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# Antimosquito Properties of 2-Substituted Phenyl/benzylamino-6-(4-chlorophenyl)-5-methoxycarbonyl-4-methyl-3,6-dihydropyrimidin-1-ium Chlorides Against *Anopheles arabiensis*

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**Abstract:** Eight novel dihydropyrimidine analogs named DHPM1-DHPM8 was synthesized in their hydrochloride salt form using one pot synthesis between methyl 2-chloro-4-(4-chlorophenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate and substituted arylamines in isopropanol. The antimosquito effect of the test compounds were assessed against the adult mosquito *Anopheles arabiensis.* For adulticidal properties the test compounds were sprayed onto ceramic tiles and screened using the cone bio-assay method. The larvicidal activity was tested by monitoring larval mortality daily and up to 3 days of exposure. Repellency properties were tested in a feeding-probe assay using unfed female *Anopheles arabiensis.* Compounds DHPM1, DHPM4, DHPM5 and DHPM6 exerted larval mortality equivalent to temephos (trade name Abate, a commercial larvicidal compound). Compounds DHPM1 to DHPM5 repelled or knocked down 92 to 98% of mosquitoes exposed to rodent skin treated with the compounds. None of the compounds showed any significant activity against the adult mosquito *Anopheles arabiensis*.

**Keywords:** Adulticidal, *Anopheles arabiensis,* antimosquito, dihydropyrimidines, larvicidal, and repellency.

## INTRODUCTION

Malaria is a widespread disease transmitted by mosquitoes and currently represents a great health problem in tropical and subtropical climates, with no part of the world immune to this risk [1]. Globally, more than two billion people live in areas threatened by malaria. The morbidity and mortality associated with the disease are commonly higher in poorly developed areas mainly in sub-Saharan Africa [2]. Annually, an estimated 1.5-2.7 million deaths are documented, 90% of them in tropical Africa. The highest burden is in children under five years of age [3].

**Malaria** is caused by protists of the genus *Plasmodium* (Apicomplexa) that have complex life cycles involving a vertebrate host, namely reptiles, birds, rodents, monkeys and humans. The main vectors are *Anopheles gambiae, Anopheles albimanus, Anopheles freeborni, Anopheles maculatus* and *Anopheles stephensi*. It begins with a bite from an infected female mosquito, which introduces the protists via its saliva into the circulatory system and ultimately to the liver where they mature and reproduce. Strategies for preventing and controlling malaria involve three different approaches. The first is to reduce human-mosquito contact. This has been achieved by impregnated bed nets, repellents, protective clothing and screens and house spraying. Another approach is to interrupt the parasite transmission by controlling or eliminating the vector population, either through environ mental modifications, chemical control (larvacides/insecticides) or biological control. The last strategy is to reduce the parasite reservoir via case detection and treatment and chemoprophylaxis [4]. The control of malaria in tropical Africa has been particularly problematic because of the high transmission rates, the overall low socio-economic level and the resistance of the vectors to current chemotherapeutics [5]. Resistance development is also an issue with insecticides [6], and thus there is an urgent need to revise vector control strategies and to develop alternative products. Chemicals derived from natural sources such as plants, considered ecologically sensitive pesticides [7], have exhibited detrimental effects on mosquitoes [8, 9]. However, even though natural products are deemed more environmentally benign than synthetic compounds, this is not always the case as biological activity of a chemical is a function of its structure rather than its origin [10].

The pyrimidine system is an important pharmacophore with ample occurrence in nature. Natural and synthetic pyrimidine derivatives have a wide range of actions including antibiotics, barbituric, inhibitors of protein synthesis in bacteria, archaea and eucarya [11]. Dihydropyrimidine (DHPM) pharmacophores possess a wide spectrum of biological activities such as anticancer by altering the mitotic spindle and arresting mitosis [12], antiviral activity [13], antihypertensive [14] related to calcium channel blocking [15], antitubercular [16], antimicrobial [17], antiinflammatory [18] and larvicidal actions [19]. Antimalarial drugs include halogenated dihydropyrimidine analogs [20] and the commercially available pyrimethamine, a folic acid antago-

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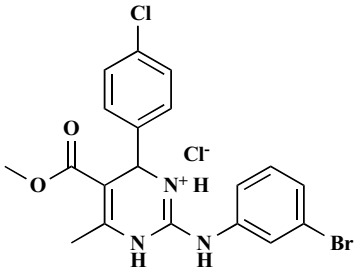
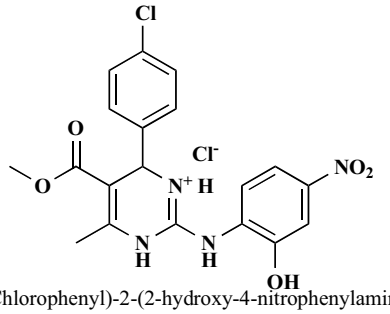
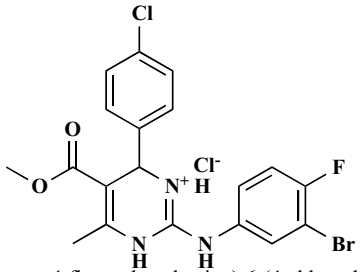


