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Original article

Synthesis and antimosquito properties of 2,6-substituted benzo[*d*]thiazole and 2,4-substituted benzo[*d*]thiazole analogues against *Anopheles arabiensis*

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ABSTRACT

A novel and efficient one pot synthesis was developed for 2,6-substituted-benzo[*d*]thiazole analogues **4a**–**k** and 2,4-substituted-benzo[*d*]thiazole analogues **4l**–**p** *via* three component condensation reaction of substituted arylaldehyde, 2-amino-6-halo/4-methyl-benzo[*d*]thiazole and 2-naphthol or 6-hydroxy quinoline in presence of 10% w/v NaCl in water by microwave method. This method enabled for short reaction times, easy work-up and significant high yields. The title compound **4b** was used for single crystal X-ray studies in order to understand its conformation and packing features. The title compounds **4a**–**p** were screened for antimosquito properties such as repellency, insecticidal and larvicidal activity against *Anopheles arabiensis* by mosquito feeding-probing assay, cone bio-assay and standard WHO larvicidal assay, respectively. Among these analogous **4b**, **4d** and **4p** exhibit the highest repellent activity comparable to the positive control DEET, and **4a** and **4k** knockdown most mosquitoes on repellent assays.

1. Introduction

Malaria is a serious and widespread parasitic disease in tropical and subtropical zones of the world. Even though the estimated incidence of malaria has been globally reduced over the last decade, this disease still causes millions of deaths each year with the highest incidence occurring in infants and young children [1]. There are also reports showing that, some mosquitoes are resistant to the current drugs that are used in the treatment of *Plasmodium falciparum* malaria [2] and to insecticides used for vector control [3]. The development of alternative drugs should aid in resistance management. Historically, the paradigm of drug development has followed an iterative cycle of screening and synthesis, involving the manipulation of individual structures. The feedstock of molecules for this process has traditionally incorporated both natural products and proprietary and commercial compound collections. The latter usually represent a collectively monumental effort of synthesis over a period of many years. The introduction of high-throughput biological screening and the accelerated discovery of new biological targets have increased the demand on synthetic chemists to produce new compounds for testing. One response to this demand has been the development of techniques to greatly increase the speed and efficiency of compound synthesis.

The chemistry of organic reactions in water is undergoing rapid growth because of various potential advantages, such as alleviation of environmental problems associated with the use of organic

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solvents, industrial applications, unique reactivity, and selectivity [4,5]. However, the solubility of organic reactants offers a great challenge, as most of these are insoluble in water. Due to their lesser solubility, organic reactions using water as solvent have been the subject of recent debate with regard to the use of terminology, and so have been classified as on water [6], in water [7] or in the presence of water [8–10]. Synthetic chemists have taken up the challenge of developing efficient aqueous phase small organic molecule-catalyzed processes [11].

2-Aminobenzo[*d*]thiazolomethylnaphthols or 5-(2-aminobenzo[*d*]thiazolomethyl)-6-hydroxyquinolines using three-component condensation reaction of an aldehyde, 2-aminobenzothiazole and 2-naphthol or 6-hydroxyquinoline have been synthesized in presence of lithium chloride in water [12] and sodium dodecyl sulphate [13], but to our knowledge there are no reports dealing with the synthesis of 5-[(6-halo & 4-methyl-benzothiazol-2-ylamino)-(substituted-phenyl)-methyl]-quinolin-6-ol, even though there are reports showing that benzothiazole derivatives exhibit diverse chemical reactivities and a broad spectrum of biological activities. Some of the important biological activities observed for benzothiazoles are anti-tumour [14], anti-inflammatory [15], analgesic [16], anti-microbial [17], anti-leishmanial [18], anti-convulsant [19], anti-malarial [20], potential anti-HIV agents [21] and selective inhibition of HIV Type 1 reverse transcriptase [22]. In continuation of our research on synthesis of bioactive compounds [23–26] and in this communication, we describe the synthesis and characterization of the title compounds **4a**–**p** (Scheme 1) and an evaluation of the antimosquito activity of these substances *in vitro* against *Anopheles arabiensis*. Quinoline and naphthol moieties were incorporated into the compounds because they have previously shown activity against insects. Naphthol is a precursor to a variety of insecticides, and is a long known component with contact insecticidal properties [27]. On the other hand, quinoline compounds gave good inhibitory activity for acetylcholinesterase (AChE) [28], and quinoline derivatives have shown insecticidal activity [29]. In order to understand the conformation and packing features of compounds, we also show the single crystal X-ray studies of compound **4b**.

2. Results and discussion

2.1. Chemistry

In continuation of our interest under green chemistry protocols for the synthesis of organic compounds [30,31], we standardized a simple, environmentally friendly and practical method for the synthesis of 2,6-substituted benzo[*d*]thiazole **4a**–**k** and 2,4-substituted benzo[*d*]thiazole **4l**–**p** *via* three component condensation reaction of substituted arylaldehyde, 2-amino-6-chlorobenzo[*d*]thiazole and 6-hydroxyquinoline or 2-naphthol under microwave irradiation in presence of water with sodium chloride. At the onset of this work, we used sodium chloride to increase the ionic strength, and then several other ionic salts such as LiCl, NaBr, NaF, $NaNO_3$, Na_2SO_4, $LiNO_3$ and Li_2SO_4 were examined (Table 1). Among the salts NaCl (10% w/v) yielded 85–93%, of title compounds **4a**–**p**.

