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RESEARCH ARTICLE

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Synthesis and characterization of a novel series of 1,4-dihydropyridine analogues for larvicidal activity against *Anopheles arabiensis*

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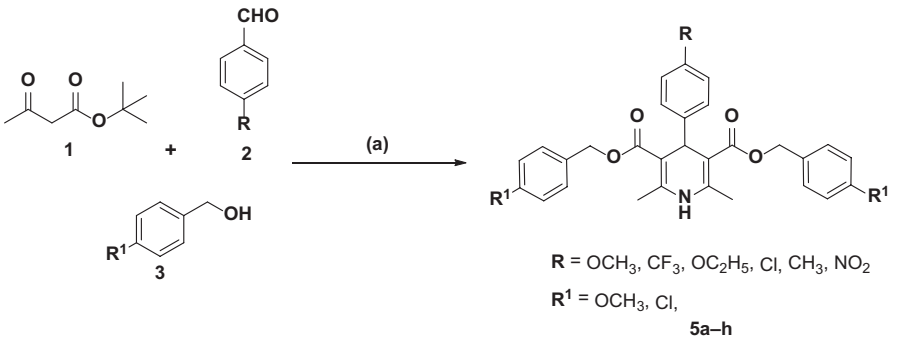
The new-fangled bis(4-substituted benzyl) 4-(4-substitued phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives were synthesized by the union of substituted aryl aldehyde, *tert*-butyl acetoacetate, ammonium carbonate with 4-substituted benzyl alcohol *via* Hantzsch ester synthesis in aqueous medium under catalyst-free conditions. The newly synthesized compounds were characterized by spectroscopic techniques such as IR, NMR (^{1}H and ^{13}C), ESI mass, elemental analysis, and single-crystal X-ray diffraction. The characterized title compounds were evaluated for the larvicidal activity against *Anopheles arabiensis* by standard WHO larvicidal assay method using Temephos as standard at 4 μg/ml. The title compounds bis(4-methoxybenzyl) 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate and bis(4-chlorobenzyl) 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate exhibited promising larvicidal activity at 65.6% and 72.2%, respectively, when compared with the standard compound at 98.9%.

KEYWORDS
crystallography, dihydropyridine, Hantzsch synthesis, larvicidal activity., multi-component reaction

1 | INTRODUCTION

1,4-Dihydropyridines (1,4-DHPs) were synthesized by the German chemist Arthur Rudolf Hantzsch in 1882,[1] by the combination of β-ketoester, aryl aldehyde, and nitrogen source under strong acidic conditions. These scaffolds have attracted medicinal chemists to synthesize several poly-functionalized dihydropyridines and these molecules have been comprehensively investigated for their potential pharmacological properties, for instance antitubercular,[2] anticancer,[3] antineurotropic,[4] neuropeptide YY_1 receptor antagonists,[5] neuroprotective,[6] platelet anti-aggregation,[7] bronchodilation,[8] antidiabetic,[9] antioxidant, and antimicrobial activities.[10] The 1,4-DHP derivatives which are commercially available are extensively used as calcium channel blockers for the treatment of cardiovascular disorder, including hypertension,[11] angina and cardiac arrhythmias.[12] Besides this, the focus on the synthesis of 1,4-DHPs with respect to Multidrug Resistance (MDR) reversal in tumour cells furnished a new aspect to their applications.[13,14] In addition, these synthetic compounds containing the dihydropyridine moiety have shown insecticidal activity against diverse insect groups, such as the mustard beetle *Phaedon cochleariaem* (Coleoptera), the fall armyworm *Spodoptera*



SCHEME 1 Synthesis of 1,4-dihydropyridine derivatives **5a–h:** Reagents and conditions; (a) $(NH_4)_2CO_3$, H_2O, Δ,70°C

frugiperda (Lepidoptera),[10] major pest rice, *Nilaparvata lugens* (Hemiptera),[11] and the bean aphid *Aphis medicaginis* (Hemiptera).[12] Dihydropyridine derivatives have shown larvicidal activity against the medically important mosquito species *Aedes aegypti* and *Culex quinquefasciatus* (Diptera).[13] In view of such promising pharmacological activities of 1,4-DHPs and in continuation of our efforts to develop novel anti-malarial agents[15–18] we herein undertake the synthesis of a series of new-fangled bis(4-substituted benzyl) 4-(4-substituted phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives **5a–h** as depicted in Scheme 1. Furthermore, it is also of interest to screen such heterocyclic compounds for the existence of polymorphs[19–22] and their implications in the observed biological property. The characterized title compounds were evaluated for larvicidal activity against *Anopheles arabiensis* by standard WHO larvicidal assay method using Temephos as a standard at 4 μg/ml.

