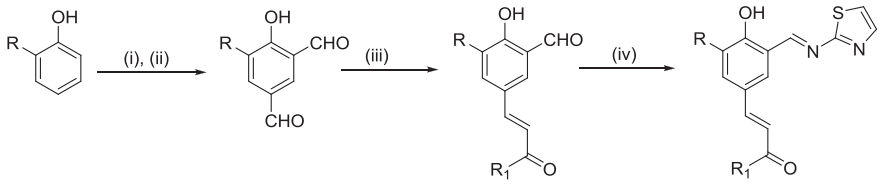
used for the control of infection [10]. The fact that even after such combination therapy there is little change in the prevalence of infection over the years indicates that we need better and safe alternatives to these agents. In view of the current scenario, an adulticidal agent is absolutely needed to destroy the adult worms, which are being implicated as major cause of development of the disease manifestations. Also, special emphasis has been laid by WHO on discovering an adulticidal (macrofilaricidal) agent since it leads to "no adult-no progeny (mf)" and thus has dual benefit. Therefore, efforts have been made to search or synthesize newer and better antifilarials for treatment of filarial infections.

In our drug development programme, we have recently reported 3, 6-epoxy [1,5] dioxocines as new class of antifilarial agents [11]. The design and synthesis of hybrid molecules encompassing two pharmacophores in one molecular scaffold is a well established approach to the synthesis of more potent drugs. Considering the antifilarial activities of chalcone [12] and thiazole derivatives [13], we were interested in synthesizing a number of new hybrid compounds bearing both chalcone and thiazole moieties. Thus the concept of molecular hybridization prompted us to synthesize chalcone–thiazole derivatives, and evaluate them for antifilarial activity *in vitro* and *in vivo* using appropriate experimental models of human filariasis.

2. Chemistry

The detailed synthetic route for the preparation of chalcone–thiazole compounds is summarized in Scheme 1. 2-Alkyl phenols were subjected to the Duff formylation in the presence of hexamethylenetetramine (HMTA) and TFA at 120 °C gave dicarbaldehydes (**2a**–**d**). These aromatic dicarbaldehydes and various substituted acetophenones were subjected to condensation in the presence of a catalytic amount of conc. HCl gave the regioselective chalcones (**3a**–**n**). In all the chalcones synthesized, the *trans* double bond was obtained exclusively. These chalcones were then condensed with 2-aminothiazoles to obtain final targeted compounds (**4a**–**n**). The structures of all the synthesized compounds were fully characterized by complete analysis of 1D and 2D NMR spectroscopy and mass spectrometry.

As a representative example the structure elucidation of **4n** was done as follows. The ^{1}H NMR spectrum of the **4n** (Fig. 2) showed signals at δ 9.32, 13.47 the former belonging to the proton of the imine (C-1″) group and the latter to a hydroxyl group (C-4). Similarly, ^{13}C NMR spectrum showed the presence of two peaks at δ_C 163.3(C-4) and 164.9 (C-1″) and all other carbon resonances were in agreement with the structure proposed. Further, in HMBC spectrum, the hydrogen of imine (H-1″) gave correlations with carbons at δ_C 163.3 (C-4), 118.6 (C-5), 132.8 (C-6) and 169.5 (C-3″) (Fig. 2) and also the hydroxyl proton at δ_H 13.47 gave correlations with δ_C



	R
1a,2a	CH_3
1b,2b	$CH(CH_3)_2$
1c,2c	$CH_2CH_2CH_3$
1d,2d	$C(CH_3)_3$

Compound	R	R_1
3a, 4a	CH_3	*p*-$CH_3C_6H_4$
3b, 4b	CH_3	*p*-$CH_3OC_6H_4$
3c, 4c	CH_3	C_4H_3O
3d, 4d	CH_3	C_4H_3S
3e, 4e	$CH(CH_3)_2$	*p*-$CH_3C_6H_4$
3f, 4f	$CH(CH_3)_2$	*p*-$CH_3OC_6H_4$
3g, 4g	$CH(CH_3)_2$	C_4H_3O
3h, 4h	$CH(CH_3)_2$	C_4H_3S
3i, 4i	$CH_2CH_2CH_3$	*p*-$CH_3C_6H_4$
3j, 4j	$CH_2CH_2CH_3$	*p*-$CH_3OC_6H_4$
3k, 4k	$C(CH_3)_3$	*p*-$CH_3C_6H_4$
3l, 4l	$C(CH_3)_3$	*p*-$CH_3OC_6H_4$
3m,4m	$C(CH_3)3$	C_4H_3O
3n, 4n	$C(CH_3)_3$	C_4H_3S

Scheme 1. Synthesis of chalcone–thiazole derivatives. Reagents and conditions: (i) HMTA, TFA, 120 °C, 4 h; (ii) aq H_2SO_4, 100 °C, 2 h; (iii) conc HCl, *p*-$R_1C_6H_4COCH_3$, dioxane, 80–90 °C, 2.5–3.5 h; (iv) 2-aminothiazole, ethanol, reflux, 3 h.

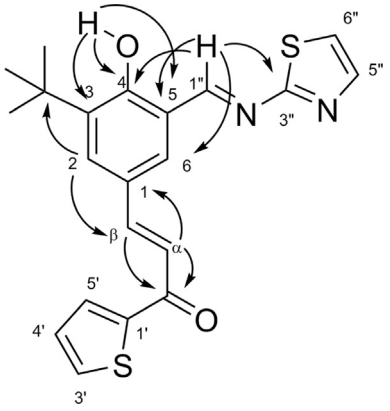


Fig. 2. Selected ^{1}H to ^{13}C-HMBC correlations of compound **4n** in $CDCl_3$.