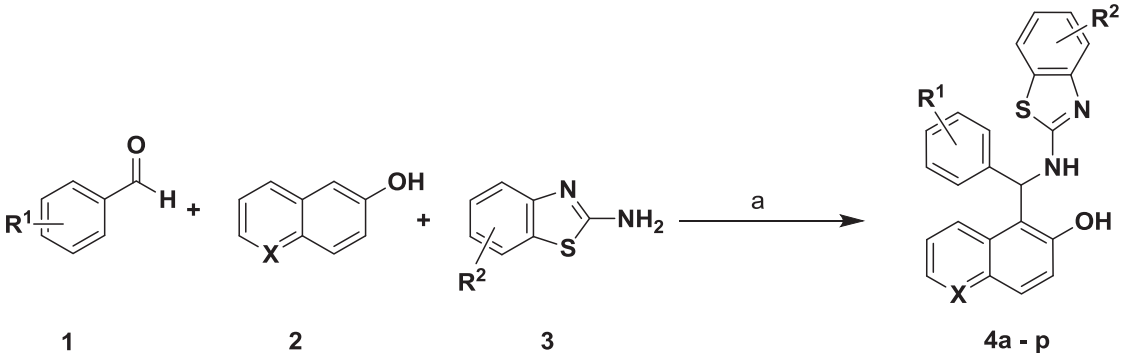
The protocol for compounds **4a**–**k** is as per the reactions used for **4a** and this is shown in Scheme 1. Compound **4a** was achieved by selecting 2-naphthol (1 mmol), *p*-hydroxy benzaldehyde (1 mmol) and 2-amino-6-chloro-benzo[*d*]thiazole (1 mmol) as the model substrate. The mixture was taken in 10 mL of 10% w/v NaCl salt in water, stirred for 10 min and heated to 90 °C and it took 4 h for completion of the reaction. The same reaction under the influence of microwave irradiation (320 W), took 10 min to yield the desired product 1-[(6-chlorobenzo[*d*]thiazol-2-ylamino)-(4-hydroxyphenyl)methyl]-naphthalen-2-ol **4a** at 89% yield (Table 2). The yields obtained with other ionic salts were lower and are tabulated in Table 1. These results encouraged us to expand this system for the preparation of several substituted benzo[*d*]thiazole analogues **4b**–**p** using various substituted benzaldehydes, 2-napthol or 6-hydroxyquinoline in the presence of 2-amino-6-chloro-benzo[*d*]thiazole or 2-amino-6-bromo-benzo[*d*]thiazole or 2-amino-4-methyl-benzo[*d*]thiazole.

The generality of the system was confirmed by using various aryl aldehydes (Table 2) having both electron-withdrawing and donating substituents. In almost all the cases, the reaction was completed within 8–10 min to afford the desired products in efficient yields. All the products were characterized by melting points, FT-IR, NMR (^{1}H & ^{13}C), LC–MS, and elemental analysis. *c*Log*P* of the compounds was calculated using ChemBioDraw Ultra 13.0v and found to be in the range of 5.3652–6.6037. Compound **4b** was taken for single crystal X-ray study to understand the confirmation and packing feature of such analogues.

IR (KBr) spectrum of the title compounds **4a**–**p** had broad Ar–OH and N–H band absorbance at 3418–3465 and 3311–3375 cm^{-1}, respectively. Compounds **4e**, **4i** and **4o** having Ar–C≡N exhibited absorbance at 2247, 2227 and 2245 cm^{-1}, respectively. The ^{1}H NMR spectrum exhibited singlet at 10.53–10.07 ppm, which was assigned to –OH. The mass spectrum showed M^+ peak of all the synthesized compounds. Elemental analysis results are in good agreement with the calculated values of the proposed title compounds **4a**–**p**.

2.2. Single crystal X-ray study

The crystallographic details of **4b** are listed in Table 3. Fig. 1 provides the thermal ellipsoid plot with atom labelling which adopts the conformation with intra-molecular N–H···O (2.22 Å, 120°) and C–H···S (2.55 Å, 115°) hydrogen bonds. The **4b** crystal structure prefers pair-wise head to head dimers with O–H···N



Scheme 1. Synthesis of title compounds **4a**–**p**. Reagents and conditions: (a) 10% w/v NaCl in water, MW.

Table 1
Comparative study on the effect of various ionic salts used for the synthesis of 1-[(6-chlorobenzo[*d*]thiazol-2-ylamino)-(4-hydroxyphenyl)-methyl]-naphthalen-2-ol[a] **4a**.

Entry	Ionic salts[b]	Time reflux (h)/MW (min)	Yield (%)[c]
1	LiCl	4.5/10	85/85
2	NaBr	4.5/10	72/74
3	NaF	4.5/10	65/69
4	$NaNO_3$	4.5/10	85/87
5	Na_2SO_4	4.5/10	80/84
6	$LiNO_3$	4.5/10	82/85
7	Li_2SO_4	4.5/10	83/86
8	NaCl	4.0/10	87/89
9	–	4.5/10	60/62

[a] 2-Amino-6-chloro-benzo[*d*]thiazole (1 mmol), 2-naphthol (1 mmol) and *p*-hydroxy benzaldehyde (1 mmol).
[b] The mixture was taken in 10 mL of 10% w/v salts in water, heated to 90 °C/same reaction condition under microwave irradiation (320 W).
[c] Isolated yield.

(1.87(3) Å, 167°) hydrogen bonds and further, the C–H···π (2.76 Å, 135°; and 2.92 Å, 145°) interactions [32,33] lead to stabilize the three dimensional random assembly as shown along *b*-axis of the unit cell (Fig. 2). It is worthwhile to mention that all the compounds (**4a**–**p**) bear the same major functional groups such as hydroxy (OH), secondary amine (NH) and sulphur groups and all the compounds may prefer similar conformation and packing features as observed in the crystal structure of **4b** with the mentioned hydrogen bonded interactions.

2.3. Pharmacology

The results of repellent activities of the different benzothiazole analogues (**4a**–**p**) are shown in Table 4. All compounds assayed exerted either repellent activity or knocked down mosquitoes and there were significant differences in repellence ($F_{18,19} = 57.90$; $p < 0.001$) and knockdown ($F_{18,19} = 55.95$; $p < 0.001$) between treatments. Compounds **4b**, **4d** and **4p** were (statistically) as repellent as the positive control *N*,*N*-diethyl-*meta*-toluamide (DEET), ranging from 95.0 to 98.3% repellence, whereas compounds **4a** and **4k** appeared to be the least repellent, which could be due to highest knockdown of all treatments (96.7%). When repellence data were corrected to exclude knockdown mosquitoes, all compounds exerted 100% repellence.

Table 2
Physicochemical constants of 2,6-substituted-benzo[*d*]thiazole analogues **4a**–**k** and 2,4-substituted-benzo[*d*]thiazole analogues **4l**–**p**.

