Tyrosinase enzyme mediated: Synthesis and larvicidal activity of 1,5-diphenyl pent-4-en-1-one derivatives against *Culex quinquefasciatus* and investigation of Ichthyotoxicity against *O. mossambicus*

SathishKumar Chidambaram

Research Department of Chemistry, Nehru Memorial College (Affiliated to Bharathidasan University),
Puthanampatti -621007, Tiruchirappalli District, Tamil Nadu

Daoud Ali

Department of Zoology, College of Sciences, King Saud University (KSU), Riyadh

Saud Alarifi

Department of Zoology, College of Sciences, King Saud University (KSU), Riyadh

Surendra Kumar Radhakrishnan

Research Department of Chemistry, Nehru Memorial College (Affiliated to Bharathidasan University),
Puthanampatti -621007, Tiruchirappalli District, Tamil Nadu

Idhayadhulla Akbar (✉ a.idhayadhulla@gmail.com)

Research Department of Chemistry, Nehru Memorial College (Affiliated to Bharathidasan University),
Puthanampatti -621007, Tiruchirappalli District, Tamil Nadu

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Abstract

Larvicidal activity of 1,5-diphenylpent-4-en-1-one derivatives were synthesized via grindstone method, tyrosinase enzyme used as a catalyst of this process. This method offers high yields with mild reaction conditions. Synthesized compounds were conformed from FTIR, ^1H NMR, ^{13}C NMR, mass spectral and elemental analysis. In this study, a total of 17 compounds (**1a–1q**) were synthesized, and their larvicidal and antifeedant activities were evaluated. Compound **1i** (1-(5-oxo-1,5-diphenylpent-1-en-3-yl)-3-(3-phenylallylidene)thiourea) was extremely active (LD_{50} : 12.09 $\mu\text{g/mL}$) against *Culex quinquefasciatus* compared with temephos and permethrin, whereas compounds **1i** at 100 $\mu\text{g/mL}$ generated 0% mortality within 24h against *Oreochromis mossambicus* in an antifeedant screening, and Ichthyotoxicity was determined as the death ratio (%) at 24 h. The compounds **1a**, **1e**, **1f**, **1j**, and **1k** were found to be highly toxic whereas the **1i** was not toxic in antifeedant screening. Therefore, **1i** was found to have a high larvicidal activity against *C. quinquefasciatus*, and was non-toxic to non-target aquatic species. Molecular docking studies also supported the finding that **1i** is a potent larvicide with more binding energy than the control (-10.0 vs. -7.6 Kcal/mol) in the 3OGN protein. The lead molecule is very important to larvicidal properties and insecticides.

Introduction

In the broadest sense, human beings are part of nature; however, our activity is often understood and interpreted as a separate and unique category from the rest of the natural phenomena. It is both the legal and moral obligation of every human to protect planet earth by undertaking activities that would prevent contaminating our planet and thereby protect it for future generations. For instance, as a chemist in chemical industries or academia, one could focus on protecting nature by employing green chemistry so as to produce various chemical and pharmaceutical active ingredients. Out of several green chemistry methodologies, grindstone chemistry technique is one of the simple methodology consist of preparing chemical compounds. Toda et al., was developed the chemical reactions carried out by simply grinding or triturating the solids together¹. We will now turn our focus towards Mannich reactions, which are a much-studied type of reaction in the organic and medicinal chemistry domains².

Mannich type reactions face significant challenges at present, major challenges such as reaction time, reaction conditions, toxicity, catalyst requirements and separating the purify of final product(s). Other challenges include synthetic methodologies such as ultrasound or microwave irradiation, the usage of Lewis acids or bases, or the usage of solubilizing agents or surfactant-type catalysts³. In addition, some of the known green trends in Mannich reactions consist of ball-milling without solvents⁴, using ionic liquid mediums⁵, using ionic liquids reinforced with nanoparticles⁶, or the application of enzymes under bio-catalytic conditions^{7,8}. However, present study focus grindstone green chemistry method was overcome the above challenges for preparation of Mannich base derivatives.