163.3 (C-4), 139.1 (C-3) and 118.6 (C-5) which further supported the proposed structure. Fig. 2 shows some of the key 2D correlations for **4n**.

3. Biological materials and methods

3.1. Evaluation of antifilarial activity

3.1.1. Parasites

Sub-periodic strain of *B. malayi* was maintained in *Mastomys coucha* and jirds (*Meriones unguiculatus*) through *Aedes aegypti* mosquitoes [14,15]. Mf and adult worms were harvested from the peritoneal cavity (p.c.) of the jirds which were exposed to infective larvae (L_3) approximately 5–6 months back. Freshly isolated live adult worms and mf were washed thoroughly in medium Hanks Balanced Salt Solution (HBSS; pH 7.2) and used for the present study.

3.2. In vitro assays

Motility and inhibition in the 3-(4,5 dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) reduction assays based on viability of the parasites were used in the present study for *in vitro* testing of the samples. Chalcone–thiazole compounds/ivermectin were dissolved in DMSO and DEC in sterile distilled water. The final concentration of DMSO in the incubation medium was kept below 0.1%. DMSO was used in place of test compounds for control. Incubation medium used was HBSS (pH 7.2) containing mixture of antibiotics (penicillin: 100 U/mL; streptomycin: 100 μg/mL).

3.2.1. Primary evaluation (in vitro efficacy)

Initially efficacy of the chalcone–thiazole compounds was assessed *in vitro* using live *B. malayi* parasites in motility and MTT reduction assays [16]. In the 1st set of experiment mf were used in motility assay; the parasites were incubated in medium containing 20 μM ivermectin, 1000 μM DEC or 40 μM chalcone–thiazole compounds for 48 h at 37°C in 5% CO_2 atmosphere. After exposure for 48 h, the effect on motility of mf was assessed. Compounds causing ≥50% inhibition in mf motility over untreated control (DMSO treated) were considered active. In the 2nd set of experiment the active compounds were tested on adult worms using both motility and MTT reduction assays [17,18].

3.2.2. Evaluation of median inhibitory concentration (IC_{50}) and cytotoxic concentration (CC_{50})

The IC_{50} of the active compounds was assessed as described elsewhere [17]. Live parasites were exposed to two fold serial dilutions of the chalcone–thiazole compounds/ivermectin and DEC starting from 0.38 to 40 μM and 15.63 to 1000 μM respectively, using triplicates of one adult female worm/ml/well and 40–50 mf/100 μL/well in 48-well or 96-well sterile cell culture plate. After assessing status of motility of the adult parasites the same treated and control parasites were processed for assessing percentage inhibition in the MTT reduction by the worms. For CC_{50} assay, VERO Cell line C1008 was incubated using a three fold serial dilutions of the test compounds and reference drugs (starting from >20 times LC100 of the compounds).

3.2.3. Assessment of in vitro efficacy

The viability of the treated worms was assessed by calculating percent inhibition in motility and MTT reduction over DMSO treated worms [17]. Briefly, parasite motility was assessed under a microscope after 48 h exposure to test substance and scored as: 0 = dead (i.e. the worms failed to gain pretreatment level of motility even after incubating the treated worms in fresh medium but without test compounds for 25–30 min at 37 °C and expressed as percentage (%) of control); 1–4 = loss of motility (1 = 75%; 2 = 50%; 3 = 25% and 4 = no loss of motility i.e. highly motile worm comparable to control). 100% inhibition in motility of female adults or mf and or ≥50% inhibition in MTT reduction ability of adult parasites was considered acceptable antifilarial activity of the test agents and subjected for further testing. The IC_{50} and CC_{50} were determined as per methods described by Mosman [19] and Page et al. [20]. Data were transferred into a graphic program (Excel); IC_{50} and CC_{50} were calculated by linear interpolation between the two concentrations above and below 50% inhibition [21]. Selectivity Index (SI) of the agents were computed by the formula as: $SI = CC_{50}/IC_{50}$.

3.3. In vivo protocol for efficacy evaluation

Initially as a standard practice and following a validated protocol, we screen synthetic compounds/plant products for antifilarial activity first *in vitro* for 'short-listing' [16,17] and then take-up the positive 'hits' for *in vivo* evaluation in rodent models. The first *in vivo* testing is done in jird model (jirds bearing intraperitoneally transplanted *B. malayi* adult worms) at a dose of 50/100 mg/kg × 5 days by s.c. route and the compound that is positive in this model is tested in the second rodent model *M. coucha* with L_3 induced *B. malayi* infection using the same dose, route and dosing schedule. If need be, the dose is escalated to 200/300 mg/kg. DEC-citrate (DEC-C) is used as the reference drug under similar conditions at recommended standard doses: 25 mg/kg, s.c. × 5 days in jirds and 50 mg/kg, i.p./s.c./p.o. in *M. coucha* [11,22,23]. Similarly ivermectin is used at 1/2 mg/kg, i.p. or s.c. in jirds/*M. coucha* [24].

3.3.1. Host-parasite systems

All the experiments in animals (*M. coucha* and jirds) were conducted in compliance with the Institutional Animal Ethics Committee guidelines for use and handling of animals. Throughout the study, the animals were housed in climate (23 ± 2 °C; RH: 60%) and photoperiod (12 h light–dark cycles) controlled animal quarters. They were fed standard rodent "maintenance diet" prepared in-house (QC analysis: carbohydrates 58.30%, protein 21.1%, fat 7.2%, crude fibre 6.6%, moisture 6.8%) supplemented with dried shrimps (for *M. coucha*) and had free access to drinking water. The treated and untreated (vehicle treated control) animals were autopsied under deep anaesthesia using sodium pentothal.

