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RESEARCH ARTICLE

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Larvicidal study of tetrahydropyrimidine scaffolds against *Anopheles arabiensis* and structural insight by single crystal X-ray studies

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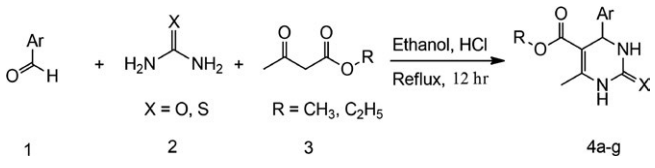
A series of methyl or ethyl 4-(substitutedphenyl/pyridyl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (HPM) analogues **4a–g** were synthesized and evaluated for larvicidal activity against *Anopheles arabiensis*. These newly synthesized compounds were characterized by spectral studies such as FT-IR, NMR (^{1}H and ^{13}C), LC-MS, and elemental analysis. The conformational features and supramolecular assembly of molecules **4a**, **4b**, and **4e** were further analyzed from single crystal X-ray study. The larvicidal activity of these tetrahydropyrimidine pharmacophore series was analyzed based on their relative substituents. Among the synthesized HPM analogous from the series, compounds **4d** and **4e** both having electron withdrawing chlorine group on phenyl ring at the fourth position of the tetrahydropyrimidine pharmacophore exhibited the most promising larvicidal activity.

KEYWORDS
characterization, larvicidal, malaria, single crystal X-ray study, synthesis, tetrahydropyrimidine analogues

1 | INTRODUCTION

The hydropyrimidine (HPM) compound is attractive to researchers in various fields because of its versatile nature, showing interesting pharmacological effects. HPM molecules are generally synthesized by multicomponent reaction, which was discovered by Italian chemist Pietro Biginelli in 1893, known as Biginelli reaction, which is a simple combination of equal molar ratio of alkyl/aryl aldehyde, β-keto ester, and urea or thiourea in the presence of acid as a catalyst in ethanol medium.[1] Owing to hetero atom present on HPM scaffolds, these multicomponent products have shown various pharmacological properties such as antimicrobial,[2] topoisomerase inhibitors,[3] antimalarial,[4–6] antitubercular,[7,8] antimosquito,[9,10] cardiovascular treatment as they act as potent calcium channel blockers,[11,12] anti-inflammatory,[13] and pancreatic neuroendocrine neoplasm therapy.[14] Copper complexes of HPM molecules are reported for the activity of human breast cancer cells.[15] Further, the guanidine-based HPM is also a natural product in marine life which control metabolite process,[16] besides these HPM derivatives play an active role as an anticancer agent.[17,18]

In spite of their small size, several mosquito species (Diptera: Culicidae) play a major role in the transmission of viral and parasitic diseases.[a,19] More than 3,400 different species of mosquito have been recorded worldwide, several of them linked to the emergence and spread of vector-borne diseases.[b,20] Among the vector-borne diseases, malaria is responsible for the largest burden in terms of fatality rate and is transmitted by only 70–80 species, all of them from genus *Anopheles*.[21] According to the global malaria report, 212 million new cases of malaria worldwide were reported in 2015, which includes 90% cases from African Region, 7% from South-East Asia Region and 2% from the Eastern Mediterranean Region (2%). It was also estimated that there were 429,000 deaths from this malaise, which is mainly counted with the 92% from WHO African Region, followed by 6% from the WHO South-East Asia Region and 2% from the WHO Eastern Mediterranean Region.[c] The use of insecticides (both to control larvae or adult mosquitoes) has been a major control method, especially for anopheline mosquito populations. However, repeated use of larvicides and insecticides causes negative effects on nontarget species and the environment, and led to insecticide resistance.[22,23] Thus, there is a growing need for alternative bioactive products within an integrated pest management strategy.



SCHEME 1 Synthetic scheme for the synthesis of hydropyrimidine analogous **4a–g**

Anopheles arabiensis belongs to the *Anopheles gambiae* species complex and is one of the most important vectors of malaria. HPM is a versatile pharmacophore that has exhibited larvicidal activity against *Anopheles arabiensis*,[9,10] *Aedesaegypti*, and *Culex quinquefasciatus*.[24] In view of such versatile pharmacological properties of HPM molecules and in continuation of our effort in search of novel heterocyclic larvicidal agents,[25–27] a new series of methyl/ethyl 4-(substitutedphenyl/pyridyl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives **4a–g** have been designed in Scheme 1. In this series of compound, the presence of hydrogen bond donors like hydroxy (–OH), hydrogen bond acceptor (pyridine –N) and halogen atom (chlorine) is explored to evaluate the potential of larvicidal activity of HPM molecules. Further single crystal structure of three molecules from this series is analyzed to understand the importance of intermolecular interaction in their molecular assembly to correlate with their larvicidal activity. Several synthetic strategies have been followed to obtain efficient yield of HPM molecules, mainly one-pot synthesis using a different catalyst such as acidic condition,[28] polyethylene glycol assisted,[29] different Lewis acid catalyst,[30] and ionic liquid under solvent-free condition.[31] However, we have followed the catalytic acidic condition for the synthesis and analysis of their physicochemical details of seven new HPM molecules **4a–g** listed in Table 1 (Scheme 1).