2 | METHODS AND MATERIALS

All the chemicals were purchased from Sigma-Aldrich Corporation (analytical grade) and were used without further purification. FT-IR spectra were registered on a Bruker IFS 55 equinox FTIR spectrophotometer as KBr discs. ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker 101 and 400 MHz spectrometer in the solvents indicated (referenced to the residual ^{1}H signals in the deuterated solvents) using TMS as internal standard. Chemical shifts are reported in ppm (δ scale), coupling constant (*J*) values are given in hertz (Hz). Mass analysis is performed on a quadruple-time of flight (Q-TOF) mass spectrometer, equipped with an ESI source (+ve). TLC analysis of reaction mixtures was performed on Merck aluminium plates coated with silica gel (60F254). Compounds were visualized by ultraviolet irradiation at 254 and 366 nm. Elemental analysis was performed on Vario Micro Cube Elemental Analyzer. Single-crystal data were collected on the Bruker D8 VENTURE diffractometer equipped with CMOS type PHOTON 100 detector using monochromated Mo Kα radiation (λ = 0.71073 Å).

2.1 | General experimental procedure for the synthesis of bis(substitutedbenzyl) 4-(substitutedphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates (5a–h)

A 25 ml round bottomed flask equipped with a reflux condenser was charged with *tert*-butyl acetoacetate (2.0 mmol) and benzyl alcohol (2.0 mmol). The precursors were finely mixed together and subjected to mechanical stirring for 30 min at 110°C (oil bath). The substituted arylaldehyde (1.0 mmol) and ammonium carbonate (1.0 mmol) were added to above mixture subsequently. The resulting reaction mixture was heated for 2 hr at 70°C with constant stirring till the reaction was complete. The progress of reaction was monitored by TLC. After completion of reaction as indicated on TLC, the content of the reaction mixture was cooled to room temperature and the crude reaction mixture was washed with chilled water (15 ml × 3), filtered and dried under vacuum. The pure product was obtained by crystallization of the crude material from methanol. Physicochemical constants of the synthesized compounds are tabulated in Table 1.

2.2 | Bis(4-methoxybenzyl)4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5a)

Appearance: White solid; yield 90%. mp 130–132°C. IR (KBr) ν/cm 3334, 3151, 2358, 1682, 1505, 1301, 824, 746. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14–7.12 (–C_6H_5, d, *J* = 8.5 Hz, 4H), 7.07–7.05 (–C_6H_5, d, *J* = 8.6 Hz, 2H), 6.81–6.79 (–C_6H_5, d, *J* = 8.6 Hz, 4H), 6.66–6.64 (–C_6H_5, d, *J* = 8.6 Hz, 2H), 5.63 (-NH, s, 1H), 5.02–4.94 (–OCH_2Ph, m, 5H), 3.79 (–OCH_3, s, 6H), 3.73 (–OCH_3, s, 3H), 2.28 (–CH_3, s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.43, 159.27, 157.91, 143.87, 140.09, 129.65, 129.07, 128.70, 113.72, 113.24, 104.28, 65.37, 55.25, 55.16, 38.67, 24.94, 19.67. ESI–MS: *m/z* 544.2 $[M + 1]^+$. Anal. Cal. for $C_{32}H_{33}NO_7$: C, 70.70; H, 6.12; N, 2.58. Found: C, 70.76; H, 6.13; N, 2.51.

TABLE 1 Synthesis of 1,4-DHPs derivatives **5a–h** in aqueous medium under catalyst-free condition[a]

Product[a]	*R*	R^1	m.p (°C)	(%)Yield[b,c]	*c*Log*P*[d]
5a	OCH_3	OCH_3	130–132	90	6.656
5b	OCH_3	Cl	122–124	92	8.244
5c	CF_3	OCH_3	142–144	89	7.62
5d	OC_2H_5	OCH_3	98–100	78	7.185
5e	Cl	OCH_3	148–150	83	7.45
5f	CH_3	OCH_3	98–100	94	7.236
5g	NO_2	OCH_3	176–178	75	6.48
5h	NO_2	Cl	156–158	78	8.068

[a]Reaction conditions: tert-Butylacetoacetate (2.0 mmol), arylaldehyde (1.0 mmol), ammonium carbonate (1.0 mmol) and benzyl alcohol (2.0 mmol) at 70°C.
[b]All of the products were characterized by spectral and physical data.
[c]Yields after purification by recrystallization method.
[d]cLogP was calculated using chembiodraw ultra 13.0v.