3.3.2. B. malayi–jird model

Male jirds of 8–10 weeks old were transplanted intraperitoneally (i.p.) with freshly isolated adult worms from peritoneal cavity (p.c.) of infected jirds. Each animal received 10 female and 5 male adult worms in the p.c. On day 2 or 3 post-adult worm transplantation (p.a.t.), the peritoneal fluid was aspirated and checked for the presence of mf. The treatment was started on day 7/8 p.a.t. The animals were killed on day 60 post initiation of treatment (p.i.t.), parasites recovered were collected, recorded their conditions and counted [25].

3.3.3. B. malayi–M. coucha model

Animals harbouring 5–7 months old *B. malayi* infection and showing progressive rise in microfilaraemia were used in the study. Peripheral blood (10 μL tail blood) taken between 12:00 noon and 1:00 PM was made into thick smears, just before initiation of treatment (day 0), on days 7/8 and 14 and thereafter at fortnightly intervals till day 84/91 p.i.t. The animals were killed on day 91 p.i.t.

3.3.4. Administration of the chalcone–thiazole compounds and reference drugs

The chalcone–thiazole compounds were pulverized to fine powder and suspended in 0.1% Tween-80 in distilled water. DEC was dissolved in distilled water and ivermectin was dissolved in 0.1% Tween-80. In jirds and *M. coucha* the test agents were administered at 100 mg/kg body weight through subcutaneous (s.c.) route, for 5 consecutive days while DEC-C was given to them at 25 and 50 mg/kg body weight, respectively, for the same duration. The suspensions/solutions of the test compounds were prepared fresh daily before administration to the animals.

3.3.5. Assessment of microfilaricidal efficacy

Microfilaricidal efficacy of each test agent was evaluated on days 7/8 and 14 p.i.t. and expressed as percent change in mf count over the pretreatment level [25,26].

3.3.6. Assessment of macrofilaricidal and worm sterilization efficacy

Macrofilaricidal/adulticidal efficacy of the chalcone–thiazole compounds was assessed and expressed as percent reduction in adult worm recovery in treated group over vehicle treated (untreated control) animals. Treated and control animals were killed on day 60 (jird) or 91 (*M. coucha*) p.i.t. to recover adult worms from the p.c. washings (jird) or heart, lung, and testes (*M. coucha*). Tissues were teased gently to avoid any damage to the parasites. Parasites were then examined under microscope for status of the motility, cell adherence on their surface, death, or calcification of the worms and recorded. All the surviving females were teased individually in a drop of saline to examine condition of intrauterine mf stages of the parasite [27]. Number of worms recovered from the treated infected animals was compared with that of control animals and percent reduction of worms calculated. Percent sterilization of female worms was determined over total live female worms recovered from treated or control animals.

3.3.7. Statistical analysis

Statistical analyses were carried out using GraphPad Prism 3.0 version software. Results were presented as mean ± S.D. of data obtained from 4 to 8 animals in two experiments. The data were subjected to Student's '*t*' test or One-way ANOVA analysis and the significance of the difference between means were determined by Tukey's Multiple Comparison Test. Values were expressed as means ± SD. '*P*' value <0.05 were considered to be significant.

4. Results and discussion

4.1. In vitro activity on mf and adult worms of B. malayi

In vitro activity of 14 chalcone–thiazole compounds (**4a**–**n**) was assessed in motility and MTT reduction assays using mf and female adult worms of *B. malayi* and the results are depicted in Table 1. Out of all compounds, two compounds **4n** and **4g** were found to be effective in killing mf (LC_{100}: 5 and 10 μM; IC_{50}: 1.8 and 3.5 μM) and adult worms (LC_{100}: 2.5 and 10 μM; IC_{50}: 0.9 and 3.2 μM); both the compounds also inhibited MTT reduction potential of adult parasites to 49 and 63%, respectively. SI values of **4n** and **4g** compounds ranged from 22 to 44 and 17 to 19, respectively, indicating acceptable safety index for *in vivo* testing. The standard drug DEC-C required several times higher concentration to kill the adult worms (LC_{100}: 1000 μM) and mf (LC_{100}: 500 μM); the IC_{50} values of the drug for respective parasite stages were 314.9 μM and 297.3 μM. However, ivermectin killed adult parasites at 5 μM and mf at

Table 1
In vitro activity of chalcone–thiazole compounds (**4a**–**n**) and the reference drugs ivermectin and DEC-C (Diethylcarbamazine–Citrate) on adult worms (AW) and microfilariae (mf) of *Brugia malayi* assessed by motility assay (MA) and MTT reduction assay.

Compounds	LC_{100}[a] (μM)	IC_{50}[b] (μM)	Mean % inhibition in MTT reduction	SI	LC_{100} (μM)	IC_{50}[b] (μM)	SI	CC_{50}[c] (μM)
	For AW in MA	For AW in MA	By AW	For AW in MA	For mf in MA	For AW in MA	For AW in MA	
4a	>40	–	NI	–	>40	–	–	–
4b	>40	–	25	–	>40	–	–	–
4c	>40	–	62	–	>40	–	–	–
4d	>40	–	–	–	>40	–	–	–
4e	>40	–	–	–	>40	–	–	–
4f	>40	–	61	–	>40	–	–	–
4g	10	3.2	49	19	10	3.5	17	60
4h	>40	–	NI	–	>40	–	–	–
4i	>40	–	NI	–	>40	–	–	–
4j	>40	–	NI	–	>40	–	–	–
4k	>40	–	NI	–	40	–	–	–
4l	>40	–	NI	–	40	–	–	–
4m	40	–	NI	–	20	7.07	28.57	220
4n	2.5	0.9	63	44	5	1.8	22	40
Ivermectin	5	3.05	5.80	75.41	2.5	1.57	146.49	230
DEC-C	1000	314.98	62.54	28.57	500	297.30	30.27	9000