2 | METHODS AND MATERIALS

All the chemicals used for the synthesis were purchased from Sigma-Aldrich/Alfa Aser and were used without advance purification. FT-IR spectra were measured on Shimadzu FT-IR spectrophotometer. The ^{1}H (400 MHz) and ^{13}C NMR (101 MHz) spectra were examined using a Bruker spectrometer in the DMSO solvents, using the TMS as an internal standard. Chemical shifts were reported in ppm δ scale, coupling constant (*J*) values are given in hertz (Hz). TLC analysis of reaction mixtures was performed on Merck aluminum plates coated with silica gel (60 F_{254}). Compounds were visualized by ultraviolet irradiation (Bio Technico India) at 254 and 366 nm. Single crystal data were collected on the Bruker D8 VENTURE diffractometer equipped with CMOS type PHOTON 100 detector using monochromated Mo Kα radiation[32] (λ = 0.71073 Å) and data were refine using SHELXL2014/7.[33]

2.1 | General procedure for synthesis of methyl/ethyl 4-(substitutedphenyl/pyridyl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate analogues 4a–g

A mixture of substituted arylaldehyde/hetero arylaldehyde (0.0125 mol), urea/thiourea (0.0125 mol), and β-ketoester (0.0125 mol), as shown in Scheme 1 was taken in a 100 ml round bottom flask and refluxed overnight (12 hr) with 30 ml of ethanol solvent with continuous stirring in the presence

TABLE 1 Physicochemical constants of the synthesized methyl/ethyl 4-(substitutedphenyl/pyridyl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate analogues **4a–g**

Comp. code	Mol formulae (mol weight)	R	Ar	X	m.p. (°C)	Yield[a,b] (%)	*c*Log*P*[c]
4a	$C_{13}H_{14}N_2O_4$ (262)	CH_3	4-OH, C_6H_4	O	139	80.55	1.4288
4b	$C_{14}H_{16}N_2O_5$ (292)	CH_3	3-OCH_3, 4-OH, C_6H_3	O	252	70.35	1.2780
4c	$C_{14}H_{16}N_2O_4S$ (308)	CH_3	3-OCH_3, 4-OH, C_6H_3	S	219	65.99	1.4076
4d	$C_{13}H_{13}ClN_2O_3$ (280)	CH_3	2-Cl, C_6H_4	O	225	85.00	2.8088
4e	$C_{13}H_{13}ClN_2O_2S$ (296)	CH_3	2-Cl, C_6H_4	S	165	90.20	2.9384
4f	$C_{12}H_{13}N_3O_3$ (247)	CH_3	Pyridyl	O	220	63.02	0.5988
4g	$C_{13}H_{15}N_3O_3$ (261)	C_2H_5	Pyridyl	O	210	62.25	1.1278

[a]All of the products were characterized by spectral and physical data. [b]Yields after purification by column chromatography/recrystallization method. [c]*c*Log*P* was calculated using ChemBioDraw Ultra 13.0v.

of concentrated hydrochloric acid as a catalyst. The reaction condition was monitored on thin layer chromatography plate, after completion of the reaction was confirmed, the contents of the reaction medium were poured into ice-cold water. The solution was taken for work-up by adding saline water, and the precipitate was observed. The precipitate obtained was filtered and washed with distilled water, dried, and recrystallized the product using methanol or ethanol solvent to get pure compound. Some of the compounds are purified using column chromatography using silica gel (silica gel 100–200 mesh pore size) and the combination of solvents ethylacetate:*n*-hexane (3:2). Further, it was attempted to crystallize in various solvents using solvent evaporation method.

2.1.1 | Methyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate monohydrate (4a)

Appearance: White solid; IR (KBR) *ν*/cm 3,591, 3,271, 3,234, 2,966, 1,703, 1,681, 1,639, 1,089. ^{1}H NMR (400 MHz, DMSO) δ = 9.33, 9.13 (N–H, s, 2H), 7.62 (C_6H_4-OH, s, 1H) 7.04–7.02 (–C_6H_4, d, *J* = 8.54 Hz, 2H), 6.70–6.67 (–C_6H_4, d, *J* = 8.54 Hz, 2H), 5.05–5.04 (C–H, d, 3.3 Hz, 1H), 3.53 (–OCH_3, s, 3H), 2.24 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 166.02, 156.68, 152.06, 148.05, 135.14, 127.29, 115.01, 99.43, 55.92, 53.23, 50.69, 18.51, 17.67.