2.3 | Bis(4-chlorobenzyl) 4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5b)

Appearance: White solid; yield 92%. mp 122–124°C. IR (KBr) ν/cm 3340, 3150, 1949, 1685, 1546, 1488, 1301, 803, 769, 737. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (–C_6H_5, d, *J* = 2.8 Hz, 2H), 7.24–7.22 (–C_6H_5, s, 2H), 7.10–7.03 (–C_6H_5, m, 5H), 6.67–6.65 (–C_6H_5, d, *J* = 8.6 Hz, 3H), 5.68 (-NH, s, 1H), 5.08–5.05 (-OCH_2Ph, d, *J* = 12.7 Hz, 2H), 4.98–4.93 (-OCH_2Ph, –C_6H_5, m, 3H), 3.75 (–OCH_3, s, 3H), 2.30 (–CH_3, s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.14, 158.08, 144.23, 139.90, 135.07, 133.64, 129.37, 129.30, 129.02, 128.59, 128.53, 113.31, 104.07, 64.78, 55.21, 38.66, 19.71. ESI–MS: *m/z* 552.5 $[M + 1]^+$. Anal. Cal. for $C_{30}H_{27}Cl_2NO_5$: C, 65.22; H, 4.93; Cl, 12.83; N, 2.54. Found: C, 65.18; H, 4.98; Cl, 12.81; N, 2.58.

2.4 | Bis(4-methoxybenzyl) 2,6-dimethyl-4-(4-(trifluoromethyl)phenyl)-1,4-dihydropyridine-3,5-dicarboxylate (5c)

Appearance: White solid; yield 89%. mp 142–144°C. IR (KBr) ν/cm 3339, 2955, 2357, 1515, 1418, 1302, 824, 738. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.32 (–C_6H_5, d, *J* = 8.1 Hz, 2H), 7.23–7.20 (–C_6H_5, d, *J* = 8.1 Hz, 2H), 7.09–7.06 (–C_6H_5, d, *J* = 8.6 Hz, 4H), 6.80–6.78 (–C_6H_5, m, 4H), 5.78 (-NH, s, 1H), 5.05 (–C_6H_5, s, 1H), 5.03–5.02 (–OCH_2Ph, d, *J* = 3.0 Hz, 2H), 4.93–4.90 (–OCH_2Ph, d, *J* = 12.1 Hz, 2H), 3.79 (–OCH_3, s, 6H), 2.30 (–CH_3, s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.09, 167.04, 159.41, 151.41, 144.59, 129.73, 128.47, 128.40, 124.81, 124.78, 113.76, 103.51, 65.51, 55.23, 39.85, 19.61. ESI–MS: *m/z* 582.6 $[M + 1]^+$. Anal. Cal. for $C_{32}H_{30}F_3NO_6$: C, 66.09; H, 5.20; F, 9.80; N, 2.41. Found: C, 66.18; H, 5.18; F, 9.84; N, 2.43.

2.5 | Bis(4-methoxybenzyl) 4-(4-ethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5d)

Appearance: White solid; yield 78%. mp 98–100°C. IR (KBr) ν/cm 3337, 2934, 1682, 1514, 1301, 822, 750.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14–7.12 (–C_6H_5, d, *J* = 8.6 Hz, 4H), 7.05–7.03 (–C_6H_5, d, *J* = 8.6 Hz, 2H), 6.81–6.79 (–C_6H_5, d, *J* = 8.6 Hz, 4H), 6.65–6.63 (–C_6H_5, d, *J* = 8.6 Hz, 2H), 5.63 (-NH, s, 1H), 4.99–4.93 (–OCH_2Ph, –C_6H_5, m, 5H), 3.98–3.93 (–OC_2H_5, q, *J* = 7.0 Hz, 2H), 3.79 (–OCH_3, s, 6H), 2.28 (–CH_3, s, 6H), 1.38–1.35 (–OC_2H_5, t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.44, 159.27, 157.30, 143.83, 139.94, 129.64, 129.05, 128.72, 113.84, 113.73, 104.31, 65.36, 63.29, 55.25, 38.67, 19.65, 14.93. ESI–MS: *m/z* 558.4 $[M + 1]^+$. Anal. Cal. for $C_{33}H_{35}NO_7$: C, 71.08; H, 6.33; N, 2.51. Found: C, 70.99; H, 6.38; N, 2.55.

2.6 | Bis(4-methoxybenzyl) 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5e)

Appearance: White solid; yield 83%. mp 148–150°C. IR (KBr) ν/cm 3338, 2954, 1682, 1514, 1301, 820, 736.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11–7.09 (–C_6H_5, d, *J* = 8.6 Hz, 4H), 7.05 (–C_6H_5, s, 4H), 6.82–6.80 (–C_6H_5, d, *J* = 8.6 Hz, 4H), 5.74 (- NH, s, 1H), 5.03–4.92 (–OCH_2Ph, –C_6H_5, m, 5H), 3.80 (–OCH_3, s, 6H), 2.28 (–CH_3, s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.19, 159.37, 146.10, 144.30, 131.66, 129.73, 129.55, 128.49, 127.94, 113.76, 103.76, 65.49, 55.28, 39.24, 19.62. ESI–MS: *m/z* 548.5 $[M + 1]^+$. Anal. Cal. for $C_{31}H_{30}ClNO_6$: C, 67.94; H, 5.52; Cl, 6.47; N, 2.56. Found: C, 67.99; H, 5.57; Cl, 6.46; N, 2.50.