[a] LC_{100} = 100% reduction in motility indicates death of parasite.
[b] IC_{50} = 50% concentration of the agent at which 50% inhibition in motility of the parasites is achieved; NI = No inhibition.
[c] CC_{50} = concentration at which 50% of cells are killed; SI = Selectivity Index (CC_{50}/IC_{50}); DEC-C = Diethylcarbamazine–citrate.

Fig. 3. Female parasite recovered from **4n** treated animal showed embryostatic effect of the compound. Uterus of female worm contained distorted eggs and the embryos were devoid of developing mf (arrows) (unstained preparation). Bar = 100 μM.

2.5 μM; IC_{50} values were found to be 3.10 and 1.60 μM, respectively. In summary, two hits were identified to be promising with >15 SI value *in vitro* and thus allowed for *in vivo* evaluation.

Structure–activity relationships (SARs) were inferred from *in vitro* activity on adult worms and of *B. malayi*. The initial examination of structure–activity relationships (SARs) revealed that the branching at position 3 of the phenyl ring is preferred and also the presence of five membered heterocycles, e.g., furan, thiophene, as the chalcone counterpart potentiates the activity. The presence of imine thiazole functionality is also a requirement for activity as the intermediates (**3a**–**n**) did not exhibit the activity. A pictorial representation the SAR is depicted in Fig. 5.

4.2. In vivo antifilarial efficacy

4.2.1. In vivo evaluation of hits (compounds **4g** and **4n**) on *B. malayi*–jird model

From *in vitro* results we considered **4g** and **4n** are most active compounds. These active compounds were tested in primary *in vivo*

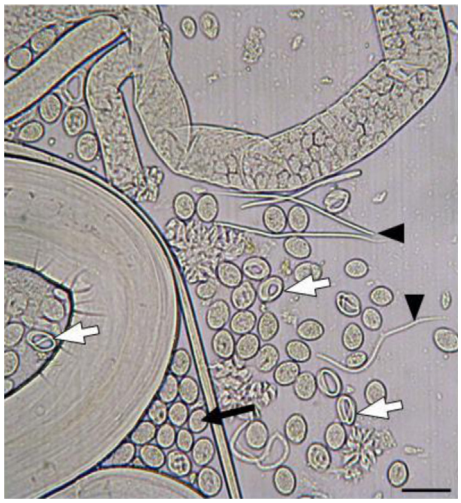


Fig. 4. Female worm from control animal showing eggs (arrows), developing mf in embryos (hollow arrows) and developed mf (arrows heads) expelled from uterus by gentle pressure on the worm during preparation, can also be seen around the worm (unstained preparation). Bar = 100 μM.

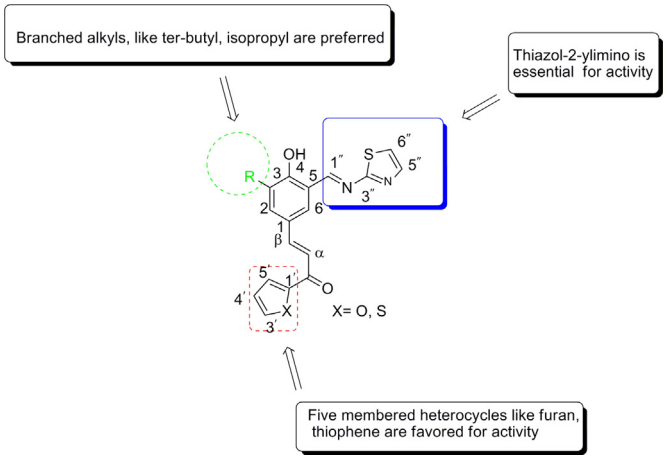


Fig. 5. SAR of synthesized hybrid.

model *B. malayi*/jird at 100 mg/kg s.c. for 5 consecutive days and the results are presented in Table 2. Compound **4n** exhibited 100% embryostatic effect (Fig. 3). Compound **4g** showed around 18% macrofilaricidal activity but did not exert any embryostatic activity. On day 7 p.i.t. mf in p.c. of treated animals were motile and active comparable to mf of vehicle treated animals (untreated control) indicating ineffective against mf immediately after treatment (data not shown). The standard drug DEC-C (25 mg/kg, s.c. × 5 days) was ineffective against both mf and adult worms in p.c.; whereas, ivermectin (1 mg/kg, s.c. × 5 days) exerted 43% embryostatic effect ($P < 0.01$; Table 2) but did not affect the mf. Parasites recovered from vehicle treated animals were healthy with almost no embryostatic activity (Table 2) as evidenced by fully developed mf (Fig. 4). The general behaviour of the jirds during 5-day course of treatment and thereafter till termination of the experiment (day 60 p.i.t.) was normal. In summary, compound **4n** showed remarkable embryostatic activity.