Product	X	R^1	R^2	Time (min)	Yield (%)[a,b]	m.p (°C)	*c*Log*P*[c]
4a	CH	4-OH	6-Cl	10	89	140–142	5.9367
4b	CH	H	6-Cl	8	93	195–197	6.6037
4c	CH	4-OCH_3	6-Cl	10	93	157–159	6.5227
4d	CH	4-NO_2	6-Cl	10	89	198–200	6.3467
4e	CH	4-CN	6-Cl	8	91	206–208	6.0367
4f	N	4-OCH_3	6-Cl	8	86	180–182	5.9512
4g	N	OH	6-Cl	9	85	138–140	5.3652
4h	N	4-NO_2	6-Cl	10	87	159–161	5.7752
4i	N	4-CN	6-Cl	8	88	158–160	5.3652
4j	CH	4-NO_2	6-Br	8	90	208–210	6.4967
4k	N	4-OCH_3	6-Br	8	88	176–178	6.1012
4l	CH	H	4-CH_3	8	93	214–216	5.6976
4m	CH	OH	4-CH_3	10	88	160–162	5.7116
4n	N	4-NO_2	4-CH_3	10	93	158–160	5.5501
4o	CH	4-CN	4-CH_3	10	92	183–185	5.8116
4p	CH	4-OCH_3	4-CH_3	10	93	188–190	6.2976

[a] All of the products were characterized by spectral and physical data.
[b] Yields were on isolated basis.
[c] *c*Log*P* was calculated using ChemBioDraw Ultra 13.0v.

Table 3
Crystal data and measurement details for compound **4b**.

Crystal data	Compound **4b**
Formula	$C_{24}H_{17}N_2OClS$
CCDC number	903760
Formula weight	416.91
Crystal morphology	Needle
Crystal size (mm)	0.17 × 0.12 × 0.03
Temperature/K	173 (2)
Radiation	MoKα
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	*P*–1
a (Å)	9.530 (2)
b (Å)	10.122 (2)
c (Å)	11.559 (2)
α (°)	73.405 (3)
β (°)	70.264 (3)
γ (°)	76.030 (2)
Volume ($Å^3$)	992.8 (3)
Z	2
Density (gm/cm^3)	1.39
μ (1/mm)	0.32
F (000)	432
θ (min, max)	2.8, 27.5
Total number of refln	3892
No. unique refln	3892
No. of parameters	263
*R*_obs, *wR*2_obs	0.038, 0.105
$\Delta\rho_{min}$ (e $Å^{-3}$), $\Delta\rho_{max}$ (e $Å^{-3}$)	−0.28, 0.23
GooF	1.144

No adverse reactions to the treatments applied were observed on any of the *Mastomys* rodents during the three days they were monitored.

The effect of the compounds on the adult mosquitoes was evaluated using a repeated measures ANOVA test. This showed that there was a significant effect of the compound ($F_{18,19} = 162.2$; $p < 0.001$), exposure time ($F_{2,38} = 1260.2$; $p < 0.001$), and their interaction ($F_{36,38} = 46.176$; $p < 0.001$) on mosquito knockdown/mortality. The results of the adulticidal assay using the different synthetic compounds are shown in Table 5. The positive control K-Othrine showed 100% knockdown/mortality from the first 30 min of exposure, while the synthetic compounds did not knockdown mosquitoes for the first 30 min, and showed significantly higher knockdown after 24 h than the negative control. The highest mean mortality (70%) was recorded in mosquitoes exposed to **4n** after 24 h. Mortality was moderate to low (<50%) for all other synthetic compounds (Table 5).

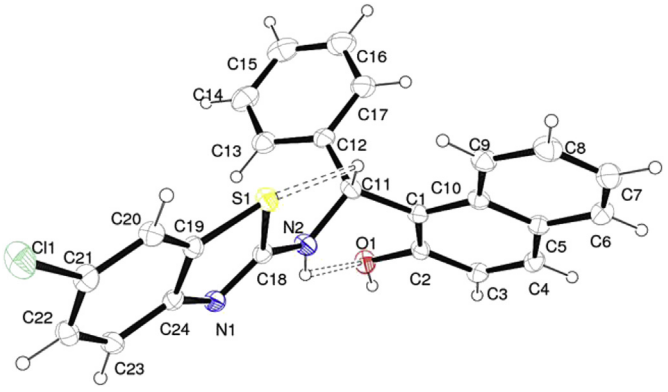


Fig. 1. Thermal ellipsoid of 50% probability plot with atom labelling, which adopts the conformation with N–H···O and C–H···S intra-molecular interactions as shown in dotted lines.

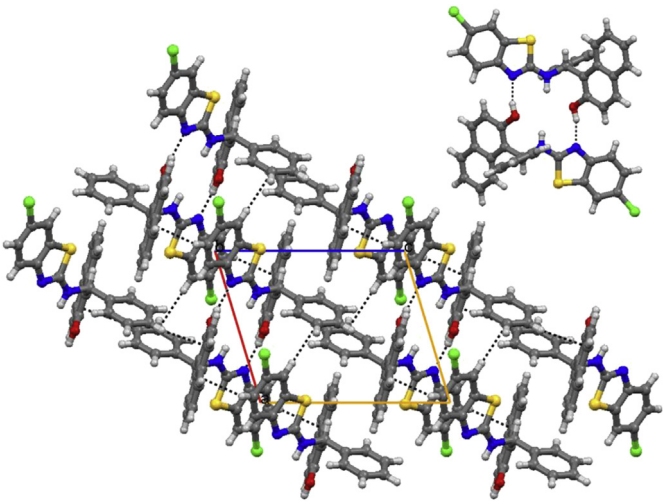


Fig. 2. The molecules form pair-wise head to head dimers with O–H···N hydrogen bonds and further, the C–H···π interactions lead to stabilize the three dimensional random assembly as shown along b-axis of the unit cell.

The results from the *A. arabiensis* larvicidal assay using the different synthetic compounds are shown in Table 6. There was a significant effect of treatment ($F_{18,19} = 178.2$; $p < 0.001$), exposure time ($F_{2,38} = 566.7$; $p < 0.001$), and their interaction ($F_{36,38} = 43.12$; $p < 0.001$) on larval mortality. Larvae treated with Temephos, a commercial insecticide used as positive control, exhibited 100% mortality at 24 h exposure time. Most benzothiazole analogues did not kill larvae until after 48 h. Although larvae exposed to most compounds (except **4a**, **4b**, **4c** and **4l**) at some point showed significantly higher mortality than the negative controls, all values were lower than the positive control. Highest mortality (43%) was recorded for *A. arabiensis* exposed for 72 h to **4n**, followed by compound **4k** and **4h** (28% at 72 h).

Benzothiazole derivatives exhibit a broad spectrum of biological activities, but there are very few reports that investigate their activity against insects. Previous studies by Zhao (2012) showed that benzothiazole showed fumigant toxicity against the stored grain pest insect *Tribolium castaneum* (Coleoptera: Tenebrionidae) [34], and Grace (1988) showed that 2-(thiocyanomethylthio) benzothiazole (TCMTB), a fungicide, exerted repellence and moderate toxicity on termites [35]. This study shows that several of the benzothiazole analogues listed in Table 6 exerted mild to moderate mortality on larvae and adult *A. arabiensis* (at least under the current test conditions); however, other effects on mosquitoes that may affect population growth (such as fertility and development time) were not evaluated.