2.7 | Bis(4-methoxybenzyl) 2,6-dimethyl-4-(*p*-tolyl)-1,4-dihydropyridine-3,5-dicarboxylate (5f)

Appearance: White solid; yield 94%. mp 98–100°C. IR (KBr) *ν*/cm 3337, 3154, 2953, 2150, 1683, 1514, 1301, 1113, 822, 737, 702.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14–7.12 (–C_6H_5, d, J = 8.6 Hz, 4H), 7.06–7.04 (–C_6H_5, d, J = 8.0 Hz, 2H), 6.95–6.93 (–C_6H_5, d, J = 7.9 Hz, 2H), 6.81–6.79 (–C_6H_5, d, J = 8.6 Hz, 4H), 5.61 (-NH, s, 1H), 5.02–4.95 (–OCH_2Ph, –C_6H_5, m, 5H), 3.79 (–OCH_3, s, 6H), 2.29 (–CH_3, s, 6H), 2.26 (–CH_3, s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.36, 159.26, 144.62, 144.00, 135.51, 129.62, 128.73, 128.62, 127.93, 113.72, 104.20, 65.36, 55.25, 39.04, 21.06, 19.70. ESI–MS: *m/z* 528.2 $[M + 1]^+$. Anal. Cal. for $C_{32}H_{33}NO_6$: C, 72.85; H, 6.30; N, 2.65. Found: C, 72.86; H, 6.37; N, 2.64.

2.8 | Bis(4-methoxybenzyl) 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (5 g)

Appearance: Pale yellow solid; yield 75%. mp 176–178°C. IR (KBr) *ν*/cm 3338, 3152, 2953, 1683, 1514, 1300, 1113, 828, 736, 707.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.88 (–C_6H_5, d, J = 8.5 Hz, 2H), 7.22–7.20 (–C_6H_5, d, J = 8.6 Hz, 2H), 7.11–7.09 (–C_6H_5, d, J = 8.5 Hz, 4H), 6.81–6.79 (–C_6H_5, d, J = 8.5 Hz, 4H), 5.85 (-NH, s, 1H), 5.09–4.81 (–OCH_2Ph, –C_6H_5, m, 5H), 3.80 (–OCH_3, s, 6H), 2.31 (–CH_3, s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.82, 159.52, 154.82, 146.20, 144.97, 129.93, 129.00, 128.19, 123.16, 113.78, 103.06, 65.68, 55.28, 40.15, 30.92, 19.60. ESI–MS: *m/z* 559.1 $[M + 1]^+$. Anal. Cal. for $C_{31}H_{30}N_2O_8$: C, 66.66; H, 5.41; N, 5.02. Found: C, 66.68; H, 5.40; N, 5.00.

2.9 | Bis(4-chlorobenzyl) 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (5h)

Appearance: Yellow solid; yield 78%. mp 156–158°C. IR (KBr) *ν*/cm 3342, 3152, 2949, 1682, 1571, 1345, 1091, 830, 772, 737. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96–7.94 (–C_6H_5, d, J = 8.6 Hz, 2H), 7.26–7.24 (–C_6H_5, d, J = 8.2 Hz, 6H), 7.11–7.09 (–C_6H_5, d, J = 8.3 Hz, 4H), 5.90 (-NH, s, 1H), 5.04–4.94 (–OCH_2Ph, –C_6H_5, m, 5H), 2.33 (–CH_3, s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.54, 154.55, 146.40, 145.33, 134.59, 134.08, 129.54, 128.89, 128.67, 123.30, 102.85, 65.17, 40.02, 19.75. ESI–MS: *m/z* 567.2 $[M + 1]^+$. Anal. Cal. for $C_{29}H_{24}Cl_2N_2O_6$: C, 61.39; H, 4.26; Cl, 12.50; N, 4.94. Found: C, 61.38; H, 4.28; Cl, 12.56; N, 4.90.