4.2.2. In vivo evaluation of compounds **4g** and **4n** on *B. malayi*–*M. coucha* model

Compounds **4n** and **4g** were evaluated in secondary model (*B. malayi*–*M. coucha*) and the results are depicted in Table 3. Compound **4n** tested at 100 mg/kg, s.c. for 5 consecutive days significantly affected adult parasites (~49%; $P < 0.001$) over control animals. The compound also exerted around 44% female sterilizing activity. Compound **4g** was found less effective showing 40% adulticidal ($P < 0.01$) with 30% female sterilizing activity. In summary, Compound **4n** possessed promising antifilarial efficacy. DEC-C (50 mg/kg) which is principally microfilaricidal caused >85% reduction in microfilarial count on day 7 p.i.t. and thereafter microfilaraemia progressively increased showing relapse by day 49 p.i.t. which further increased and crossed the pretreatment level (data not shown). DEC-C treatment in infected animals caused around 24% macrofilaricidal effect. In these animals the percent female sterilization was comparable to the female worms recovered from control animals (Table 3). Vehicle treated animals (untreated control) showed no effect on microfilariae (mf) (data not shown) or adult worms (Table 3).

The general behaviour of **4g-** and **4n-**treated animals during the 5-day course of treatment till termination of the experiment i.e. day 60 (jird)/91 (*M. coucha*) p.i.t. was normal indicating that apparently the compounds were safe.

As described above, of the two compounds **4n** showed both micro- and macrofilaricidal activity *in vitro* and macrofilaricidal and embryostatic activity *in vivo*. The reference drug DEC (principally a

Table 2
Antifilarial activity of chalcone–thiazole compounds (**4g** and **4n**) and reference drugs ivermectin and DEC-C against *Brugia malayi* in jirds (*Meriones unguiculatus*).

Antifilarial agent	Dose mg/kg s.c. × 5 days (*n*)	Worms recovered			Sterilized female worm count (%)
		Male	Female	Total (% reduction over untreated)	
4g	100 (5)	1.67 ± 0.58	7.67 ± 0.58	9.33 ± 1.15 (18.16)	0 (0)
4n	100 (6)	1.33 ± 1.15	10 ± 0	11.33 ± 1.15	10 ± 0[b,***] (100)
Ivermectin[a]	1 (4)	2.75 ± 0.96	6.25 ± 1.50	9.00 ± 2.52 (21.05)	2.75 ± 0.96** (43.21 ± 5.15)
DEC-C[a]	25 (6)	2.60 ± 0.55	6.60 ± 1.14	9.20 ± 1.48 (19.30)	0.60 ± 0.89 (7.86 ± 11.41)
Untreated Control	– (5)	3.20 ± 1.10	8.203 ± 0.84	11.40 ± 1.52	0.80 ± 0.45 (9.80 ± 5.63)

Values are mean ± SD.
n = number of animals; DEC-C: Diethylcarbamazine-Citrate.
$^{**}P < 0.01$ (Ivermectin vs Untreated control); $^{***}P < 0.001$ (**4n** vs **4g**/Ivermectin/DEC-C/Untreated control; Ivermectin vs **4g**/DEC-C). Statistics: Tukey's Multiple Comparison Test.
[a] Reference drugs.
[b] Distorted and disintegrated microfilarial stages in uteri.

Table 3
Antifilarial activity of chalcone–thiazole compounds (**4g** and **4n**) and reference drug DEC-C against *Brugia malayi* in *Mastomys coucha*. Values are Mean ± SD.

Antifilarial agent	Dose in mg/kg, s.c. × 5 days (n)	No. Live worms			Sterilized female worms count (%)
		Male	Female	Total (% over untreated)	
4g	100 (8)	7.88 ± 3.76	14.88 ± 4.09	22.75 ± 6.18** (40.91%)	4.88 ± 3.52 (30)
4n	100 (8)	6.60 ± 4.04	13.20 ± 2.77	19.80 ± 4.60*** (48.57%)	5.80 ± 1.48 (44)
DEC-C[a]	50 (6)	6.00 ± 1.79	23.33 ± 4.60	29.33 ± 4.80 (23.82)	6.8 ± 5.70 (29)
Untreated Control	– (5)	16.83 ± 5.19	21.67 ± 6.15	38.50 ± 10.39	5.17 ± 2.64 (23)

n = number of animals.
$^{**}P < 0.01$; $^{***}P < 0.001$ (vs untreated control; Statistic: Tukey's Multiple Comparison Test).
[a] DEC-C showed >85% microfilaricidal activity on day 7 post initiation of treatment.

microfilaricide), a piperazine derivative also showed these effects *in vitro* but only at a very high dose; *in vivo* it showed around 24% macrofilaricidal (at usual effective dose of 50 mg/kg × 5 days) activity in *B. malayi–M. coucha* model. At a higher dose of 100 mg/kg DEC showed no increase in its microfilaricidal efficacy, but there was further increase in macrofiaricidal efficacy to the tune of 45% [28]. Ivermectin (a macrocyclic lactone), another principally microfilaricide, showed microfilaricidal activity *in vitro* and in addition, some macrofilaricidal (~20%) and embryostatic (~40%) efficacy *in vivo* [24]. Our chalcone–thiazole derivative 4n which is different from the existing antifilarials has shown micro- and macrofilaricidal activity *in vitro*, and macrofilaricidal along with embryostatic activity *in vivo*. Thus, compound 4n was found superior than the control drugs and provides a new structural clue for the development of novel antifilarial lead molecules. The detailed mechanism studies of potent hybrids and further structural modifications are currently underway.

5. Conclusion

In summary, a series of chalcone–thiazole derivatives were designed, synthesized and evaluated for their *in vitro* as well as *in vivo* activity against filarial nematode, *B. malayi*. Interestingly, of the two hits identified *in vitro*, the most potent compound **4n** seems to possess remarkable embryostatic with moderate macrofilaricidal activity *in vivo* compared to standard drugs ivermectin and DEC. Furthermore, the lead compound showed good SI as evidenced by *in vitro* cytotoxicity test and did not adversely affect the health and general behaviour of the treated animals as well. Hence these studies provide new prototype for the development of new class of antifilarial agents.