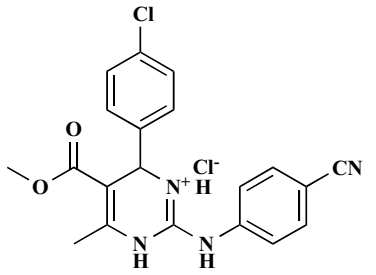
2-(3-Bromophenylamino)-6-(4-chlorophenyl)-5
-(methoxycarbonyl)-4-methyl-
3,6-dihydropyrimidin-1-ium chloride (**DHPM1**)



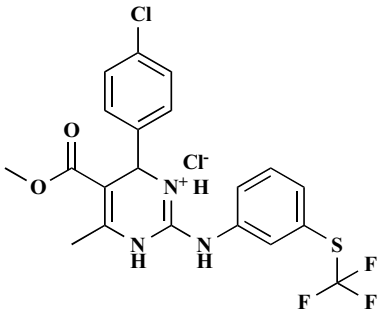
6-(4-Chlorophenyl)-2-(2-hydroxy-4-nitrophenylamino)-
5-(methoxycarbonyl)-4-methyl-
3,6-dihydropyrimidin-1-ium chloride (**DHPM2**)



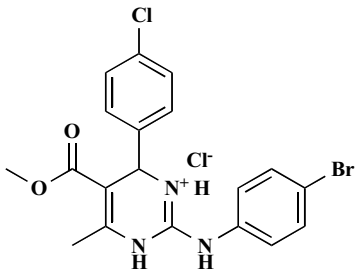
2-(3-Bromo-4-fluorophenylamino)-6-(4-chlorophenyl)-
5-(methoxycarbonyl)-4-methyl-
3,6-dihydropyrimidin-1-ium chloride (**DHPM3**)



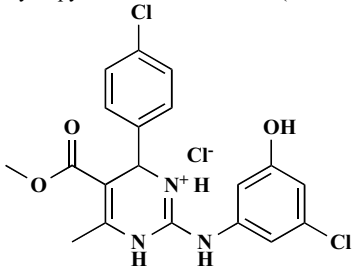
6-(4-Chlorophenyl)-2-(4-cyanophenylamino)-
5-(methoxycarbonyl)-4-methyl-
3,6-dihydropyrimidin-1-ium chloride (**DHPM4**)



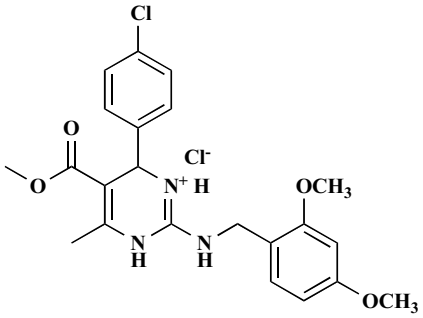
6-(4-Chlorophenyl)-5-(methoxycarbonyl)-4-methyl-
2-(3-(trifluoromethylthio)phenylamino)-
3,6-dihydropyrimidin-1-ium chloride (**DHPM5**)



2-(4-Bromophenylamino)-6-(4-chlorophenyl)-5-
(methoxycarbonyl)-4-methyl-
3,6-dihydropyrimidin-1-ium chloride (**DHPM6**)



2-(3-Chloro-5-hydroxyphenylamino)-6-(4-chlorophenyl)-
5-(methoxycarbonyl)-4-methyl-
3,6-dihydropyrimidin-1-ium chloride (**DHPM7**)



6-(4-Chlorophenyl)-2-(2,4-dimethoxybenzylamino)-5-
(methoxycarbonyl)
-4-methyl-3,6-dihydropyrimidin-1-ium chloride (**DHPM8**)

**Fig. (1).** Chemical structure and IUPAC names of the test compounds.

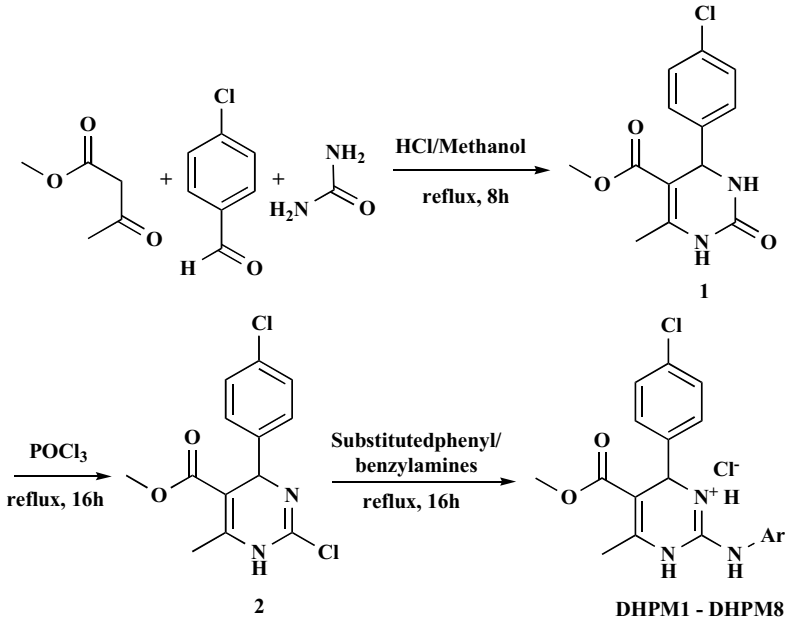
nist used for treatment and prevention of malaria or with a sulfonamide to treat toxoplasmosis.