The *c*Log*P* value of a compound (the logarithm of its partition coefficient between *n*-octanol and water log ($c_{octanol}/c_{water}$)), is a measure of the compound's hydrophilicity, and is one criterion used to assess the potential toxicity or activity of drug candidates [36]. The *c*Log*P* values for all the synthetic compounds assessed were close to 5.0, indicating that the compounds are moderately lipophilic, as expected of compounds with biological activity.

Table 4
Repellent activity against *Anopheles arabiensis* of benzothiazole analogues **4a**–**p**.

Compound code	Repelled	Knocked-down[a]
4a	3.3 ± 4.7^{AB}	96.7 ± 3.3^{A}
4b	95.0 ± 7.1^{CF}	5.0 ± 5.0^{BC}
4c	18.3 ± 2.3^{D}	81.7 ± 1.7^{D}
4d	95.0 ± 2.3^{CF}	5.0 ± 1.7^{B}
4e	76.7 ± 4.7^{E}	23.3 ± 3.3^{E}
4f	11.7 ± 2.3^{D}	88.3 ± 1.7^{AD}
4g	11.7 ± 7.1^{D}	88.3 ± 5.0^{AD}
4h	16.7 ± 4.7^{D}	83.3 ± 3.3^{D}
4i	91.7 ± 2.3^{F}	8.3 ± 1.7^{C}
4j	90.0 ± 4.7^{F}	10.0 ± 3.3^{C}
4k	3.3 ± 4.7^{AB}	96.7 ± 3.3^{A}
4l	90.0 ± 4.7^{F}	10.0 ± 3.3^{C}
4m	43.3 ± 4.7^{D}	56.7 ± 3.3^{D}
4n	8.3 ± 2.3^{AB}	91.7 ± 1.7^{A}
4o	70.0 ± 4.7^{E}	30.0 ± 3.3^{E}
4p	98.3 ± 2.3^{CF}	1.7 ± 1.7^{B}
Acetone	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}
DEET	100.0 ± 0.0^{C}	0.0 ± 0.0^{B}

Values are mean ± SE.
[A–F]Means without a common letter differ significantly ($p < 0.05$).
[a] Knockdown, the rapidly and normally reversible paralysis.

Table 5
Adulticidal activity of benzothiazole analogues **4a**–**p**.

Compound code	Adulticidal activity		
	Knockdown		Mortality
	30 min	60 min	24 h
4a	0.0 ± 0.0^{A}	20.0 ± 3.3^{K}	31.7 ± 1.7^{M}
4b	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	13.3 ± 3.3^{DE}
4c	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	13.3 ± 0.0^{DE}
4d	0.0 ± 0.0^{A}	26.7 ± 3.3^{K}	41.7 ± 5.0^{H}
4e	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	10.0 ± 3.3^{CD}
4f	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	31.7 ± 1.7^{M}
4g	8.3 ± 1.7^{BC}	36.7 ± 3.3^{L}	55.0 ± 1.7^{I}
4h	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	45.0 ± 1.7^{GH}
4i	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	15.0 ± 1.7^{E}
4j	0.0 ± 0.0^{A}	20.0 ± 3.3^{F}	48.3 ± 1.7^{G}
4k	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	21.7 ± 1.7^{F}
4l	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	11.7 ± 1.7^{CDE}
4m	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	23.3 ± 3.3^{FK}
4n	5.0 ± 1.7^{AB}	38.3 ± 5.0^{HL}	70.0 ± 3.3^{J}
4o	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	11.7 ± 1.7^{CDE}
4p	0.0 ± 0.0^{a}	3.3 ± 3.3^{A}	15.0 ± 1.7^{E}
Water	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
Acetone	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
K-Othrine	100.0 ± 0.0^{N}	100.0 ± 0.0^{N}	100.0 ± 0.0^{NI}

Values are mean ± SE.
[A–N]Means of treatment mortality without a common letter differ significantly ($p < 0.05$).

Table 6
Larvicidal activity of benzothiazole analogues **4a**–**p**.

Compound code	Larvicidal activity (h)		
	24	48	72
4a	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
4b	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
4c	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	1.7 ± 1.7^{A}
4d	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	5.0 ± 1.7^{B}
4e	0.0 ± 0.0^{A}	8.3 ± 1.7^{C}	11.7 ± 1.7^{D}
4f	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	15.0 ± 1.7^{E}
4g	1.7 ± 1.7^{A}	26.7 ± 3.3^{GH}	35.0 ± 1.7^{I}
4h	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	28.3 ± 1.7^{H}
4i	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	13.3 ± 3.3^{DE}
4j	0.0 ± 0.0^{A}	20.0 ± 3.3^{F}	25.0 ± 1.7^{G}
4k	0.0 ± 0.0^{A}	16.7 ± 3.3^{EF}	28.3 ± 1.7^{H}
4l	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
4m	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	18.3 ± 1.7^{F}
4n	8.3 ± 1.7^{C}	36.7 ± 3.3^{I}	43.3 ± 3.3^{J}
4o	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	15.0 ± 1.7^{E}
4p	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
Water	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
Acetone	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
Temephos	100.0 ± 0.0^{K}	100.0 ± 0.0^{K}	100.0 ± 0.0^{K}

Values are mean ± SE.
[A–K]Means of treatment mortality without a common letter differ significantly ($p < 0.05$).

Lipophilic components may influence the structure of biological membranes, affect transport and distribution processes, ligand–receptor interactions or modify cellular processes [37]. The *c*Log*P* values found may explain the low larvicidal activity due to the components relatively low solubility in water. On the other hand, the high knockdown detected in adult mosquitoes in close contact with the compounds (as in the repellent assays) suggest a contact toxicity mode of action.

The wide variety of biological effects shown by benzothiazole derivatives suggest a complex mechanism of action, probably acting in different metabolic pathways. For example, the antifilarial activity of benzothiazole derivatives in *Litomosoides carinii* was shown (*in vitro*) to be due to the blocking of the respiratory chain [38]. Other benzothiazole derivatives affect the nervous system and exhibit acetylcholinesterase (AChE) inhibitory potential [39]. The specific mechanisms of action of the compounds assessed in the present study in *Anopheles* are still unknown. Due to the relevance of the present findings, further studies aimed at elucidating the mechanism of action of the most promising repellent compounds would be worthy.