6. Experimental

6.1. General information

All reagents were commercial and were used without further purification. Chromatography was carried on silica gel (60–120 and 100–200 mesh). All reactions were monitored by thin-layer chromatography (TLC), and silica gel plates with fluorescence F254 were used. Melting points were taken in open capillaries on a Complab melting point apparatus and are presented uncorrected. Infrared spectra were recorded on a Perkin–Elmer FT-IR RXI spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Supercon Magnet DPX-200 and DRX-300 spectrometers (operating at 300 and 400 MHz for ^{1}H and 75 and 100 MHz for ^{13}C) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million. Electrospray ionization mass spectra (ESIMS) were recorded on Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded on 6520 Agilent Q Tof LC MS/MS (Accurate mass).

6.2. General procedure for the synthesis of compounds (*2a–d*) and chalcones (*3a–n*)

Synthesis of dicarbaldehyde substrates and the regioselective *para* condensed chalcones were achieved by our previously reported protocol [29].

6.3. General procedure for the synthesis of compounds (*4a–n*)

A mixture of appropriate *para* condensed chalcones **3a–n** (1 equiv) and 2-aminothiazole (1 equiv) in ethanol were refluxed for 3 h. The solvent was evaporated under vacuum and the solid

was purified directly with column chromatography to obtain the respective compounds **4a**–**n** in excellent yields.

*6.3.1. (E)-3-(4-hydroxy-3-methyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-p-tolylprop-2-en-1-one (**4a**)*

Yellow solid, yield: 75%; mp 170–171 °C; IR (KBr): 3584, 3018, 1657, 763 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 12.92 (s, 1H), 9.25 (s, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.76 (d, J = 15.6 Hz, 1H), 7.69 (d, J = 3.4 Hz, 1H), 7.65 (s, 1H), 7.58 (s, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.32–7.28 (m, 3H), 2.44 (s, 3H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): 189.7, 169.6, 164.4, 162.0, 143.6, 143.3, 141.6, 135.8, 134.4, 132.6, 129.4, 128.6, 127.7, 126.5, 120.0, 119.1, 117.8, 21.7, 15.7; ESI-MS (m/z): 363.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{21}H_{18}N_2O_2S$ $(M + H)^+$ 363.1167, found: 363.1153.

*6.3.2. (E)-3-(4-hydroxy-3-methyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(4-methoxy phenyl)prop-2-en-1-one (**4b**)*

Yellow solid, yield: 75%; mp 168–169 °C; IR (KBr): 3574, 3045, 1684, 778 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 12.91 (s, 1H), 9.25 (s, 1H), 7.76 (d, J = 15.5 Hz, 1H), 7.69 (d, J = 3.5 Hz, 1H), 7.65 (s, 1H), 7.57 (s, 1H), 7.45 (d, J = 15.5 Hz, 1H), 7.28 (d, J = 3.4 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 3.89 (s, 3H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) : 188.4, 169.7, 164.4, 163.4, 161.9, 142.9, 141.6, 134.4, 132.6, 131.2, 130.8, 127.7, 126.5, 119.8, 119.1, 117.8, 113.9, 55.5, 15.7; ESI-MS (m/z): 379.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{21}H_{19}N_2O_3S$ $(M + H)^+$ 379.1116, found: 379.1104.

*6.3.3. (E)-1-(furan-2-yl)-3-(4-hydroxy-3-methyl-5-((E)-(thiazol-2-ylimino)methyl)phenyl)prop-2-en-1-one **(4c)***

Yellow solid, yield: 80%; mp 188–189 °C; IR (KBr): 3629, 3021, 1593, 761 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 12.94 (s, 1H), 9.24 (s, 1H), 7.82 (d, J = 15.7 Hz, 1H), 7.69 (d, J = 3.3 Hz, 1H), 7.65 (s, 2H), 7.58 (s, 1H), 7.38–7.32 (m, 2H), 7.28 (d, J = 3.3 Hz, 1H), 6.60–6.59 (m, J = 1.6 Hz, 1H), 2.23 (s, 3H); ESI-MS (m/z): 339.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{18}H_{15}N_2O_3S$ $(M + H)^+$ 339.0803, found: 339.0798.

*6.3.4. (E)-1-(furan-2-yl)-3-(4-hydroxy-3-methyl-5-((E)-(thiazol-2-ylimino)methyl)phenyl)prop-2-en-1-one (**4d**)*

Yellow solid, yield: 74%; mp 183–184 °C; IR (KBr): 3654, 3075, 1576, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 12.95 (s, 1H), 9.27 (s, 1H), 7.89 (d, J = 3.0 Hz, 1H), 7.82 (d, J = 15.4 Hz, 1H), 7.72–7.69 (m, 2H), 7.66 (s, 1H), 7.60 (s, 1H), 7.36–7.28 (m, 2H), 7.22–7.19 (m, 1H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 181.8, 169.6, 164.3, 162.2, 145.7, 143.0, 141.7, 134.5, 133.8, 132.8, 131.7, 128.3, 127.8, 126.2, 119.7, 119.1, 117.9, 15.7; ESI-MS (m/z): 355.1 $(M + H)^+$; HRMS (m/z): calcd for $C_{18}H_{15}N_2O_2S_2$ $(M + H)^+$ 355.0575, found: 355.0587.