In continuation of our research work on the synthesis of bioactive molecules [21, 22], and due to the encouragement from the wide spectrum of pyrimidine derivatives, and specially DHPM for various pharmacological properties, we designed and synthesized 3,6-dihydropyrimidine analogs (Fig. **1**), according to Lipinski´s rule [23] for promising drug discovery and development, that vary at different nucleophilic and electrophilic substitutions on arylamine group.

## MATERIALS AND METHODS

### Chemistry

The synthetic strategy employed to produce **DHPM1**-**DHPM8** are illustrated in (Scheme **1**).




**Scheme 1.** Synthetic routes to 3,6-dihydropyrimidine analogs DHPM1-DHPM8.

All the chemicals were obtained from Aldrich and Merck chemical company. 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 (6:4) as solvent system and visualization with UV-light. Melting points were determined on a Büchi Melting Point B-545 apparatus. The IR spectra were recorded on a Nicolet 6700 FT-IR spectrometry. $^{1}H$ and $^{13}C$ NMR spectra were recorded on Bruker AVANCE III 400 MHz instruments in DMSO as a solvent. Chemical shifts ($\delta$) 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; m, multiplet. Mass spectra were recorded using LC-MS-Agilent 1100 series with MSD (Ion trap) using 0.1% aqueous trifluoroacetic acid in acetonitrile system on C18-BDS column. Elemental analysis was performed on Thermo Finningan FLASH EA 1112 CHN analyzer.

### Synthesis of methyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1)

A solution of methyl acetoacetate (0.12 mol), 4-chlorobenzaldehyde (0.1 mol) and urea (0.1 mol) was refluxed in the presence of concentrated hydrochloric acid (0.05 mol) for 8 h in 5 mL of EtOH (Scheme **1**). Completion of reaction was monitored on thin layer chromatography. The reaction mixture was then cooled to room temperature and the pure precipitate was collected by filtration. The solid obtained was washed with cold EtOH, dried and recrystalized using EtOH solvent. Yield 66%, mp 205-206 °C.

### Synthesis of methyl 2-chloro-4-(4-chlorophenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate (2)

A solution of compound **1** (1 mol) in $POCl_3$ (5 mol) was refluxed for 16 h (Scheme **1**). The reaction was monitored on TLC. Unreacted $POCl_3$ was evaporated completely and the remaining residue was taken in ethyl acetate and washed with 10% sodium bicarbonate solution followed by water and finally brine solution. The ethyl acetate layer was dried over sodium sulphate and evaporated to obtain a solid which was recrystallized using EtOH solvent. Yield 72%, mp 218-219 °C.

### General Procedure for the Synthesis of DHPM1-DHPM8

A solution of compound **2** (1 mmol) and substituted phenyl/benzylamines (1 mmol) in isopropanol (10 mL) was refluxed for 16 h (Scheme **1**). The reaction completion was monitored by TLC. The reaction medium was cooled to room temperature; the product obtained was filtered, washed with cold isopropanol and dried to get the pure product. The product obtained was purified by column chromatography using ethyl acetate and *n*-hexane (6:4) as eluent (60-120 silica gel). Compounds **DHPM1-DHPM8** were achieved as hydrochloride salts. The physicochemical constants of the title compounds are tabulated in (Table **1**).

## PHARMACOLOGY

### Antimosquito Activity

#### *Larvicidal Assays*

The *Anopheles arabiensis* used were from a colonized strain from Zimbabwe which had been reared according to the WHO [24] guidelines in an insectary simulating the temperature (27.5 °C), humidity (70%) and lighting (12/12) of a malaria endemic environment. One mL of test compound (1 mg/mL) was added to 250 mL of distilled water producing a final concentration of 4 µg/mL. Thirty 3$^{rd}$ instar larvae were placed in the container. A negative control was set up using a solvent (acetone) and distilled water and a positive control included Temephos (Mostop; Agrivo; Fig. **2**), an effective emulsifiable concentrate larvicidal (an organo-phosphate) used by the malarial control program as a larvicidal. Each container was monitored for larval mortality at 24 h intervals for a period of three days and fed (specially made cat food

**Table 1. Physicochemical Characteristics of 2-substituted phenyl/benzylamino-6-(4-chlorophenyl)-5-methoxycarbonyl-4-methyl-3,6-dihydropyrimidin-1-ium chlorides DHPM1-DHPM8 [16]**