2.1.2 | Methyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b)

Appearance: Yellow solid; IR (KBR) *ν*/cm 3,390, 3,253, 3,122, 2,954, 2,840, 1,716, 1,666, 1,639, 1,517, 1,236, 1,091, 1,035, 775. ^{1}H NMR (400 MHz, DMSO) δ = 9.13, 8.90 (N–H, s, 2H), 7.60 (C_6H_3–OH, s, 1H), 6.82–6.81 (–C_6H_3, d, *J* = 2 Hz, 1H), 6.71–6.69 (–C_6H_3, d, *J* = 8.1 Hz, 1H), 6.61–6.59 (–C_6H_3, d, *J* = 2.01 Hz, 1H), 5.06 (sp3 C–H, d, *J* = 3.3 Hz, 1H), 3.73 (–$COOCH_3$, s, 3H), 3.55 (–OCH_3, s, 3H), 2.25 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 166.2, 152.72, 148.95, 147.68, 146.40, 136.38, 118.47, 115.68, 111.30, 99.64, 55.74, 53.87, 51.09, 18.40.

2.1.3 | Methyl 4-(4-hydroxy-3-methoxyphenyl) -6-methyl-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (4c)

Appearance: Yellow solid; IR (KBR) *ν*/cm 3,471, 3,332, 3,174, 2,997, 1,677, 1,569, 1,515, 1,276, 1,193, 1,031, 761. ^{1}H NMR (400 MHz, DMSO) δ = 10.27, 9.56 (–N–H, s, 2H), 9.00 (–OH, s, 1H), 6.81, 6.73, 6.71 (C_6H_3, 3H), 5.10–5.09 (–CH, d, *J* = 3.68, 1H), 3.73 (–OCH_3, s, 3H), 3.57 (–$COOCH_3$, s, 3H), 2.28 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 174.40, 166.16, 148.01, 146.68, 145.33, 134.60, 118.76, 115.81, 111.36, 101.32, 56.13, 54.09, 51.40, 17.60.

2.1.4 | Methyl 4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4d)

Appearance: Yellow solid; IR (KBR) *ν*/cm 3,365, 3,226, 3,095, 2,952, 1,704, 1,643, 1,568, 1,425, 1,218, 1,085, 956, 756. ^{1}H NMR (400 MHz, DMSO) δ = 9.30, 7.70 (N–H, s, 2H), 7.42, 7.40, 7.31, 7.28 (C_6H_4, d, 2H, m, 2H, *J* = 6.9 Hz), 5.63–5.62 (–CH, d, *J* = 2.69, 1H), 3.46 (–$COOCH_3$, s, 3H), 2.31 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 165.97, 151.79, 149.94, 142.00, 132.12, 129.93, 129.58, 129.12, 128.28, 98.12, 51.95, 51.14, 18.24.

2.1.5 | Methyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4e)

Appearance: Yellow solid; IR (KBR) *ν*/cm 3,393, 3,222, 3,174, 3,097, 2,991, 1,695, 1,566, 1,467, 1,344, 1,031, 1,031, 769. ^{1}H NMR (400 MHz, DMSO) δ = 10.4, 9.59 (–N–H, s, 2H), 7.44–7.42 (C_6H_4, d, *J* = 7.84, 1H), 7.3–7.28 (C_6H_4, m, *J* = 7.84, 3H), 5.64–5.63 (–CH, d, *J* = 2.69, 1H), 3.49 (–OCH_3, s, 3H), 2.33 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 174.28, 165.71, 146.37, 140.98, 132.20, 130.04, 129.69, 128.40, 100.06, 59.78, 51.91, 51.20, 17.47.

2.1.6 | Methyl 6-methyl-2-oxo-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4f)

Appearance: Brown solid; IR (KBR) *ν*/cm 3,400, 3,220, 3,087, 2,952, 2,906, 1,728, 1,633, 1,596, 1,421, 1,232, 1,091,

775. ^{1}H NMR (400 MHz, DMSO) δ = 9.35, 7.89 (–N–H, s, 2H), 8.54–8.53 (C_6H_4N, d, J = 5.94, 2H), 7.25–7.24 (C_6H_4N, d, J = 6.09, 2H), 5.17–5.16 (–CH, d, J = 3.54, 1H), 3.56 (–OCH_3, s, 3H), 2.26 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 166.1, 153.33, 152.46, 150.37, 150.19, 121.79, 98.39, 53.67, 51.31, 17.89.

2.1.7 | Ethyl 6-methyl-2-oxo-4-(pyridin-4-yl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4g)

Appearance: Brown solid; IR (KBR) ν/cm 3,442, 3,207, 3,068, 2,979, 2,802, 1,706, 1,685, 1,452, 1,230, 833. ^{1}H NMR (400 MHz, DMSO) δ = 9.59, 8.10 (–N–H, s, 2H), 8.84–8.83 (C_6H_4N, d, J = 6.66, 2H), 7.80–7.79 (C_6H_4N, d, J = 6.57, 2H), 5.384–5.375 (–CH, d, J = 3.4, 1H), 4.02–4.01 (–CH_2, d, J = 6.05, 2H), 1.11 (–CH_3, s, J = 6.05, 3H), 2.29 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 165.3, 161.54, 160.61, 151.94, 143.89, 124.65, 96.99, 60.09, 53.85, 21.04, 18.36.