*6.3.5. (E)-3-(4-hydroxy-3-isopropyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-p-tolylprop-2-en-1-one (**4e**)*

Yellow solid, yield: 64%; mp 150–151 °C; IR (KBr): 3635, 3078, 1642, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 13.02 (s, 1H), 9.24 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.68–7.66 (m, 2H), 7.58 (d, J = 1.8 Hz, 1H), 7.42 (d, J = 15.6 Hz, 1H), 7.31–7.27 (m, 3H), 3.47–3.38 (m, 1H), 2.43 (s, 3H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) : 189.9, 169.6, 164.6, 161.3, 143.7, 143.6, 141.6, 137.9, 135.8, 132.3, 130.8, 129.4, 128.7, 126.6, 120.1, 119.1, 118.1, 26.9, 22.3, 21.7; ESI-MS (m/z): 391.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{23}H_{23}N_2O_2S$ $(M + H)^+$ 391.1480, found: 391.1474.

*6.3.6. (E)-3-(4-hydroxy-3-isopropyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(4methoxy phenyl)prop-2-en-1-one (**4f**)*

Yellow solid, yield: 70%; mp 180–181 °C; IR (KBr): 3670, 3176, 1593, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 13.02 (s, 1H), 9.25 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 15.5 Hz, 1H), 7.70–7.67 (m, 3H), 7.58 (d, J = 1.8 Hz, 1H), 7.45 (d, J = 15.5 Hz, 1H), 7.28 (d, J = 3.6 Hz, 1H), 6.99 (d, J = 8.7 Hz, 2H), 3.89 (s, 3H), 3.51–3.40 (m, 1H), 1.33 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 188.6, 169.7, 164.6, 163.4, 161.2, 143.3, 141.6, 137.8, 132.2, 131.3, 130.8, 130.7, 126.7, 119.9, 119.0, 118.1, 113.9, 55.5, 26.9, 22.3; ESI-MS (m/z): 407.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{23}H_{23}N_2O_3S$ $(M + H)^+$ 407.1429, found: 407.1420.

*6.3.7. (E)-1-(furan-2-yl)-3-(4-hydroxy-3-isopropyl-5-((E)-(thiazol-2-ylimino)methyl)phenyl)prop-2-en-1-one (**4g**)*

Yellow solid, yield: 76%; mp 183–184 °C; IR (KBr): 3732, 3134, 1655, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 13.05 (s, 1H), 9.26 (s, 1H), 7.85 (d, J = 15.7 Hz, 1H), 7.69–7.66 (m, 3H), 7.61 (s, 1H), 7.38–7.33 (m, 2H), 7.29 (d, J = 3.4 Hz, 1H), 6.61–6.60 (m, 1H), 3.48–3.39 (m, 1H), 1.32 (d, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 178.0, 169.6, 164.6, 161.5, 153.9, 146.4, 143.2, 141.6, 137.9, 132.4, 130.9, 126.3, 119.2, 119.1, 118.1, 117.3, 112.6, 26.9, 22.2; ESI-MS (m/z): 367.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{20}H_{19}N_2O_3S$ $(M + H)^+$ 367.1116, found: 367.1124.

*6.3.8. (E)-3-(4-hydroxy-3-isopropyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**4h**)*

Yellow solid, yield: 76%; mp 160–161 °C; IR (KBr): 3654, 3172, 1652, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 13.12 (s, 1H), 9.34 (s, 1H), 7.96 (d, J = 2.9 Hz, 1H), 7.90 (d, J = 15.6 Hz, 1H), 7.77–7.74 (m, 3H), 7.68 (s, 1H), 7.42–7.33 (m, 2H), 7.28–7.25 (m, 1H), 3.56–3.47 (m, 1H), 1.40 (d, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 181.9, 169.6, 164.5, 161.4, 145.7, 143.3, 141.6, 137.9, 133.7, 132.3, 131.7, 130.9, 128.3, 126.3, 119.6, 119.1, 118.1, 26.9, 22.2; ESI-MS (m/z): 383.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{20}H_{19}N_2O_2S_2$ $(M + H)^+$ 383.0888, found: 383.0883.

*6.3.9. (E)-3-(4-hydroxy-3-propyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-p-tolylprop-2-en-1-one (**4i**)*

Yellow solid, yield: 62%; mp 149–150 °C; IR (KBr): 3610, 3192, 1652, 767 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 12.94 (s, 1H), 9.25 (s, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.69 (d, J = 3.4 Hz, 1H), 7.62 (s, 1H), 7.59 (s, 1H), 7.44 (d, J = 15.6 Hz, 1H), 7.32–7.28 (m, 3H), 2.72 (t, J = 7.3 Hz, 2H), 2.44 (s, 3H), 1.75–1.68 (m, 2H), 1.01 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) : 189.8, 169.7, 164.5, 161.9, 143.6, 143.5, 141.7, 135.8, 134.0, 132.7, 132.1, 129.4, 128.7, 126.5, 120.1, 119.1, 118.1, 31.8, 22.7, 21.7, 14.1; ESI-MS (m/z): 391.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{23}H_{23}N_2O_2S$ $(M + H)^+$ 391.1480, found: 391.1478.