TABLE 2 Single-crystal data collection and refinement for title compound **5h**

Data	Compound 5h
Formula	$C_{29}H_{24}Cl_2N_2O_6$
Formula weight	567.40
Temperature/K	110 (2)
Wavelength (Å)	0.71073
Solvent system, temperature	Methanol, 25°C
CCDC number	1461676
Crystal system	Monoclinic
Space group	$P2_1/m$
a (Å)	6.4679 (10)
b (Å)	19.640 (3)
c (Å)	10.3089(17)
α (°)	90
β (°)	104.241(6)
γ (°)	90
V(Å^3)	1269.3(4)
Z′, Z	1/2, 2
Density(g/cm^3)	1.485
μ (mm^{-1})	0.305
F (000)	588
θ (min, max)	2.287, 30.028
$h_{min,max}$, $k_{min,max}$, $l_{min,max}$	−9 7, −27 27, −13 14
No. of ref.	29392
No. of unique ref./obs. Ref.	3786/3093
No. parameters	197
R_{all}, R_{obs}	0.0559, 0.0410
$wR2_{all}$, $wR2_{obs}$	0.1054, 0.0976
$\Delta\rho_{min,max}$ (eÅ^{-3})	−0.495, 0.409
G. O. F.	1.045

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

Single crystals of the compound **5h** were grown from slow evaporation of methanol at room temperature (25°C). Single-crystal data were collected on the Bruker D8 VENTURE diffractometer equipped with CMOS type PHOTON 100 detector using monochromated Mo Kα radiation (λ = 0.71073 Å). Unit cell measurement, data collection, integration, scaling, and absorption corrections for the crystal data collected were performed using Bruker Apex II software.[23] Data reduction was carried out by BRUKER SAINT Suite.[24] The crystal structure was solved by direct methods using SIR 2014[25] and refined by the full matrix least squares method using SHELXL 2014[26] present in the program suite WINGX (version 2014.1).[27] Absorption correction was applied using

TABLE 3 List of intermolecular interactions in title compound **5h**

Motifs	D–H···A	Symmetry	Geometry		
			D···A/Å	H···A/Å	∠D–H···A/°
I	N2-H2A···O2	$x-1, -y+1/2, z-1$	2.993 (2)	1.99	175
II	C11 -H11A···O3	$-x, -y, -z$	3.234 (2)	2.47	126
III	C17 -H17···O3	$x+1, y, z$	3.483 (2)	2.63	135
IV	C14-H14···O1	$x+1, y, z$	3.452 (2)	2.81	118
V	C17-H17···O3	$-x, -y, -z$	3.444 (2)	2.71	125
VI	C14 -H14···O1	$x+1, -y+1/2, z$	3.452 (2)	2.81	118
VII	C18-H18B···O2	$x-1, -y+1/2, z-1$	3.560 (2)	2.73	134
VIII	C18-H18B···Cl1	$x-2, y, z-1$	3.616 (2)	2.99	117
IX	C11-H11B···Cl1	$-x+1, -y, -z+1$	3.711 (2)	2.96	127
X	*C3(π) ···C14(π)*	$x-1, -y+1/2, z$	3.262 (2)	–	–
XI	H14···H18A	$x-1, y, z-1$	2.09	–	–
XII	H3···H6	$x-1, y, z$	2.11	–	–

SADABS.[28] All non-hydrogen atoms were refined anisotropically and all hydrogen atoms (except H-atom bonded to N_2) were positioned geometrically and refined using a riding model with $U_{iso}(H) = 1.2U_{eq}$. The H-atom bonded to the ring nitrogen was taken directly from the difference Fourier peak. ORTEP was generated using MERCURY 3.5.1 (CCDC) program.[29] Geometrical calculations were made using PARST[30] and PLATON.[31] Crystallographic and refinement data of the title compound **5h** were tabulated in Table 2.

2.11 | Larvicidal activity

The *Anopheles arabiensis* mosquitoes used were from a colonized strain from Zimbabwe. *A. arabiensis* had been reared according to the WHO (1975) guidelines[32] in an insectary simulating the temperature (27.5°C), humidity (70%), and lighting (12/12) of a malaria endemic environment. About 1 ml of the test compound (1 mg/ml) was added to distilled water (250 ml), the final concentration was made to 4 µg/ml. Third instar mosquito larvae about 30were placed in the container. A negative control was set up using a solvent (acetone) and distilled water. A positive control included Temephos (Mostop; Agrivo), an effective emulsifiable organophosphate larvicidal used in the malaria control program. Each container was monitored to determine the larval mortality at 24 hr intervals for a period of 2 days. The larva was fed with specially made cat food with reduced oil/fat content at regular intervals. Bioassays were triplicated and the percentage of mortality was calculated relative to the initial number of the exposed larvae. The larvicidal results are tabulated in Table 4.

TABLE 4 Mortality of *Anopheles larvae* exposed to test compounds **5a–h** at 4 µg/ml (1 mg/250 ml) and their negative (acetone) and positive (Temephos) controls

Compound	Mortality	
	24 hr	48 hr
5a[a]	24.4	27.8
5b[bc]	10.0	12.2
5c[bc]	7.8	11.1
5d[ab]	21.1	23.3
5e[c]	3.3	3.3
5f[bc]	11.1	12.2
5g[d]	63.3	65.6
5h[d]	70.0	72.2
Acetone[c]	7.8	10.0
Temephos[e]	97.8	98.9

Adjusted means are shown. Adjusted standard errors were 2.4.
[a–e]Compounds not sharing a letter differ significantly ($p < .05$).