| Comp Code | Ar | M. F (Mol. Wt.) | Yield[a] (%) | m.p. (°C) |
|---|---|---|---|---|
| **DHPM1** | 3-Br-$C_6H_4$ | $C_{19}H_{18}BrCl_2N_3O_2$ (471.2) | 65 | 217-218 |
| **DHPM2** | 2-OH,4-$NO_2$-$C_6H_3$ | $C_{19}H_{18}Cl_2N_4O_5$ (453.3) | 67 | 172-173 |
| **DHPM3** | 3-Br,4-F-$C_6H_3$ | $C_{19}H_{17}BrCl_2FN_3O_2$ (489.2) | 71 | 236-237 |
| **DHPM4** | 4-CN-$C_6H_4$ | $C_{20}H_{18}Cl_2N_4O_2$ (417.3) | 63 | 116-117 |
| **DHPM5** | 3-SCF3-$C_6H_4$ | $C_{20}H_{18}Cl_2F_3N_3O_2S$ (492.3) | 69 | 220-221 |
| **DHPM6** | 4-Br-$C_6H_4$ | $C_{19}H_{18}BrCl_2N_3O_2$ (471.2) | 66 | 227-228 |
| **DHPM7** | 3-Cl,5-OH-$C_6H_3$ | $C_{19}H_{18}Cl_3N_3O_3$ (442.7) | 67 | 198-199 |
| **DHPM8** | 2,4-$OCH_3$-benzyl | $C_{21}H_{23}Cl_2N_3O_4$ (452.3) | 71 | 221-222 |

[a] All the yields are on isolated basis.

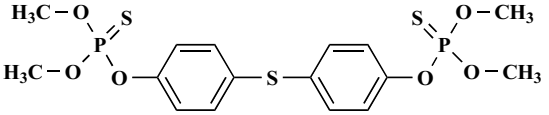


**Fig. (2).** Chemical structure of Temephos (IUPAC: *O,O,O',O'*-tetramethyl *O,O'*-(thiobis(4,1-phenylene)) bis(phosphorothioate).

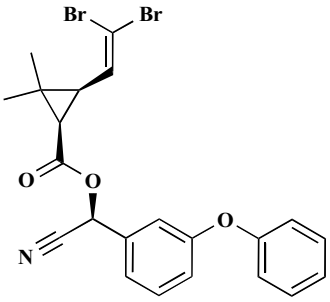


**Fig. (3).** Chemical structure of K-Othrine® (IUPAC: (*S*)-Cyano (3-phenoxyphenyl)methyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylate).

with reduced oil/fat content) at regular intervals. The percentage mortality was calculated relative to the negative control.

### *Insecticidal Assay*

Insecticidal activity assessment was conducted in accordance with WHO protocol [24]. One mL of test compound solution (1 mg/mL) was sprayed onto a clean, dry, non-porous ceramic tile using a pre-calibrated Potter's Tower apparatus [25]. The tiles were then air dried and the assay was initiated within 24 h of spraying. A cone was fixed over the sprayed tile and thirty nonblood-fed, 2-5 day-old susceptible adult *A.arabiensis* mosquitoes were introduced into the cone. The effect of the test compounds was measured by determining the knockdown rate, which was based on temporary paralysis of the mosquitoes during a 60 min exposure period, and mortality 24 h post-exposure. Deltamethrin (15 g/L; K-Othrine®, Fig. **3**) was used as a positive control and the negative controls were acetone and distilled water negative controls. All bioassays were duplicated to ensure validity of results. The WHO criteria [26] was adapted in which only the compounds that induced mortality greater than 75% were considered as potential insecticide candidates for further research and development.

### *Repellence Assays*

#### *Animal Preparation*

The standard WHO guidelines were adapted to test the synthetic compounds **DHPM1-DHPM8** [26]. The rodent *Mastomys coucha* (12 weeks old males weighing 65g) was used for the screening of the test compounds. Ethical approval for the use of live animals in this study was obtained from the Ethics Committee of the South African Medical Research Council. Animals were put into groups of four and each test substance was tested on two animals, whilst the remaining two animals were used as negative and positive controls respectively. 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 (Fig. **4**), and acetone was used as negative control. Adult rodents were weighed individually and injected intraperitonially 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 the abdomens of each of two rodents. The percentage repellency was taken as the mean of the number of bites relative to the untreated negative control.

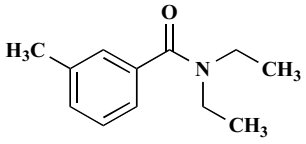


**Fig. (4).** Chemical structure of DEET (IUPAC: *N,N*-Diethyl-3-methylbenzamide).

### *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* fe-

males 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 anesthetic and monitored for 3 days for adverse reactions to the applied components.

### *Statistical Analysis*

Larvicidal mortality was subjected to a repeated measures analysis of variance (ANOVA) that examined the main effects of treatment (compounds and controls), time after application of treatment (1, 2 and 3 days; the repeated measure) and their interaction. Adult knockdown and mortality data were also subjected to repeated measures ANOVA that examined the main effects of treatment (compounds and controls), time after application of treatment (30 min, 60 min and 24 h; the repeated measure) and their interaction. One-way analysis of variance followed by least significant difference (LSD). Fisher test was used to compare the mean repellence, knockdown, and combination of repellence plus knockdown for the synthetic compounds and controls on adult *A. arabiensis* mosquitoes. LSD Fisher test was used for post hoc analyses. Before ANOVA testing, data were transformed to ranks [27] to fit better the assumptions of the test. In all cases, a value of $p < 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