*6.3.10. (E)-3-(4-hydroxy-3-propyl-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(4-methoxy phenyl)prop-2-en-1-one (**4j**)*

Yellow solid, yield: 66%; mp 145–146 °C; IR (KBr): 3652, 3234, 1667, 761 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 12.91 (s, 1H), 9.24 (s, 1H), 8.04 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 15.5 Hz, 1H), 7.68 (d, J = 3.4 Hz, 1H), 7.61 (s, 1H), 7.57 (s, 1H), 7.44 (d, J = 15.5 Hz, 1H), 7.27 (d, J = 3.4 Hz, 1H), 6.98 (d, J = 8.7 Hz, 2H), 3.88 (s, 3H), 2.71 (t, J = 7.3 Hz, 2H), 1.75–1.68 (m, 2H), 1.01 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 188.5, 169.7, 164.5, 163.4, 161.8, 143.0, 141.6, 133.9, 132.6, 132.6, 132.1, 131.3, 130.8, 126.6, 119.9, 119.1, 118.1, 113.9, 55.5, 31.8, 22.7, 14.1; ESI-MS (m/z): 407.2 $(M + H)^+$; HRMS (m/z): calcd for $C_{23}H_{23}N_2O_3S$ $(M + H)^+$ 407.1429, found: 407.1419.

*6.3.11. (E)-3-(3-tert-butyl-4-hydroxy-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-p-tolylprop-2-en-1-one (**4k**)*

Yellow solid, yield: 72%; mp 210–211 °C; IR (KBr): 3655, 3157, 1735, 762 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.3 (s, 1H), 9.26 (s, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.78 (d, J = 15.6, 1H), 7.74 (d, J = 2 Hz, 1H), 7.69 (d, J = 3.5 Hz, 1H),7.62 (s, 1H), 7.42 (d, J = 15.6 Hz, 1H), 7.31 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 3.4 Hz, 1H), 2.44 (s, 3H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): 188.6, 169.5, 164.9, 163.4, 163.0, 143.5,

141.6, 138.9, 132.6, 131.6, 131.3, 130.8, 126.2, 119.8, 119.0, 118.6, 113.9, 55.5, 35.1, 29.3; ESI-MS (*m/z*): 405.2 $(M + H)^+$; HRMS (*m/z*): calcd for $C_{24}H_{25}N_2O_2S$ $(M + H)^+$ 405.1637, found: 405.1648.

*6.3.12. (E)-3-(3-tert-butyl-4-hydroxy-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (**4l**)*

Yellow solid, yield: 76%; mp 199–200 °C; IR (KBr): 3756, 3112, 1743, 735 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 13.38 (s, 1H), 9.26 (s, 1H), 8.04 (d, $J = 8.9$ Hz, 2H), 7.78 (d, $J = 15.5$ Hz, 1H), 7.74 (d, $J = 2.0$ Hz, 1H), 7.69 (d, $J = 3.5$ Hz, 1H), 7.62 (d, $J = 2.0$ Hz, 1H), 7.43 (d, $J = 15.5$ Hz, 1H), 7.29 (d, $J = 3.4$ Hz, 1H), 6.99 (d, $J = 8.9$ Hz, 2H), 3.90 (s, 3H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) : 188.6, 169.5, 164.9, 163.4, 163.0, 143.5, 141.6, 138.9, 132.6, 131.6, 131.3, 130.8, 126.2, 119.8, 119.0, 118.6, 113.9, 55.6, 35.2, 29.3; ESI-MS (*m/z*): 421.2 $(M + H)^+$; HRMS (*m/z*): calcd for $C_{24}H_{25}N_2O_3S$ $(M + H)^+$ 421.1586, found: 421.1598.

*6.3.13. (E)-3-(3-tert-butyl-4-hydroxy-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(furan-2-yl)prop-2-en-1-one (**4m**)*

Yellow solid, yield: 68%; mp 180–181 °C; IR (KBr): 3617, 3166, 1650, 762 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 13.42 (s, 1H), 9.27 (s, 1H), 7.86 (d, $J = 15.7$ Hz, 1H), 7.75 (d, $J = 1.5$ Hz, 1H), 7.69 (d, $J = 3.4$ Hz, 1H), 7.66 (s, 1H), 7.64 (s, 1H), 7.37–7.33 (m, 2H), 7.29 (d, $J = 3.4$ Hz, 1H), 6.61–6.59 (m, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 178.0, 169.5, 164.8, 163.3, 153.9, 146.4, 143.5, 141.6, 139.0, 132.8, 131.8, 125.9, 119.0, 118.6, 117.3, 112.6, 35.2, 29.3; ESI-MS (*m/z*): 381.2 $(M + H)^+$; HRMS (*m/z*): calcd for $C_{21}H_{21}N_2O_3S$ $(M + H)^+$ 381.1273, found: 381.1269.

*6.3.14. (E)-3-(3-tert-butyl-4-hydroxy-5-((E)-(thiazol-2-ylimino) methyl)phenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**4n**)*

Yellow solid, yield: 74%; mp 180–182 °C; IR (KBr): 3564, 3245, 1662, 763 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.74 (s, 1H), 7.48 (s, 1H), 7.36 (s, 1H), 3.84 (s, 3H), 3.43 (s, 3H), 3.34–3.22 (m, 1H), 3.14 (t, $J = 5.8$ Hz, 2H), 2.59–2.50 (m, 5H), 2.12–2.06 (m, 2H), 1.66–1.61 (m, 2H), 1.22 (d, $J = 6.9$ Hz, 3H), 0.79 (t, $J = 6.5$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) : 182.0, 169.5, 164.9, 163.3, 145.8, 143.6, 141.7, 139.1, 133.8, 132.8, 131.8, 131.7, 128.3, 125.8, 119.6, 119.1, 118.6, 35.2, 29.3; ESI-MS (*m/z*): 397.2 $(M + H)^+$; HRMS (*m/z*): calcd for $C_{21}H_{21}N_2O_2S_2$ $(M + H)^+$ 397.1044, found: 397.1050.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.029.

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