2.2 | Crystal growth and single crystal X-ray crystallographic study

The slow evaporation method was used for growing of single crystals of synthesized compounds **4a**, **4b**, and **4e** in different solvents at room temperature. For obtaining a good quality single crystal of the synthesized compound, 5 to 10 mg of the compound was taken in a 5-ml beaker and dissolved with a minimum amount of different and/or combination of solvents. The beaker closed with parafilm and two to three small holes are made on it, which was kept for slow evaporation at room temperature. After 5–7 days, well-shaped crystals were obtained successfully for the case of **4a**, **4b**, and **4e** which were analyzed using a polarizing optical microscope. Block-shaped and colorless single crystal was obtained from methanol in case of compound **4a** and **4b**, whereas **4e** preferred plate-shaped crystal from ethanol. The crystal data were collected on the Bruker VENTURE diffractometer using monochromated Mo Kα radiation (λ = 0.71073 Å). The data collection, integration, unit cell measurement, scaling, and absorption corrections for the crystal data collected were performed using Bruker Apex II software[34]; data reduction was carried out by Bruker SAINT Suit.[35] The crystal structures were solved using direct methods using SIR 2014[36] and refined by the full matrix least squares method using SHELXL 2014[37] present in the program suite WinGX (version 2014).[38] Absorption correction was applied using SADABS. The thermal ellipsoid plot in the asymmetric unit are drawn using ORTEP program,[39] and molecular packings were generated using Mercury 3.5.1 (CCDC) program.[40] Treatment of hydrogen atoms must be mentioned. Geometrical calculations were taken using PARST[41] and PLATON.[42] Crystallographic and refinement data of the **4a**, **4b**, and **4e** were listed in Table 2, whereas their intermolecular interactions are listed in Table 3.

2.3 | Larvicidal activity

Larvicidal activity of the test compounds was screened using *Anopheles arabiensis* from a colonized strain from Zimbabwe, reared according to the WHO[43] guidelines. The insectary reproduced conditions of a malaria-endemic environment: 27.5°C temperature, 70% humidity, and a daily cycle of 12-hr light: 12-hr dark. One milliliter of the test compound (1 mg/ml) was added to distilled water (249 ml) to produce a final concentration of 4 μg/ml (1 mg/250 ml). Third instar mosquito larvae in groups of 30 were placed in the container. Throughout the experiment, the larvae were fed with specially made cat food with reduced oil/fat content at regular intervals. The negative control was set up using acetone and water, while the positive control included the emulsifiable organophosphate larvicidal Temephos (Mostop; Agrivo), a product used in the malaria control program. The percentage mortality was estimated as the number of dead larval recorded per container after 24 and 48 hr relative to the initial number of exposed larvae. Bioassays were triplicated.

2.4 | Data analysis

Differences in larval mortality between larvicide treatments were assessed with general linear mixed models.[44] The dependent variable was *A. arabiensis* mortality. Fixed effects were the test compounds (**4a–4g**, acetone, or Temephos) and observation period (24 and 48 hr), while mosquito groups (i.e., containers) were the random effects. LSD Fisher test was used for post hoc analyses, and in all cases, a probability (p) value <0.05 was considered to indicate statistically significant effects of treatment. Results are presented as the mean plus/minus the standard error.

3 | RESULT AND DISCUSSION

3.1 | Calculation of partition coefficient

ChemBioDraw Ultra 13.0v was used to calculate *c*Log*P* and the results for test compounds **4a–g** were in the range of 0.5988–2.9384. Compound **4f** with pyridine substitution at the fourth position of the hydropyrimidine pharmacophore exhibited lowest *c*Log*P* at 0.5988, whereas compound **4d** having electron withdrawing chlorine group at the second position of phenyl which is at the fourth position of HPM pharmacophore revealed the highest *c*Log*P* at 2.9384.

TABLE 2 Single crystal data collection and refinement for title compounds **4a**, **4b**, and **4e**