### Chemistry

The synthesis of 3,6-dihydropyrimidine analogs **DHPM1-DHPM8** was achieved as illustrated in (Scheme **1**). Starting from the first intermediate methyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **1** which has been synthesized by Biginelli reaction by employing Lewis acid according to the procedure described in our previous paper [28]. The purification of the product was accomplished using ETOH by recrystallization method and the yield of the product was 66%. Synthesis of the second intermediate, methyl 2-chloro-4-(4-chlorophenyl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate **2** was achieved by treating compound **1** with phosphoryl chloride for 16 h to yield 72% of compound **2** after purification by recrystallization using EtOH as solvent. Title compounds **DHPM1-DHPM8** were achieved as hydrochloride salt by refluxing equimolar proportion of compound **2** and mono/disubstituted phenyl/benzylamines in isopropanol medium. The yield of the compounds was found to be in satisfactory yield at 63-71% after purification by column chromatography. The structures of novel 3,6-dihydro-pyrimidine analogs **DHPM1-DHPM8** were characterized by IR, NMR ($^1$H and $^{13}$C), and elemental analysis [16].

**2-(3-Bromophenylamino)-6-(4-chlorophenyl)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM1)**

Appearance: white solid; yield 65%. mp 217-218 °C. IR (KBr) νcm$^{-1}$ 3199, 3068, 1707, 1671, 1582, 1475, 787, 676. $^1$H-NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.41 (s, 3H), 3.62 (s, 3H), 5.43 (s, 1H), 7.19-7.51 (m, 8H), 10.20 (s, 1H), 11.14 (s, 2H). $^{13}$C-NMR (400 MHz, DMSO-d$_6$) $\delta$ 17.53, 51.68, 103.31, 122.17, 123.19, 126.87, 128.46, 128.93, 129.70, 131.67, 132.98, 136.24, 140.00, 144.90, 148.71, 164.61. Anal. calcd for $C_{19}H_{18}BrCl_2N_3O_2$ (471.2): C, 48.43; H, 3.85; N, 8.92%; found C, 48.39; H, 3.84; N, 8.93%.

**6-(4-Chlorophenyl)-2-(2-hydroxy-4-nitrophenylamino)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM2)**

Appearance: yellow solid; yield 67%. mp 172-173 °C. IR (KBr) νcm$^{-1}$ 3356, 3193, 3067, 1707, 1674, 1588, 1492, 769. $^1$H-NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.40 (s, 3H), 3.61 (s, 3H), 5.39 (s, 1H), 7.17 (d, $J$ = 9.00 Hz, 1H), 7.34 (d, $J$ = 8.48 Hz, 2H), 7.45 (d, $J$ = 8.44 Hz, 2H), 8.10-8.15 (m, 2H), 9.75 (s, 1H), 10.21 (s, 1H), 11.01 (s, 1H), 11.93 (s, 1H). $^{13}$C-NMR (400 MHz, DMSO-d$_6$) $\delta$ 19.87, 50.77, 52.68, 99.77, 116.06, 116.92, 120.21, 128.20, 128.42, 129.57, 131.99, 138.23, 143.60, 148.79, 151.05, 157.11, 163.69, 165.94. Anal. calcd for $C_{19}H_{18}Cl_2N_4O_5$ (453.3): C, 50.35; H, 4.00; N, 12.36%; found: C, 50.36; H, 4.04; N, 12.35%.

**2-(3-Bromo-4-fluorophenylamino)-6-(4-chlorophenyl)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM3)**

Appearance: white solid; yield 71%. mp 236-236 °C. IR (KBr) cm$^{-1}$ 3191, 3032, 1718, 1676, 1583, 1487, 769, 699. $^1$H-NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.41 (s, 3H), 3.59 (s, 3H), ν5.35 (s, 1H), 7.32-7.77 (m, 7H), 9.63 (s, 1H), 10.68 (s, 1H), 11.25 (s,1H). $^{13}$C-NMR (400 MHz, DMSO-d$_6$) $\delta$ 17.57, 51.56, 51.97, 102.85, 116.22, 116.44, 120.50, 120.76, 122.59, 122.69, 128.70, 128.79, 129.38, 131.35, 131.44, 132.88, 140.16, 144.58, 149.52, 160.10, 162.58, 164.60. Anal. calcd for $C_{19}H_{17}BrCl_2FN_3O_2$ (489.2): C, 46.65; H, 3.50; N, 8.59%; found C, 46.65; H, 3.51; N, 8.58%.

**6-(4-Chlorophenyl)-2-(4-cyanophenylamino)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM4)**

Appearance: yellow solid; yield 63%. mp 116-117 °C. IR (KBr) νcm$^{-1}$ 3194, 3063, 2226, 1673, 1562, 1489, 770. $^1$H-NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.38 (s, 3H), 3.60 (s, 3H), 5.41 (s, 1H), 7.35 (t, $J$ = 8.62 Hz, 4H), 7.44 (d, $J$ = 8.48 Hz, 2H), 7.80 (d, $J$ = 8.60 Hz, 2H), 9.88 (s, 1H), 10.11 (s, 1H), 10.58 (s, 1H). $^{13}$C-NMR (400 MHz, DMSO-d$_6$) $\delta$ 18.22, 51.37, 52.13, 102.30, 106.66, 118.81, 122.48, 128.10, 128.37, 128.80, 132.60, 133.70, 141.23, 147.73, 165.05. Anal. calcd for $C_{20}H_{18}Cl_2N_4O_2$ (417.3): C, 57.57; H, 4.35; N, 13.43%; found C, 57.58; H, 4.35; N, 13.44%.