2.12 | Data analysis

General linear-mixed models (Infostat software)[33] were used to determine the differences between treatments registered in the larval mortality (larvicide assays). Dependent variables were the *A. arabiensis* mortality, and the fixed effects includethe test compound (test compounds **5a–h**, acetone, and Themephos) and observation period (24 and 48 hr). Random effects were mosquito groups (i.e., container in larvicide tests). Bonferroni test was used for post hoc analyses. In all cases, a value of $p < .05$ was considered statistically significant. Throughout the text, the results are presented as the adjusted mean plus/minus the standard error.

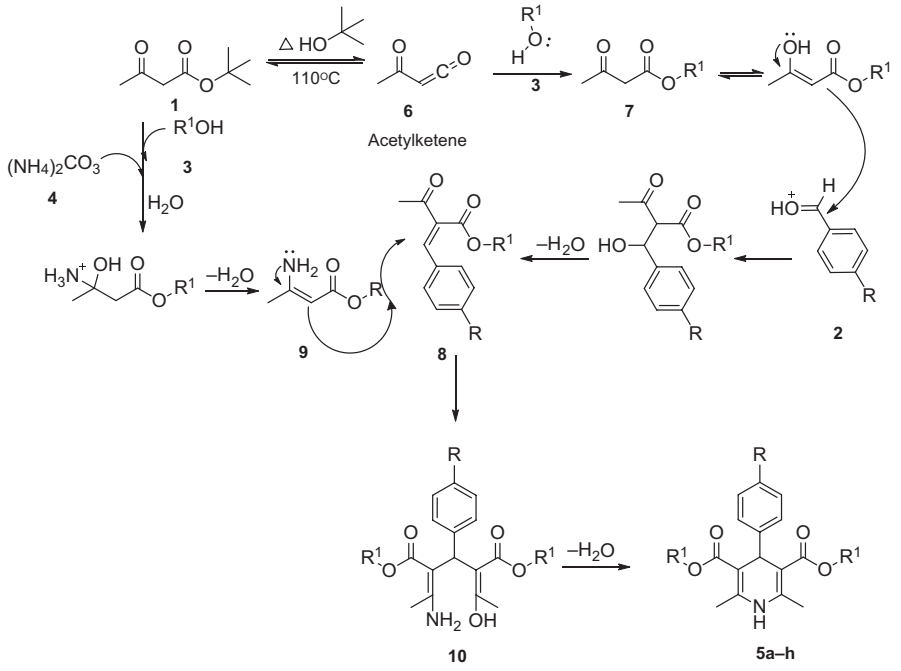


FIGURE 1 Proposed reaction mechanism for the construction of 1,4-DHPs analogues **5a–h**

3 | RESULTS AND DISCUSSION

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

In the present study, the synthesis of a series of novel 1,4-dihydropyridine analogues is depicted in Scheme 1 and are obtained by a one-pot four component cyclocondensation reaction of *tert*-butylacetoacetate, substituted benzyl alcohol with a wide range of aryl aldehydes and ammonium carbonate in aqueous medium at 70°C. To optimize the reaction conditions, 4-methoxy aryl aldehyde, *tert*-butylacetoacetate, 4-methoxy benzylalcohol and ammonium carbonate were taken as model substrates and the reaction was carried out by varying the mole ratios. It was observed that when *tert*-butylacetoacetate, 4-methoxy benzaldehyde, 4-methoxy benzyl alcohol and ammonium carbonate, was taken in the ratio of 2: 1: 2: 1, in aqueous medium, at 70°C under catalyst-free conditions, high yields of the desired product was obtained. Based on the reports in the literature, a plausible reaction mechanism for the observed symmetrical 1,4-DHPs ester derivatives **5a–h** is depicted in Figure 1. In *trans*-esterification transformation, *tert*-butylacetoacetate **1** undergoes *trans*-esterification reaction through acetylketene intermediate **6** to form new acetoacetate **7** with substituted aromatic alcohol **3**. The new acetoacetate undergoes reaction with substituted arylaldehydes **2** to form an intermediate **8** *via* Knoevenagel condensation. The Knoevenagel condensation intermediate is reacted with α, β-unsaturated amine ester (enamine ester, **9**), which was generated by the reaction of **7** and **4** followed by dehydration to form adduct acyclic product **10**. The intramolecular cyclization followed by dehydration occurred during