Data	Compound 4a	Compound 4b	Compound 4e
Formula	$C_{13}H_{14}N_4O_4{\cdot}H_2O$	$C_{14}H_{16}N_2O_5$	$C_{13}H_{13}N_2O_2ClS$
Formula weight	280.30	292.3	296.8
Temperature/K	110(2)	110	110
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	Triclinic	Triclinic	Monoclinic
Space group	$P-1$	$P-1$	$P2(1)/n$
a (Å)	5.7934(2)	7.2612(6)	4.8764(3)
b (Å)	10.5193(4)	8.8318(8)	9.8774(5)
c (Å)	11.8476(5)	11.7644(10)	27.2041(13)
α (°)	67.829(1)	103.743(4)	90.000
β (°)	88.076(2)	94.156(4)	91.999(3)
γ (°)	76.924(1)	111.293(4)	90.000
V (Å^3)	650.23(4)	672.23(17)	1309.52(2)
Z', Z	1,2	1, 2	1, 4
Density (g/cm^3)	1.43	1.44	1.51
μ (mm^{-1})	0.111	0.111	0.450
F (000)	296.0	308.0	616.0
θ (min, max)	3.3, 30.6	2.7, 26.0	2.2, 26.0
$h_{min,\ max,}$ $k_{min,\ max,}$ $l_{min,\ max.}$	−8 8, −13 15, −16 16	−8 8, −10 10, −14 14	−6 4, −12 12, −33 33
No. of refl.	18,863	7,605	9,337
No of unique refl./ Obs. refl.	3,965, 3,499	2,624, 1,777	2,553, 2,193
No. parameters	200	254	196
$R_{all,}$ R_{obs}	0.065, 0.058	0.082, 0.053	0.075, 0.066
wR_{all}, wR_{obs}	0.157, 0.152	0.142, 0.128	0.133, 0.130
$\Delta\rho_{min,\ max}$ (eÅ^{-3})	−0.381, 0.730	−0.327, 0.300	−0.403, 0.468
G.O.F.	1.067	1.032	1.267

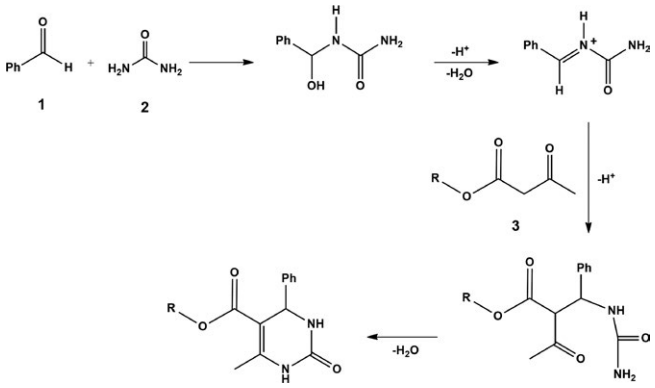
3.2 | Synthesis and reaction mechanism for the formation of the title compounds

In the current study, the synthesis of a series of new HPM correspondent is depicted in Scheme 1 and is obtained by one pot three component cyclocondensation reaction between aryl/hetero-aryl aldehyde, urea/thiourea and β-keto ester in alcoholic medium by reflux method. To obtain the maximum yield, the reaction mixture was poured into saline cold water. It was observed that pyridine aldehyde HPM gave low yield only because of slight solubility in water. The possible reaction mechanism for the synthesized **4a–g** analogues is depicted in Scheme 2. The purity of the synthesized compounds **4a–g** was established by HPLC and structural elucidation by FT-IR, ^{1}H NNR, ^{13}C NMR and Mass spectral studies. The successfully obtained good single crystals of **4a**, **4b**, and **4e** were analyzed by single crystal X-ray diffractometer (SCXRD) study. The FT-IR spectra of synthesized derivatives show a peak in the range of 3,271–3,200 and 1,705–1,640 cm^{-1} which confirm the presence of secondary amine (N–H) in the heterocyclic ring system and ester/amide group, respectively, in the compound. The ^{1}H NMR of the synthesized compound **4a** exhibited the singlet peaks at δ = 2.24 and δ = 3.53 for methyl substituent on pyrimidine ring and methyl of ester functional groups, respectively. For compounds **4a–g**, secondary amino group (–NH–) in the pyrimidine nucleus is observed in the range of δ = 9.33–10.40 ppm. The appearance of singlet in the range δ = 5.06–5.64 corresponds to the methine C–H peak of HPM nucleus. The ^{13}C NMR of **4a–g** exhibits the characteristic carbonyl group at δ = 165–168 range. The molecular mass of the title compounds **4a–g** is in agreement with molecular ion peak in LC-MS.