3. Conclusion

In conclusion, we introduced 1-[(6-halo or 4-methyl-benzo[*d*] thiazol-2-ylamino)-phenyl-methyl]-naphthalen-2-ol derivatives and 5-[(6-halo or 4-methyl-benzo[*d*]thiazol-2-ylamino)-phenyl-methyl]-quinolin-6-ol derivatives under microwave conditions with 10% w/v NaCl. The reaction was completed with high yield, within 8–10 min with no liberation of toxic compounds (Green Chemistry) *via* three component condensation reaction of substituted arylaldehyde, 2-amino-6-halo or 4-methyl-benzo[*d*] thiazole and 2-naphthol or 6-hydroxyquinoline in water with sodium chloride. The conformation of **4b** was confirmed by single crystal X-ray method.

Benzothiazole analogues **4b**, **4d** and **4p** showed the highest repellent activity, comparable to the positive control DEET, and analogues **4a** and **4k** had the highest repellency. The death of mosquitoes (adulticidal activity) was low to moderate (11–55%) and was achieved after 24 h exposure, except for **4n** that killed 70% of the mosquitoes. Compound **4n** was also very effective as a repellent as it knocked down 92% females after 2 min of exposure time and repelled the remaining ones. However, these differences in knockdown/mortality effects may be related to differences in analogue concentration upon mosquito exposure, because in repellent assays the compounds were applied to the rodents ventral surface just before the beginning of the assay, while for adulticidal testing tiles were prepared and allowed to dry up to 24 h prior to the assessments. Regarding larvicidal activity, toxicity slightly but significantly increased with exposure time for most analogues except **4a**–**d** that did not show any significant mortality. Results suggest that benzothiazole potential for insecticidal development is low; however, repellent activity should be further assessed.

4. Experimental section

4.1. Methods and materials

All the chemicals were obtained from Aldrich and Merck chemical company and were used without further purification. Reactions were monitored by Thin Layer Chromatography (TLC). TLC was performed on Merck 60 F-254 silica gel plates with ethyl acetate and *n*-hexane (6.5:3.5) as solvent system and visualization with UV-light or iodine chamber. Melting points were determined on a Büchi Melting Point B-545 apparatus. IR and NMR (^{1}H & ^{13}C) spectra were recorded on Shimadzu-IR and Bruker AMX (400 MHz) spectrophotometers, respectively. The IR spectra were taken as KBr pellets. NMR (^{1}H & ^{13}C) spectra was recorded in DMSO solvent. Chemical shifts (δ) were indicated in parts per million downfield from tetramethylsilane and the coupling constants (J) are recorded in Hertz. Splitting pattern is abbreviated as follows; s, singlet; d, doublet and m, multiplet. LC–MS was performed on an Agilent Technologies 1200 series instrument. Elemental analysis was performed on Thermo Finningan FLASH EA 1112 CHN analyzer. *c*Log*P* of the compounds was calculated using ChemBioDraw Ultra 13.0v.

4.2. General procedure for the synthesis of 1-[(6-halo or 4-methyl-benzo[d]thiazol-2-ylamino)-phenyl-methyl]-naphthalen-2-ol **4a**–**k** and 5-[(6-halo or 4-methyl-benzo[d]thiazol-2-ylamino)-phenyl-methyl]-quinolin-6-ol derivatives **4l**–**p**

To a suspension of 2-naphthol or 6-hydroxyquinoline (1 mmol), arylaldehyde (1 mmol) and 2-amino-6-halo or 4-methyl-benzo[*d*] thiazole (1 mmol), 10 mL of 10% w/v sodium chloride in water was added. The reaction mixture was stirred for 2 min in order to prevent aggregation of solid substances and then heated with microwave irradiation using domestic microwave oven [5 cycles of 2 min each, with cooling periods of 20 s, at 320 W and additional 5 mL of water] for 10 min, the reaction time is shown in Table 2. The progress and completion of reaction were monitored by TLC. The reaction vessel was then cooled to room temperature; the precipitate obtained was filtered and washed with cold water. The crude products were purified by recrystallization from acetone or methyl *tert*-butyl ether in 85–93% yields.

4.2.1. 1-((6-Chlorobenzo[d]thiazol-2-ylamino)-(4-hydroxyphenyl)-methyl)-naphthalen-2-ol (**4a**)

Appearance: white powder. IR (KBr): ν cm^{-1} 3423, 3371, 1668, 1564, 1475. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.16–7.35 (m, 13H, 11H$_{arom}$ and *CH*NH), 7.77–7.85 (m, 2H$_{arom}$), 8.92 (s, 1H, OH), 10.16 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 53.09, 118.32, 118.42, 118.94, 120.52, 122.37, 123.67, 124.64, 125.49, 125.98, 126.18, 128.09, 128.53, 129.57, 132.07, 132.52, 142.27, 151.04, 153.17, 166.84. LCMS (m/z): 433 (M^+). Anal. calcd for $C_{24}H_{17}N_2O_2ClS$: C, 66.58; H, 3.96; N, 8.19%. Found: C, 66.51; H, 3.98; N, 8.11%.

4.2.2. 1-[(6-Chloro-benzo[d]thiazol-2-ylamino)-phenyl-methyl]-naphthalen-2-ol (**4b**)

Appearance; white powder. IR (KBr): ν cm^{-1} 3451, 3375, 1596, 1549, 1449. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.57–7.30 (m, 11H, 9H$_{arom}$ and *CH*NH), 7.58–7.70 (m, 5H$_{arom}$), 10.07 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.19, 108.58, 114.88, 115.80, 118.53, 120.50, 121.97, 122.30, 124.44, 125.50, 126.05, 127.23, 129.23, 132.06, 132.54, 134.54, 151.12, 151.71, 153.05, 155.23, 155.78, 163.27, 167.10. LCMS (m/z): 417 (M^+). Anal. calcd for $C_{24}H_{17}N_2OClS$: C, 69.14; H, 4.11; N, 6.72%. Found: C, 69.10; H, 4.21; N, 6.68%.