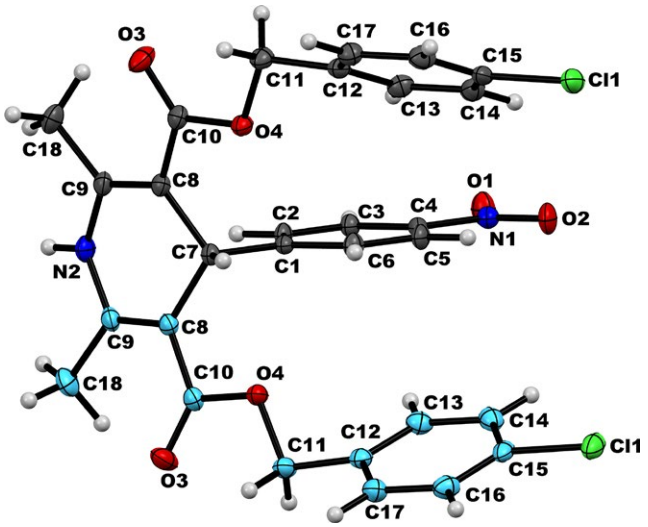


FIGURE 2 *ORTEP* of **5h** drawn with 50% ellipsoidal probability showing the atom numbering scheme (different colour of C-atoms showing symmetrically generated molecule by mirror plane) [Colour figure can be viewed at wileyonlinelibrary.com]

the course of the formation of the final desired Hantzsch symmetrical 1,4-DHPs ester derivatives **5a–h**. The structure of the synthesized compounds **5a–h** was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra, elemental analysis and one such compound was characterized by single-crystal X-ray study. The IR spectra of the synthesized compounds reveal the formation of the compounds **5a–h** with a secondary amino group (–NH–), corresponding to the 1,4-dihydropyridine nucleus, in the range of 3,334–3,342/cm^{-1}. ^{1}H-NMR of the compounds **5a–h** exhibited the appearance of a singlet in the range of δ = 2.26–2.33 ppm, corresponding to the presence