3.3 | Analysis of the crystal structure of compounds 4a, 4b, and 4e

HPM molecules have the ability to form strong hydrogen bondings, whereas in the presence of halogen and other functional groups such as pyridyl, methoxy, and hydroxy, the molecular packings are influenced by other weak intermolecular interactions including electrostatic and van der Waal's interactions. **4a**, **4b**, and **4e** crystal structures evidence the importance of such intermolecular interaction in their molecular assemblies. The compound **4a** crystallizes as a monohydrate with one HPM molecule and one H_2O molecule in the asymmetric unit ($Z' = 1$) in the centrosymmetric space group $P-1$ (Figure 1a). The molecule forms a hydrate and the stability of the molecule is enhanced due to the existence of three hydrogen bonds formed by water molecule with the hydroxyl group through O4-H10···O5 (distance = 1.79 Å, bond angle = 172°) with ester group through O5-H2O···O2 (2.03(4) Å, 143.(3)°) and amide group through O5-H3O···O1 (1.88(4) Å, 166(3)°). The crystal structure consists of a dimer through two different hydrogen bonds N1-H1N···O1 (2.03(3) Å, 177.1(3)°), and N2-H2N···O4 (2.05(3) Å, 169(2)°) with R_2^2 (8) graph set motif (Figure 1b).[45] The dihedral angles between the planes of the six-membered tetrahydropyrimidine ring with its 4-hydroxyphenyl and ester substituents are 86.78(0.10)° and 7.9(0.13)°, respectively. It is interesting to note that this monohydrated form exists as a new polymorph with two molecules in the asymmetric unit with $Z' = 2$ in the triclinic system with similar intermolecular hydrogen bonds, the detailed crystal structural analysis of $Z' = 2$ form is already reported.[46]

The compound **4b** crystallizes in the centrosymmetric triclinic space group $P-1$ with two molecules in the unit cell ($Z = 2$, $Z' = 1$). The dihedral angles between the planes of the six-membered tetrahydropyrimidine ring with its 4-hydroxy-3-methoxy phenyl ring and ester substituents are 84.47° and 11.74°, respectively. The ORTEP shows that one asymmetric molecule is present and exhibits two intramolecular hydrogen bonds, that is, O4-H4O···O5 (2.14(5) Å), C7-H7A···O3 (2.24(3) Å). The other intermolecular interactions N1-H1N···O2 (2.08(3) Å) and N2-H2N···O1 (2.00(3) Å) are



SCHEME 2 Proposed reaction mechanism for the synthesis of title compounds methyl/ethyl 4-(substitutedphenyl/pyridyl)-6-methyl-2-oxo/thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate analogues **4a–g**

favorable for dimer formation with each other and form $R_2^2(8)$ graph set motif. Further, the oxygen atom of the ester functional group forms C–H···O (2.01(5), 2.55(2), 2.24(3) Å) and N–H···O type of interaction which provides additional stability to the crystal packing (Figure 2b).

The compound **4e** crystallizes in the centrosymmetric triclinic space group $P\ 2_1/n$ with four molecules in unit cell ($Z = 4, Z' = 1$). The ORTEP shows that one asymmetric molecule is present and exhibits one intramolecular hydrogen bond C7-H7C···O2 (2.24(5) Å) which provides rigidity to the molecule (Figure 3a). The dihedral angles between the planes of the six-membered tetrahydropyrimidine ring with its 2 chloro phenyl ring and ester substituents are 73.41° and 8.31°, respectively. The crystal structure forms a dimer through N1-H1N···S1 (2.57(4) Å) hydrogen bond with $R_2^2(8)$ graph set motif. Again additional stabilization is achieved through C10-H10···O1 (2.59(5) Å), C7-H7A···S1 (2.87(5) Å) and C13-H13···O1 (2.49(5) Å) type of interactions (Figure 3b).

It is interesting to note that **4a** prefers two different types of dimers through (N1-H1N···O1 and N2-H2N···O4) hydrogen bonds, which is stabilized by hydrogen bonding with water molecules (Figure 1b), whereas additional methoxy substitution of **4b** prefers only one type of dimer(N2-H2N···O1) as well as additional molecular chain through hydrogen bonds (N1-H1N···O2, Figure 2b). However, **4e** forms only dimer with N1-H1N···S1 hydrogen bonds (Table 2; Figure 3b). It is worthy to mention that common dimermotif of all these crystal structures of **4a**, **4b**, and **4e** exhibit same type of hydrogen bonding graph set motif, that is, $R_2^2(8)$.