4.2.3. 1-((6-Chlorobenzo[d]thiazol-2-ylamino)-(4-methoxyphenyl)-methyl)-naphthalen-2-ol (**4c**)

Appearance: white powder. IR (KBr): ν cm^{-1} 3465, 3330, 1625, 1550, 1455, 1340. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.66 (s, 3H, OCH_3), 6.81–7.56 (m, 11H, 9H$_{arom}$ and *CH*NH), 7.76–8.24 (m, 3H$_{arom}$), 10.40 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.68, 55.00, 108.30, 113.68, 118.50, 118.99, 120.58, 120.96, 121.70, 124.69, 125.56, 127.26, 130.50, 131.91, 132.42, 133.54, 134.10, 143.66, 146.62, 147.10, 151.07, 153.32, 155.44, 157.87, 166.65. LCMS (m/z): 447 (M^+). Anal. calcd for $C_{25}H_{19}N_2O_2ClS$: C, 67.18; H, 4.28; N, 6.27%. Found: C, 67.13; H, 4.35; N, 6.23%.

*4.2.4. 1-((6-Chlorobenzo[d]thiazol-2-ylamino)-(4-nitrophenyl)-methyl)-naphthalen-2-ol (**4d**)*

Appearance: yellow powder. IR (KBr): ν cm^{-1} 3428, 3361, 1586, 1542, 1340. ^1H NMR (400 MHz, DMSO-d_6): δ 7.20–7.48 (m, 9H, 7H_{arom} and *CH*NH), 7.80–7.84 (m, 6H_{arom}), 10.28 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.97, 117.48, 118.27, 119.20, 120.65, 122.60, 123.39, 124.96, 125.57, 126.68, 127.15, 128.69, 130.20, 130.60, 131.89, 132.64, 145.96, 150.88, 153.28, 166.70. LCMS (*m*/*z*): 462 (M^+). Anal. calcd for $C_{24}H_{16}N_3O_3ClS$: C, 62.40; H, 3.49; N, 9.10%. Found: C, 62.31; H, 3.55; N, 9.07%.

*4.2.5. 4-((6-Chlorobenzo[d]thiazol-2-ylamino)-(2-hydroxynaphtha len-1-yl)-methyl)-benzonitrile (**4e**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3440, 3375, 2247, 1647, 1552, 1450, ^1H NMR (400 MHz, DMSO-d_6): δ 7.17–7.41 (m, 9H, 7H_{arom} and *CH*NH), 7.72–7.83 (m, 6H_{arom}), 10.26 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.99, 108.90, 117.55, 118.26, 118.53, 119.18, 120.49, 120.62, 122.57, 124.91, 125.56, 126.34, 126.62, 126.94, 128.19, 128.67, 130.10, 131.91, 132.09, 132.64, 133.13, 148.62, 150.85, 153.28, 166.71. LCMS (*m*/*z*): 442 (M^+). Anal. calcd for $C_{25}H_{16}N_3OClS$: C, 67.94; H, 3.65; N, 9.51%. Found: C, 67.81; H, 3.64; N, 9.43%.

*4.2.6. 5-((6-Chlorobenzo[d]thiazol-2-ylamino)-(4-methoxyphenyl)-methyl)-quinolin-6-ol (**4f**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3453, 3368, 1603, 1525, 1448, 1321. ^1H NMR (400 MHz, DMSO-d_6): δ 3.68 (s, 3H, OCH_3), 6.84 (d, J = 8.56 Hz, 1H_{arom}), 7.13–7.21 (m, 4H, 2H_{arom} and *CH*NH), 7.28–7.39 (m, 2H_{arom}), 7.46–7.58 (m, 1H_{arom}), 7.76–7.89 (m, 2H_{arom}), 8.25 (s, 1H_{arom}), 8.63 (s, 1H_{arom}), 8.94 (s, 1H_{arom}), 10.43 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.65, 54.97, 108.27, 113.65, 118.47, 118.96, 120.55, 120.92, 121.34, 121.67, 124.66, 125.53, 127.23, 130.48, 132.40, 133.52, 134.06, 143.63, 146.58, 151.04, 153.31, 155.41, 157.83, 166.62. LCMS (*m*/*z*): 448 (M^+). Anal. calcd for $C_{24}H_{18}N_3O_2ClS$: C, 64.35; H, 4.05; N, 7.91%. Found: C, 64.18; H, 4.68; N, 7.42%.

*4.2.7. 5-((6-Chlorobenzo[d]thiazol-2-ylamino)-(4-methoxyphenyl)-methyl)-quinolin-6-ol (**4g**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3456, 3320, 1675, 1548, 1452. ^1H NMR (400 MHz, DMSO-d_6): δ 7.20–7.49 (m, 9H, 7H_{arom} and *CH*NH), 7.73–7.94 (m, 5H_{arom}), 9.02 (s, 1H, OH), 10.53 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.80, 108.27, 109.12, 117.52, 119.24, 120.67, 121.34, 121.90, 125.00, 125.60, 127.00, 129.83, 131.12, 132.20, 133.14, 134.06, 143.52, 147.07, 148.06, 150.80, 153.46, 155.40, 166.55. LCMS (*m*/*z*): 434 (M^+). Anal. calcd for $C_{23}H_{16}N_3O_2ClS$: C, 63.66; H, 3.72; N, 9.68%. Found: C, 63.58; H, 3.81; N, 9.62%.

*4.2.8. 5-((6-Chlorobenzo[d]thiazol-2-ylamino)-(4-nitrophenyl)-methyl)-quinolin-6-ol (**4h**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3451, 3371, 1596, 1549, 1446, 1345. ^1H NMR (400 MHz, DMSO-d_6): δ 7.19–7.63 (m, 9H, 7H_{arom} and *CH*NH), 7.83–8.17 (m, 5H_{arom}) 10.37 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.75, 108.27, 117.49, 119.29, 120.49, 120.69, 121.61, 123.48, 125.04, 125.62, 127.22, 130.60, 131.22, 132.61, 143.55, 146.09, 146.90, 147.07, 150.26, 150.78, 153.36, 166.54. LCMS (*m*/*z*): 463 (M^+). Anal. calcd for $C_{23}H_{15}N_4O_3ClS$: C, 59.68; H, 3.27; N, 12.10%. Found: C, 59.61; H, 3.32; N, 12.02%.

*4.2.9. 4-((6-Chlorobenzo[d]thiazol-2-ylamino)-(6-hydroxyquinolin-5-yl)-methyl)-benzonitrile (**4i**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3439, 3359, 2227, 1549, 1446. ^1H NMR (400 MHz, DMSO-d_6): δ 6.92.7.36 (m, 9H, 7H_{arom} and *CH*NH), 7.75–8.36 (m, 5H_{arom}), 10.49 (d, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.73, 108.28, 115.03, 118.53, 120.49, 121.34, 121.93, 124.46, 125.50, 127.27, 128.39, 129.26, 130.32, 132.54, 134.10, 142.97, 147.05, 151.06, 151.69, 155.41, 163.27, 167.11. LCMS (*m*/*z*): 443 (M^+). Anal. calcd for $C_{24}H_{15}N_4OClS$: C, 65.08; H, 3.41; N, 12.65%. Found: C, 65.02; H, 3.48; N, 12.69%.