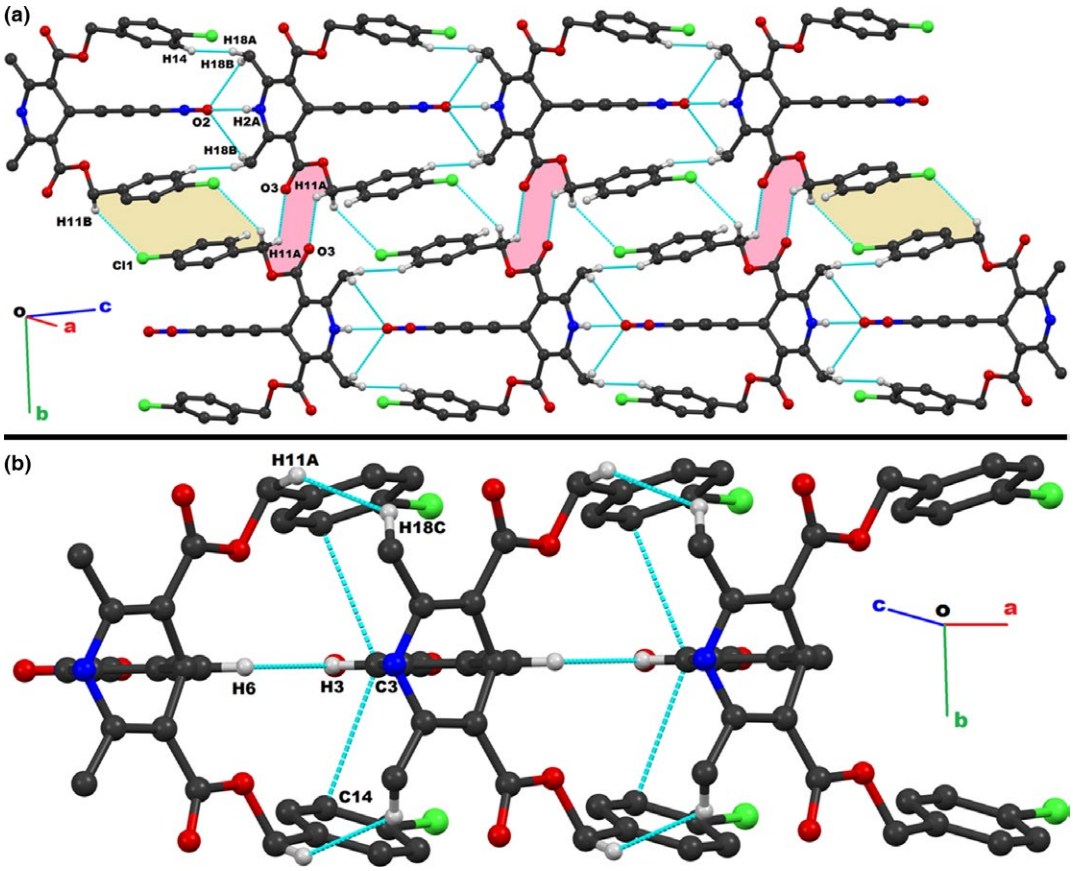


FIGURE 3 (a) Packing network of **5h** molecule down the bc plane associated with N–H···O=N, C–H···O=C and C–H···Cl hydrogen bonds along with weak H···H contacts. (b) Packing of molecules down the ab plane associated with weak π···π stacking and H···H contacts. All non-interacting hydrogen atoms have been omitted for clarity [Colour figure can be viewed at wileyonlinelibrary.com]

of two methyl groups on 1,4-dihydropyridine nucleus. ^{13}C-NMR of **5a–h** exhibits the characteristic carbonyl group at δ = 166.54–168.09 ppm range. Elemental analysis results are in good agreement with the calculated values of the proposed title compounds **5a–h**. The IR, NMR (^{1}H and ^{13}C), results on mass and elemental analysis are discussed in detail in the experimental section.

3.2 | Analysis of the crystal structure of the compound 5h

Single-crystal data collection and subsequent structure solution of the title compound results indicate that the compound **5h** crystallizes in the centrosymmetric monoclinic space group $P2_1/m$ with half-molecule in the asymmetric unit ($Z = 2$). The mirror plane symmtery generates the other half of the molecule and the overall conformation of the molecule is "*wing shaped*.". The presence of the bulky ester and *nitro* benzene moiety which is almost perpendicular to dihydropyridine ring results in a twist boat conformation for the six-membered dihydropyridine ring. The ORTEP of the compound are shown in Figure 2. The strong intermolecular N–H···O=N and weak C–H···O=N hydrogen bonds (involving H2A, O2 and H18B, O2) form a molecular chain along the [101] crystallographic direction associated with H···H contacts (involving H14, H18A) (Figure 3a).

Such molecular chains are connected via C–H···O=C (involving H11A, O3) and C–H···Cl (involving H11B, Cl1) hydrogen bonding dimers (shaded regions in Figure 3a). Furthermore, molecules are stabilized by π···π stacking (involving C3, C14) along the *b*-crystallographic axis followed by weak H···H contacts along crystallographic *a*- axis (Figure 3b). From the crystal structure analysis, it is to be noted that several intermolecular weak hydrogen bonds (such as C–H···O, C–H···Cl), π···π stacking and H···H contact plays an important role[20,34] along with strong directional N–H···O=N hydrogen bond towards the overall crystal packing of the compound **5h**. The intermolecular interactions of the compound are listed in Table 3.

3.3 | Larvicidal activity

Table 4 summarizes the results of the assessments of the larvicidal activity in a series of title compounds **5a–h**. Data analysis using general linear-mixed models showed that there were significant effects of treatment ($p < .0001$) and

exposure time ($p = .001$) but not their interaction ($p = .93$) on the larval mortality. Overall mortality showed a small but statistically significant ($p = .001$) increase with time (31.7 ± 0.8% at 24 hr and 33.7 ± 0.8% at 48 hr of exposure). Out of eight compounds from the series, highest mortality were exerted by molecules with a nitro group at the *para* position of the phenyl ring, which is at the fourth position of the 1,4-dihydropyridine ring in the case of compounds **5g** (64.4%) and **5h** (adjusted average mortality 71.1%), which were lower than the positive control obtained from Temephos (98.3%). Compounds with methoxy, trifluoromethyl, chloro and methyl on **5b**, **5c**, **5e** and **5f,** respectively, did not differ from the negative control acetone ($p > .05$). Larvae exposed to compounds **5a** and **5d**, with R = methoxy and ethoxy and R^1 = methoxy on the 1,4-dihydropyridine ring showed a low-mortality rate (ranging from 22% to 26%), although significantly higher than the negative control. These results indicate a moderate larvicidal activity of dihydropyridine **5g** and **5h** that merit further research for their potential application as anti-malarial agents.

4 | CONCLUSIONS

In conclusions, we have established a simple, environmental friendly and straightforward one-pot method for the synthesis of 1,4-dihydropyridine analogues *via* the Hantzsch ester reaction under catalyst-free conditions in aqueous medium at 70°C. It was of importance to screen all the eight synthesized compounds for their larvicidal activity, compounds **5g** and **5h** emerged as potential biocidal agent in comparison with the standard compound Temephos. Furthermore, the crystal structure analysis of highly potent compound **5h** revealed that the intermolecular N–H···O, C–H···O, C–H···Cl hydrogen bonds along with H···H and π···π stacking interactions govern the overall crystal packing in the solid state.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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