**6-(4-Chlorophenyl)-5-(methoxycarbonyl)-4-methyl-2-(3-(trifluoromethylthio) phenylamino)-3,6-dihydropyrimidin-1-ium chloride (DHPM5)**

Appearance: pale yellow solid; yield 69%. mp 220-221 °C. IR (KBr) νcm$^{-1}$ 3191, 3043, 1714, 1671, 1585, 1472, 1088, 766. $^1$H-NMR (400 MHz, DMSO-d$_6$) $\delta$ 2.42 (s, 3H), 3.62 (s, 3H), 5.44 (s, 1H), 7.36 (d, $J$ = 8.48 Hz, 2H), 7.42-7.63 (m, 6H), 10.29 (s, 1H), 11.09 (s, 2H). $^{13}$C-NMR (400

MHz, DMSO-$d_6$) $\delta$ 17.56, 25.44, 51.61, 51.69, 103.32, 124.31, 124.33, 126.67, 127.85, 128.43, 128.91, 130.80, 131.28, 132.97, 133.78, 136.35, 140.03, 148.62, 164.65. Anal. calcd for $C_{20}H_{18}Cl_2F_3N_3O_2S$ (492.3): C, 48.79; H, 3.69; N, 8.53%; found C, 48.80; H, 3.68; N, 8.54%.

**2-(4-Bromophenylamino)-6-(4-chlorophenyl)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM6)**

Appearance: pale yellow solid; yield 66%. mp 227-228 °C. IR (KBr) $\nu cm^{-1}$ 3184, 3042, 1707, 1671, 1571, 1488, 771, 688. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.41 (s, 3H), 3.61 (s, 3H), 5.40 (s, 1H), 7.16 (d, $J$ = 8.64 Hz, 2H), 7.34 (d, $J$ = 8.48 Hz, 2H), 7.47 (d, $J$ = 8.44 Hz, 2H), 7.64 (d, $J$ = 8.64 Hz, 2H), 9.93 (s, 1H), 10.76 (s, 1H), 11.13 (s, 1H). $^{13}$C-NMR (400 MHz, DMSO-$d_6$) $\delta$ 19.49, 50.84, 52.39, 99.86, 114.98, 123.88, 128.25, 128.54, 131.88, 131.99, 140.90, 143.41, 148.00, 150.94, 165.80. Anal. calcd for $C_{19}H_{18}BrCl_2N_3O_2$ (471.2): C, 48.43; H, 3.85; N, 8.92%; found C, 48.45; H, 3.86; N, 8.94%.

**2-(3-Chloro-5-hydroxyphenylamino)-6-(4-chlorophenyl)-5-(methoxycarbonyl) -4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM7)**

Appearance: white solid; yield 67%. mp 198-199 °C. IR (KBr) $\nu cm^{-1}$ 3646, 3198, 3062, 1719, 1677, 1581, 1481, 787. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.40 (s, 3H), 3.58 (s, 3H), 5.32 (s, 1H), 6.77-7.32 (m, 5H), 7.43 (d, $J$ = 8.48 Hz, 2H), 9.41 (s, 1H), 10.30 (s, 2H), 10.80 (s, 1H). $^{13}$C-NMR (400 MHz, DMSO-$d_6$) $\delta$ 17.68, 51.46, 52.03, 102.35, 115.15, 116.31, 118.94, 128.63, 128.67, 130.67, 132.72, 140.60, 145.33, 148.79, 157.38, 164.78. Anal. calcd for $C_{19}H_{18}Cl_3N_3O_3$ (442.7): C, 51.55; H, 4.10; N, 9.49%; found C, 51.57; H, 4.08; N, 9.50%.

**6-(4-Chlorophenyl)-2-(2,4-dimethoxybenzylamino)-5-(methoxycarbonyl)-4-methyl-3,6-dihydropyrimidin-1-ium chloride (DHPM8)**

Appearance: pale yellow solid; yield 71%. mp 221-222 °C. IR (KBr) $\nu cm^{-1}$ 3222, 3029, 1714, 1680, 1585, 1468, 762. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.37 (s, 3H), 3.60 (s, 3H), 3.74 (s, 6H), 4.30 (d, $J$ = 14.72 Hz, 1H), 4.45 (d, $J$ = 14.32 Hz, 1H), 5.43 (s, 1H), 6.41 (d, $J$ = 7.68 Hz, 1H), 6.54 (s, 1H), 7.17 (d, $J$ = 8.36 Hz, 1H), 7.30 (d, $J$ = 8.48 Hz, 2H), 7.41 (d, $J$ = 8.40 Hz, 2H), 8.50 (s, 1H), 10.26 (s, 1H), 11.11 (s, 1H). $^{13}$C-NMR (400 MHz, DMSO-$d_6$) $\delta$ 17.69, 51.29, 51.46, 55.23, 55.42, 98.47, 104.28, 115.86, 128.42, 128.70, 129.60, 132.71, 140.53, 149.67, 158.01, 160.64, 164.78. Anal. calcd for $C_{22}H_{25}Cl_2N_3O_4$ (466.3): C, 56.66; H, 5.40; N, 9.01%; found C, 56.68; H, 5.36; N, 9.07%.