*4.2.10. 1-((6-Bromobenzo[d]thiazol-2-ylamino)-(4-nitrophenyl)-methyl)-naphthalen-2-ol (**4j**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3441, 3375, 1605, 1555, 1452, 1375. ^1H NMR (400 MHz, DMSO-d_6): δ 7.24–7.48 (m, 9H, 7H_{arom} and *CH*NH), 7.61–7.94 (m, 6H_{arom}), 10.30 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.97, 112.57, 117.48, 118.29, 119.73, 122.59, 123.19, 123.39, 126.51, 126.68, 127.15, 128.30, 128.69, 130.19, 133.19, 145.96, 150.88, 151.16, 153.30, 166.68. LCMS (*m*/*z*): 508 (M^+). Anal. calcd for $C_{24}H_{16}N_3O_3BrS$: C, 56.93; H, 3.18; N, 8.30%. Found: C, 56.88; H, 3.26; N, 8.21%.

*4.2.11. 5-((6-Bromobenzo[d]thiazol-2-ylamino)-(4-methoxyphenyl)-methyl)-quinolin-6-ol (**4k**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3417, 3365, 1578, 1562, 1447, 1326. ^1H NMR (400 MHz, DMSO-d_6): δ 3.68 (s, 3H, OCH_3), 6.83 (d, J = 12.52, 2H_{arom}), 7.13–7.49 (m, 5H, 3H_{arom} and *CH*NH), 7.84–7.90 (m, 3H_{arom}), 8.12 (d, J = 8.15 Hz, 1H_{arom}), 8.63 (s, 2H_{arom}), 8.96 (s, 1H_{arom}), 10.09 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.65, 54.96, 108.27, 112.02, 113.65, 118.45, 119.05, 121.34, 121.91, 123.23, 127.24, 128.24, 129.26, 130.34, 133.06, 134.06, 143.00, 146.59, 147.06, 151.35, 152.02, 155.41, 157.84, 167.10. LCMS (*m*/*z*): 492 (M^+). Anal. calcd for $C_{24}H_{18}N_3O_2BrS$: C, 58.54; H, 3.68; N, 8.53%. Found: C, 58.49; H, 3.72; N, 8.48%.

*4.2.12. 1-((4-Methylbenzo[d]thiazol-2-ylamino)-(phenyl)-methyl)-naphthalen-2-ol (**4l**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3453, 3370, 1585, 1545, 1435. ^1H NMR (400 MHz, DMSO-d_6): δ 2.42 (s, 3H, CH_3), 6.84–7.81 (m, 14H, 12H_{arom} and *CH*NH), 8.04 (s, 1H_{arom}) 8.85 (s, 1H_{arom}), 10.17 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 52.97, 117.48, 118.26, 119.20, 120.64, 120.81, 122.61, 123.19, 123.39, 124.96, 125.57, 126.69, 127.14, 128.51, 128.69, 130.20, 131.88, 132.64, 145.96, 150.78, 150.87, 153.26, 166.69. LCMS (*m*/*z*): 397 (M^+). Anal. calcd for $C_{25}H_{20}N_2OS$: C, 75.73; H, 5.08; N, 7.07%. Found: C, 75.67; H, 5.12; N, 6.98%.

*4.2.13. 1-((4-Hydroxyphenyl)-((4-methylbenzo[d]thiazol-2-ylamino)-methyl)-naphthalen-2-ol) (**4m**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3438, 3346, 1595, 1549, 1268. ^1H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, CH_3), 6.64–8.78 (m, 16H, 14H_{arom} and *CH*NH), 10.13 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 53.15, 108.58, 114.83, 118.23, 118.52, 118.56, 120.64, 120.68, 122.27, 122.57, 125.93, 126.05, 126.09, 127.02, 127.37, 127.48, 128.40, 129.16, 129.23, 129.93, 131.84, 150.88, 153.33, 155.83, 165.49. LCMS (*m*/*z*): 413 (M^+). Anal. calcd for $C_{25}H_{20}N_2O_2S$: C, 72.79; H, 4.89; N, 6.79%. Found: C, 72.67; H, 4.95; N, 6.68%.

*4.2.14. 5-((4-Methylbenzo[d]thiazol-2-ylamino)-(4-nitrophenyl)-methyl)-quinolin-6-ol (**4n**)*

Appearance: yellow powder. IR (KBr): ν cm^{-1} 3440, 3373, 1704, 1593, 1549, 1339. ^1H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 6.77–7.54 (m, 8H, 6H_{arom} and *CH*NH), 7.91–8.68 (m, 5H_{arom}), 8.98 (s, 1H_{arom}), 10.48 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 17.98, 52.80, 108.27, 118.34, 120.64, 121.11, 121.37, 121.68, 123.34, 126.14, 127.35, 130.59, 130.96, 134.06, 143.40, 146.07, 146.85, 150.19, 150.64, 153.50, 165.02. LCMS (*m*/*z*): 443 (M^+). Anal. calcd for $C_{24}H_{18}N_4O_3S$: C, 65.14; H, 4.10; N, 12.66%. Found: C, 65.03; H, 4.21; N, 12.59%.

*4.2.15. 4-((2-Hydroxynaphthalen-1-yl)-(4-methylbenzo[d]thiazol-2-ylamino)-methyl)-benzonitrile (**4o**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3418, 3365, 2245, 1652, 1548, 1438. ^1H NMR (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3), 6.79–7.42 (m, 13H, 11H_{arom} and *CH*NH), 7.70–7.75 (m, 2H_{arom}), 10.10 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 17.96, 52.95, 113.38, 118.17, 118.42, 118.91, 120.67, 122.23, 123.73, 125.98, 126.03, 127.01, 127.28, 128.36, 128.46, 129.20, 129.90, 132.15, 133.66, 150.79, 153.26, 157.70, 165.40. LCMS (*m*/*z*): 422 (M^+). Anal. calcd for $C_{26}H_{19}N_3OS$: C, 74.08; H, 4.54; N, 9.97%. Found: C, 74.16; H, 4.61; N, 9.88%.