TABLE 3 Intermolecular interactions of compounds **4a**, **4b**, and **4e**

Sample	D-X···A	D-X (Å)	X···A (Å)	D···A (Å)	<D-X···A (Å)	Symmetry code
4a	N1-H1N···O1	0.86(3)	2.03(3)	2.893(2)	177(3)	−x,1−y,−z
	N2-H2N···O4	0.89(3)	2.05(3)	2.922(2)	169(2)	1−x,1−y,1−z
	O4-H10···O5	0.84	1.79	2.626(2)	172	x, y, z
	O5-H2O···O2	0.90(4)	2.03(4)	2.797(3)	143(3)	1−x,−y,1−z
	O5-H3O···O1	0.90(4)	1.88(4)	2.761(2)	166(3)	−x,1−y,1−z
	C7-H7C···O2	0.98	2.32(3)	2.828(3)	111	x, y, z
4b	N1-H1N···O2	0.93(3)	2.08(3)	2.986(3)	164(2)	−1+x,y,z
	N2-H2N···O1	0.89(3)	2.00(3)	2.893(3)	179(3)	2−x,1−y,1−z
	O4-H4O···O5	0.96(5)	2.14(5)	2.646(3)	111(4)	x,y,z
	O4-H4O···O3	0.96(5)	2.01(5)	2.878(3)	150(4)	2−x,−y,−z
	C6-H6B···O1	0.98(3)	2.55(2)	3.516(4)	166(2)	2−x,−y,1−z
	C7-H7A···O3	1.00(3)	2.24(3)	2.810(3)	115(2)	x,y,
	C12-H12···O4	1.01(3)	2.59(3)	3.290(4)	126(2)	3−x,1−y,−z
	C13-H13···O1	0.97(2)	2.48(2)	3.427(3)	165(2)	1+x,y,z
4e	N1-H1N···S1	0.81(4)	2.57(4)	3.368(4)	166(4)	1−x,2−y,−z
	C4-H4···N1	1.00(4)	2.58(4)	3.366(6)	135(3)	−1+x,y,z
	C7-H7A···S1	0.89(5)	2.87(5)	3.653(5)	148(4)	1−x,2−y,−z
	C7-H7C···O2	0.98(5)	2.24(5)	2.813(6)	116(4)	x,y,z
	C10-H10···O1	0.97(5)	2.59(5)	3.227(5)	124(3)	−1/2−x,1/2+y, 1/2−z
	C13-H13···O1	0.99(5)	2.49(5)	3.407(5)	154(4)	1+x,y,z

3.4 | Larvicidal activity

There were significant effects of treatment ($p < 0.0001$) and exposure time ($p < 0.001$), but not a significant interaction between these two variables ($p = 0.66$). Larval mortality was higher after 48 hr exposure (58.52 ± 1.08 after 24 hr; 63.83 ± 1.1 after 48 hr) (Table 4). Mortality of larvae exposed

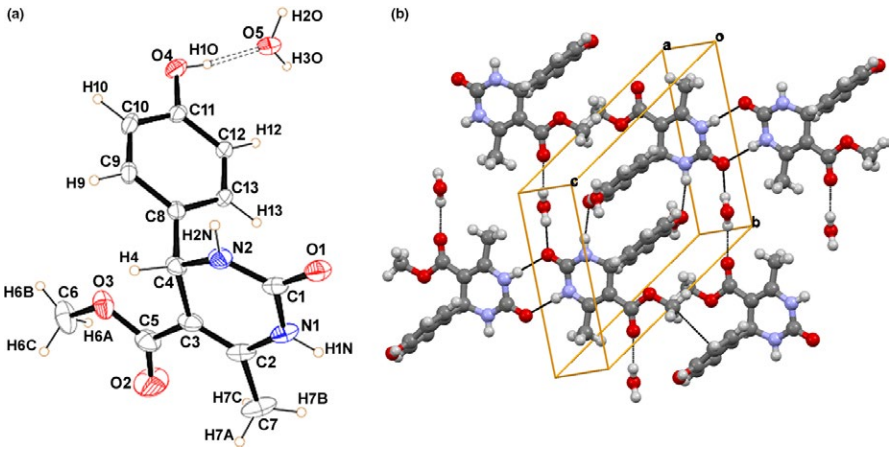


FIGURE 1 (a) The asymmetric unit of the title compound **4a** with 50% probability ellipsoids (Left: The double-dashed lines indicate hydrogen bonds with water molecule) and (b) crystal packing through intermolecular hydrogen bonds shown in unit cell (Right)

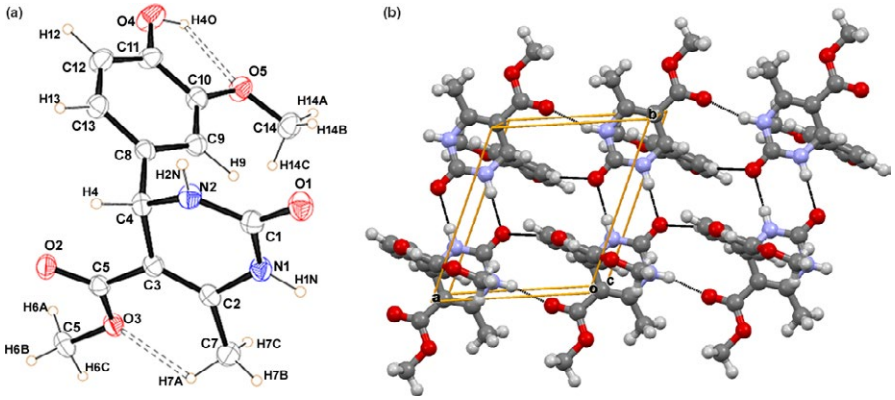


FIGURE 2 (a) The asymmetric unit of compound **4b** with 50% probability thermal ellipsoids and intramolecular interactions as double-dashed lines (left), and (b) dimer formation through hydrogen bonds in their molecular assembly (right)