## PHARMACOLOGY

### Larvicidal Activity

There was a significant effect of treatment on larval mortality ($F_{10,20}$ = 142.81; p <0.001), significant differences between treatment times ($F_{2,40}$ = 16.95; p <0.01) but no significant interaction between treatment and exposure times ($F_{20,40}$ = 1.82; p >0.05). The highest activity was noted with **DHPM6** showing 100% mortality after 24 h of exposure, which was the same as for the positive control temephos, followed by **DHPM1**, **DHPM4** and **DHPM5**. Compounds **DHPM1 and DHPM6** have an electron withdrawing bromine group respectively at *meta* and *para* position of the phenyl ring which is connected to dihydropyrimidine through secondary amine bride, which may be responsible for its toxic effect. Even though post hoc analysis showed that mortality of larvae exposed to **DHPM4** and **DHPM5** increased with time, and were as high as **DHPM6** after 2 and 3 days, respectively (Table **2**), the difference found among

**Table 2. Mortality of *Anopheles arabiensis* Larvae Exposed to Chemically Synthesized Compounds DHPM1-DHPM8 at 4 µg/mL and their Negative Controls (Water and Acetone)**

| Comp. Code | Assessment Time (h) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| **DHPM1** | 95.56±1.1 $^{A}$ | 100.0±0.0 $^{B}$ | 100.0±0.0 $^{B}$ |
| **DHPM2** | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **DHPM3** | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **DHPM4** | 95.0±1.0 $^{A}$ | 98.7±1.3 $^{AB}$ | 98.7±1.3 $^{AB}$ |
| **DHPM5** | 96.0±2.0 $^{A}$ | 96.7±3.3 $^{A}$ | 100.0±0.0 $^{B}$ |
| **DHPM6** | 100.0±0.0 $^{C}$ | 100.0±0.0 $^{C}$ | 100.0±0.0 $^{B}$ |
| **DHPM7** | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **DHPM8** | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **Water** | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **Acetone** | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **Temephos**$^{C}$ | 100.0±0.0 $^{B}$ | 100.0±0.0 $^{B}$ | 100.0±0.0 $^{B}$ |

$^{A-C}$ Assessment times and treatments not sharing a capital letter differ significantly (p<0.05).

**Table 3. Insecticidal Effects (Knockdown and Mortality) against *Anopheles arabiensis* Exposed to Synthetic Compounds DHPM1-DHPM8 at 1 mg/mL, positive control (K-Othrine) and Negative Controls (Water and Acetone)**

| Comp. Code | Knockdown | | Mortality |
|---|---|---|---|
| | 30 min | 60 min | 24 h |
| **DHPM1** | 20.7±2.9 $^{A}$ | 43.0±15.0 $^{B}$ | 77.3±7.2 $^{C}$ |
| **DHPM2** | 2.0±2.0 $^{D}$ | 2.0±2.0 $^{D}$ | 7.7±3.9 $^{DE}$ |
| **DHPM3** | 1.0±1.0 $^{D}$ | 2.0±0.0 $^{D}$ | 13.3±3.3 $^{AE}$ |
| **DHPM4** | 1.0±1.0 $^{D}$ | 1.0±1.0 $^{D}$ | 10.7±2.3 $^{E}$ |
| **DHPM5** | 2.0±1.0 $^{D}$ | 3.0±1.7 $^{D}$ | 7.3±1.3 $^{DE}$ |
| **DHPM6** | 25.0±14.3 $^{A}$ | 56.3±11.9 $^{B}$ | 75.3±4.7 $^{C}$ |
| **DHPM7** | 0.0±0.0 $^{D}$ | 0.0±0.0 $^{D}$ | 19.5±3.5 $^{AE}$ |
| **DHPM8** | 1.0±1.0 $^{D}$ | 1.0±1.0 $^{D}$ | 18.0±6.2 $^{AE}$ |
| **Water** | 0.0±0.0 $^{D}$ | 0.0±0.0 $^{D}$ | 0.0±0.0 $^{AE}$ |
| **Acetone** | 0.0±0.0 $^{D}$ | 0.0±0.0 $^{D}$ | 0.0±0.0 $^{DE}$ |
| **K-Othrine** | 100.0±0.0 $^{F}$ | 100.0±0.0 $^{F}$ | 100.0±0.0 $^{F}$ |

$^{A-F}$ Treatments and pos-treatment times not sharing a capital letter differ significantly ($p<0.05$).

**Table 4. Percentage Repellence and Knockdown of *Anopheles arabiensis* after 2 min Exposure to Chemically Synthesized Compounds DHPM1-DHPM8 at 1 mg/mL**