*4.2.16. 1-((4-Methoxyphenyl)-(4-methylbenzo[d]thiazol-2-ylamino)-methyl)-naphthalen-2-ol (**4p**)*

Appearance: white powder. IR (KBr): ν cm^{-1} 3430, 3311, 1623, 1579, 1549, 1250. ^1H NMR (400 MHz, DMSO-d_6): δ 2.40 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 6.82–6.90 (m, 3H, 1H_{arom} and *CH*NH), 7.00 (d, J = 7.32 Hz, 1H_{arom}), 7.18–7.26 (m, 5H_{arom}), 7.35–7.46 (m, 2H, H_{arom}), 7.74–8.83 (m, 4H_{arom}), 10.15 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6): δ 18.02, 53.04, 54.96, 113.45, 118.24, 118.51, 118.97, 120.73, 122.29, 126.10, 127.07, 127.35, 128.43, 129.27, 129.97, 132.22, 133.73, 150.86, 153.38, 157.76, 165.47. LCMS (*m*/*z*): 427 (M^+). Anal. calcd for $C_{26}H_{22}N_2O_2S$: C, 73.21; H, 5.20; N, 6.57%. Found: C, 73.16; H, 5.29; N, 6.51%.

4.3. Single crystal X-ray crystallographic study

Single crystals of **4b** were grown from solvent acetonitrile *via* slow evaporation method at room temperature. A particular size of single crystal **4b** (Table 3) was taken for X-ray study on a Nonius Kappa-CCD diffractometer using graphite monochromated MoKα radiation (λ = 0.71073 Å). Data collection was carried out at 173 (2) K temperature using liquid Nitrogen (N_2) cryo-system attached with Oxford Cryostat. The strategy for the data collections was evaluated using the Bruker Nonius "Collect" program. Data were scaled and reduced using DENZO-SMN software [40,41]. The crystal structure solution was worked out by full matrix least-squares method using SHELXL97 [42] and absorption correction performed using SADABS [43]. All the non-hydrogen atoms were located in difference Fourier maps, the hydrogen atoms were fixed geometrically and refined isotropically. Graphical presentations were drawn using Ortep-3 and Mercury [44,45].

4.4. Pharmacology

4.4.1. Repellence assays

4.4.1.1. Animal preparation. Repellent activity of the synthesized compounds was assessed by topical application on the ventral surface of test rodents and subsequent exposure of the treated area to unfed female mosquitoes. The percentage of repellency was determined as the number of bites relative to the untreated negative control. The standard WHO guidelines were adapted for use in this trial [46]. The rodent *Mastomys coucha* was used for the screening of the test compounds **4a**–**p**. Ethical approval for the use of live animals in this study was obtained from the Ethics Committee of the South African Medical Research Council. Acetone was used as a solvent for the preparation of stock solution at 1 mg/mL. Laboratory grade DEET was used as the positive control and plane acetone was used as negative control. Adult rodents were weighed individually and injected intraperitoneally with a 1 mL solution of sodium pentobarbital (60 mg/L) per 0.225 kg of body weight. Once anaesthetized, rodents were shaved on the ventral surface and 1 mL of test sample solution was applied to their abdomens.

4.4.1.2. Probing activity assay. Paper cups (500 mL) were modified by replacing the base of the cup with mosquito netting held in place with a rubber band and covering the mouth of the cup with transparent plastic film. 30 unfed four day old *A. arabiensis* females were introduced into the cup and held in contact with the treated ventral surface of each anaesthetized rodent. Mosquito activity was observed through the transparent plastic film. At the end of a 2 min exposure period the number of mosquitoes probing (attempting to feed on the anaesthetized mouse, through the netting) was recorded. The rodent was then returned to the animal facility, allowed to recover from the anaesthetic and monitored for 3 days for adverse reactions to the applied components.

4.4.2. Adulticidal assays

1 mL of test sample solution was sprayed onto a clean, dry, non-porous ceramic tile using a pre-calibrated Potter's Tower apparatus [47]. This instrument allowed for even application of precise amounts of test solutions to the tiles, following which they were air dried. Assays were initiated within 24 h of spraying. The assay was conducted in accordance with WHO protocol [48] in which a standard bioassay cone was fixed over the sprayed tile and thirty, nonblood-fed, 2–5 day-old susceptible adult mosquitoes (*A. arabiensis*) were introduced into the cone. All bioassays were duplicated to ensure validity of results. The effect of the test samples **4a**–**p** was measured by determining the knockdown rate, constituting temporary paralysis of the mosquitoes during the sixty-minute exposure period, and post-exposure mortality within 24 h. In order to establish whether any test samples warranted further investigation, stringent WHO criteria [48] was adapted for the screening of test samples. Therefore only potentially active compounds which resulted in mortality greater than 60% were considered as potential insecticide candidates for further research and development.

4.4.3. Larvicidal assays

1 mL volume of the test samples concentration was added to a vessel containing thirty-third instar mosquito larvae in 250 mL of distilled water, producing a final concentration of 40 mg/mL. The target species was a colonized strain of *A. arabiensis* from Zimbabwe which had been reared according to the WHO guidelines [48] in an insectary simulating the temperature (27.5 °C), humidity (70%) and lighting (12/12) of a malaria endemic environment. The negative control trials included acetone and distilled water, whilst Temephos (Mostop; Agrivo), an effective emulsifiable concentrate larvicidal was used as a positive control. Each container was monitored for larval mortality at 24 h intervals for a period of three days and fed (specially made cat food with reduced oil/fat content) at regular intervals. The percentage mortality was calculated and compared to the negative control.

4.5. Statistical analysis

One-way analysis of variance (ANOVA) followed by least significant difference (LSD) Fisher test was used to compare the mean repellence time for the plant extracts and controls on adult *A. arabiensis* mosquitoes. Results from the adult knockdown and mortality data were subjected to repeated measures ANOVA that examined the main effects of treatment (benzothiazole analogues and controls), time after application of treatment (30 min, 60 min and 24 h; the repeated measure) and their interaction. Larvicidal data were also analyzed with repeated measures ANOVA that examined the main effects of treatment (benzothiazole analogues and controls), time after application of treatment (24, 48 and 72 h; the repeated measure) and their interaction. LSD Fisher test was used for post hoc analyses. Before ANOVA testing, data were transformed to ranks [49] to fit better the assumptions of the test.

In all cases, a value of $p < 0.05$ was considered statistically significant.

Competing interests

The authors declare that they have no competing interests.

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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.2013.04.061.

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