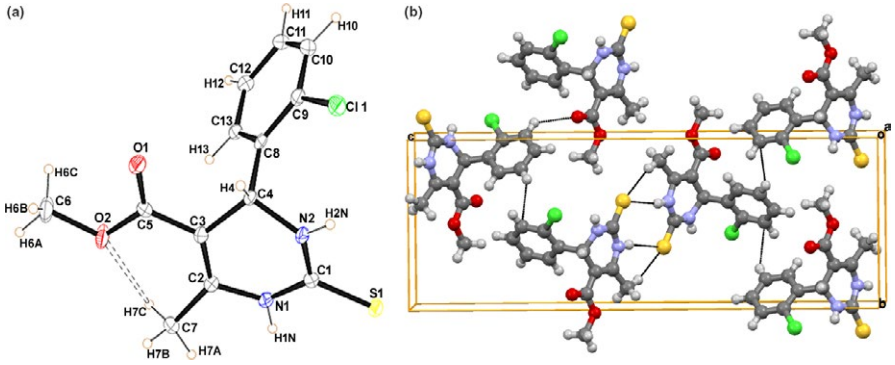


FIGURE 3 (a) The asymmetric unit of compound **4e** with 50% probability ellipsoids and intramolecular interactions as double-dashed lines (left), and (b) Molecular assembly depicts dimer formation through N–H···S hydrogen bonds and other weak interactions (Right)

to any of the compounds was higher than negative controls. Highest mortality resulted from exposure to compound **4e** (which was as strong as Temephos after 48 hr exposure) followed by **4d**, **4a**, and **4c**. Lowest mortality was recorded for treatment with **4f**, resulting in less than 20% mortality (Table 4).

Studies on the interaction of HPMs with artificial membranes suggest that their bioactivity may lead to interact with the lipid molecules which can modulate the organization of the biological membranes and membrane proteins functionality.[47] Besides, the larvicidal activity of test compounds depends on their relative substituents on phenyl ring at fourth position of the HPM pharmacophore. Results presented herein show that compounds **4d** and **4e**, both having electron withdrawing chlorine group at *ortho* position of phenyl ring at fourth position of the HPM pharmacophore, were the most lethal and promising larvicides. Compounds **4d** and **4e** were lipophilic in nature with partition coefficient value (*c*Log*P*) of 2.8088 and 209,384, respectively, which helped the molecules for better cell permeability. Test compound **4f** with pyridyl moiety and having polar property of *c*Log*P* at 0.5988 exhibited low larvicidal activity. However, compounds **4a** and **4c** having similar partition coefficient value (*c*Log*P*) of 1.4288 and 1.4076, respectively, exhibited moderate similar larvicidal activity after 48 hr. From this experiment, it is confirmed that compounds which are lipophilic property are known to show significant larvicidal activity.

TABLE 4 Mortality of *Anopheles arabiensis* larvae exposed to test compounds **4a–g** at 4 μg/ml (1 mg/250 ml)

Treatment	24 hr	48 hr
4a^{AB}	71.11 ± 3.2^{abc}	74.44 ± 3.2^{abc}
4b^{C}	57.78 ± 3.2^{de}	65.56 ± 3.2^{cd}
4c^{B}	67.78 ± 3.2^{bc}	75.56 ± 3.2^{ab}
4d^{A}	77.78 ± 3.2^{af}	80 ± 3.2^{af}
4e^{D}	86.67 ± 3.2^{fg}	90 ± 3.2^{gh}
4f^{E}	11.11 ± 3.2^{i}	24.44 ± 3.2^{j}
4g^{F}	48.89 ± 3.2^{e}	55.56 ± 3.2^{e}
Acetone^{G}	7.78 ± 3.2^{i}	10 ± 3.2^{i}
Temephos^{H}	97.78 ± 3.2^{h}	98.89 ± 3.2^{h}

$^{A–H}$Different capital letters indicate significant differences between treatments. $^{a–h}$Different letter indicates significant differences between treatments and exposure times.

4 | CONCLUSIONS

Seven new molecules (**4a–g**) with HPM pharmacophore were screened for larvicidal activity, and it is worthy to mention that the electron withdrawing group such as chlorine

at ortho position on phenyl ring at fourth position of HPM pharmacophore exhibits a significant lethal effect on *A. arabiensis* larvae compared to other electron releasing substituent on phenyl ring. Further, molecules with thiourea moiety enhance the larvicidal activity in comparison with urea moiety due to electronic withdrawing C=S group on HPM pharmacophore. Single crystal structure studies of **4a**, **4b**, and **4e** support this co-relation that the role functional group by preferring a different type of interactions and crystal packing and hence variation in their larvicidal activity properties.

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ENDNOTES

[a1] http://wwwmosquitoorg/mosquito-borne-diseases visited on June 1, 2017.

[b2] https://wwwintechopencom/books/the-importance-of-biological-interactions-in-the-study-of-biodiversity/global-impact-of-mosquito-biodiversity-human-vector-borne-diseases-and-environmental-change visited on June 09, 2017.

[c3] http://wwwwhoint/malaria/publications/world-malaria-report-2016/report/en/ visited on June 10, 2017.

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