| Comp. Code | Repelled* | Knockdown* | Corrected Repellence | Repelled or Knocked |
|---|---|---|---|---|
| **DHPM1** | 61.0±30.5 | 33.3±33.3 | 91.7±1.7 $^{AB}$ | 94.4±2.9 $^{AB}$ |
| **DHPM2** | 59.7±30.1 | 33.3±33.3 | 90.0±6.7 $^{B}$ | 93.3±5.1 $^{AB}$ |
| **DHPM3** | 58.7±29.5 | 38.4±31.0 | 96.7±3.3 $^{A}$ | 92.2±4.8 $^{AB}$ |
| **DHPM4** | 64.3±32.0 | 35.3±32.4 | 100.0±0.0 $^{A}$ | 97.8±2.2 $^{AB}$ |
| **DHPM5** | 61.0±30.9 | 33.3±33.3 | 91.7±8.3 A$^{B}$ | 94.4±5.5 $^{AB}$ |
| **DHPM6** | 54.3±27.2 | 33.3±33.3 | 81.7±1.7 $^{B}$ | 87.8±6.2 $^{B}$ |
| **DHPM7** | 18.00±2.0 | 0.00±0.00 | 18.0±2.0 $^{BC}$ | 18.3±1.7 $^{C}$ |
| **DHPM8** | 54.3±27.2 | 33.3±33.3 | 81.7±1.7 $^{B}$ | 87.8±6.2 $^{B}$ |
| **Water** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **Acetone** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 $^{C}$ | 0.0±0.0 $^{C}$ |
| **DEET** | 100.0±0.0 | 0.0±0.0 | 100.0±0.0 $^{A}$ | 100.0±0.0 $^{A}$ |

$^{A-C}$ Within same column, treatments not sharing a capital letter differ significantly ($p<0.05$).
* No significant differences ($p<0.05$) between treatments.

these groups (less than 5%) suggest that these compounds have the same lethal effect. All other compounds did not significantly differ from negative controls.

## INSECTICIDAL ACTIVITY

A repeated measures ANOVA for adult *A. arabiensis* knockdown and/or mortality showed significant effects of treatment ($F_{10,21} = 66.27$; $p < 0.0001$), time after application of treatment ($F_{2,42} = 72.81$; $p < 0.0001$) and their interaction ($F_{20,42} = 4.59$; $p < 0.001$). Knock down and mortality of **DHPM1** and **DHPM6** treatments were significantly higher compared to negative controls from the first 30 min after exposure. Mortality of mosquitoes exposed to **DHPM7** and **DHPM8** were significantly higher than **DHPM3** and **DHPM4** after 24 h. However, mortality due to K-othrine (1 mg/mL) was significantly higher than any of the other products assayed (Table **3**). For all other treatments, although there were increased mortalities after 24 h, they were not significantly different than the water or acetone treated controls.

## REPELLENT ACTIVITY

Most components assessed were either highly repellent or knocked down mosquitoes at 1 mg/mL. ANOVA did not show significant effects of component on repellence ($F_{10,19}$ = 1.66; p = 0.16), or knockdown ($F_{10,19}$ = 0.46; p = 0.90) when tested independently. When repellence data were corrected to exclude knocked down individuals, significant effects of compound were observed ($F_{10,13}$ = 6.56; p < 0.01). Components **DHPM1, DHPM4** and **DHPM5** were as repellent as DEET. Significant differences were also detected when knockdown and repellent effects were combined ($F_{10,19}$ = 5.99; p <0.001) (Table **4**). Most components exerted a moderate to high knockdown/repellent activity, except **DHPM7** that showed 18% repellence. No adverse reactions to the applied components were observed on any of the *Mastomys* rodents during the days they were monitored.

Although there are several reports of a variety of pharmacological properties of various substituted dihydropyrimidines [12,14,17,18,29,30], little has been published about their insecticidal and mosquito repellent properties. Recently, a group of dihydropyrimidine derivatives proved toxic against the mosquito *Culex quinquefasciatus* larvae [31]. The most toxic compound against *C. quinquefasciatus* larvae had a bromine group similar to compounds **DHPM1** and **DHPM6** that proved toxic against *A. arabiensis* (Table **2**). The results presented in (Tables **2** to **4**) indicate a potential for a group of halogenated dihydropirimidine analogs not only for larvicidal but also for repellent properties against the malaria vector *A. arabiensis*. In fact, all components assayed showed some level of activity against this mosquito, except **DHPM7** that was only mildly repellent, probably related to the hydroxy group at *meta* position on phenyl ring involved in the intramolecular hydrogen bonding in the molecule. Components **DHPM1, DHPM4**, **DHPM5**, and **DHPM6** were the most bioactive because they were both larvicidal and repellent. Based on mortality values, these compounds were as toxic as temephos, a product widely used for mosquito control, and merit to be further evaluated for their larvicidal potential. Regarding repellency, compounds **DHPM1**, **DHPM4**, and **DHPM5** were as effective as DEET. Considering that no adverse reactions to the applied components were observed on any of the *Mastomys* rodents during the 3 days they were monitored, potential of these products as repellent sources should be further evaluated.

## CONCLUSION

Herein we describe the rational design, synthesis and characterization of 3,6-dihydropyrimidine analogs **DHPM1-DHPM8**, which have been evaluated for their *in vitro* larvicidal, adulticidal and repellent properties against the malaria vector *A. arabiensis*. The test compounds **DHPM1**, **DHPM4**, **DHPM5** and **DHPM6** were considered as larvicide candidates for further research and development because they exerted 100% mortality after 3 days of exposure or less. Compounds **DHPM1** to **DHPM5** were as repellent as the positive standard control DEET during the exposure time and should be further evaluated for their repellent potential. Adulticidal activity on the other hand was considered only mild to moderate.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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## ABBREVIATIONS

DHPM = Dihydropyrimidine

EtOH = Ethanol

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