Mosquitoes are a very important transmission vector for several diseases, particularly malaria^{9,10}. These types of diseases have an economic and social impact all over the world. Among mosquito species, *Culex quinquefasciatus* is particularly associated with various disease vectors in several regions. Control of mosquitos presents a substantial challenge, and currently mosquito inhibitors such as Permethrin¹¹, organophosphates¹², fenthion^{13,14}, chlorpyrifos^{15–17}, temephos^{18,19}, diflubenzuron²⁰ and methoprene²¹ are used; Fig. 1 details the compositions of commercial insecticides. However, the usage of chemical insecticides causes bigger challenges and various potential environmental problems, such as widespread development of resistance and disrupted natural biological control systems^{22,23}. These problems require overcoming new mosquito larvae inhibitors, and give rise to a strong need to improve green methodologies so as to address these challenges; this need can be met through Mannich base condensation reactions. Mannich base synthesis is one of the best tools for the preparation of green synthesis. For this reason, we selected 1,5-diphenylpent-4-en-1-one derivatives, which are environmentally safe and match well with 1,3-diaryl-2-propen-1-ones (chalcones, Fig. 1) and with mosquito larvicidal properties²⁴. In this study, we sought to develop a simple and efficient grindstone chemistry methodology that can overcome the above identified challenges and limitations so as to obtain novel water-soluble and nontoxic Mannich base derivatives.

Results And Discussion

Chemistry

A one-pot multicomponent of title compounds was achieved using grindstone green chemistry method. The synthetic route outline was represented in Scheme 1. The proposed mechanism for the formation of Mannich base derivative was displayed in Scheme 2. Copper containing materials like coppertriflate²⁵, copperacetate²⁶, copperbromide²⁷ and copper nanoparticles²⁸ plays a vital role in Mannich base reaction. One-pot multicomponent Mannich reaction was synthesized via various enzymes catalyzed such as, trypsin²⁹, lipase^{30,31} and protease³². Present study copper containing tyrosinase enzyme was used as a catalyst for the synthesis of *N*-Mannich base (**1a–1q**) derivatives. The title compounds were synthesized using the catalysts trypsin, lipase, protease, CuCl₂·2H₂O, and tyrosinase enzyme with yields of 64%, 72%, 68%, 84%, and 92%, respectively. The use of the Tyrosinase enzyme green catalyst, instead of CuCl₂·2H₂O, increased the yield of the Mannich derivatives to 92% and reduced the reaction time. The optimization of reaction conditions and catalysts were presented in Tables 1 and 2. The obtained compounds were analyzed via FT-IR, ¹H, and ¹³C NMR spectra. The key assignments of the compounds showed significant bands at 3170.23–3176.54, 2595.45–2599.98 and 1710.68–1716.70 cm⁻¹ in the IR spectrum, conforming towards the -NH, -C = N and -C = O groups, respectively. The ¹H NMR showed signals at δ 8.03–9.70, 3.82–4.81 and 2.40–2.98 ppm, indicating -NH, 4-CH, and -CH₂ protons, respectively. The ¹³C NMR showed peaks at δ 197.4–197.6, 48.4–59.2, and 48.0–50.6 ppm, which conforms to -C = O, -CH, and -CH₂ atoms, respectively. The mass spectra and elemental analysis were used to satisfy with the conformation of all compounds.

Table 1
Catalyst optimization for compound **1a**

Entry	Catalyst	Yield (%)	Time (min)
1	No enzyme	06	30
2	Trypsin from bovine pancreas	64	8
3	Lipase from <i>Candida antarctica</i>	72	12
4	Protease from <i>Streptomyces griseus</i>	68	10
5	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	84	5
6	Tyrosinase from mushroom	92	2

Table 2. Optimization of reactants and yield with final product for compounds **1a–1q**.

Compounds	R	Final Products	Yield (%)
1a	NH ₂ -NH ₂		92
1b			86
1c			88
1d			85
1e			88
1f			86
1g			91
1h			80
1i			87
1j			88
1k			85
1l			84
1m			82
1n			82
1o			80
1p			89
1q	H ₂ C-NH ₂		86

Biological activity

A total of 17 compounds (**1a–1q**) were tested against second instar *C. quinquefasciatus* larvae and the toxicity of the title compounds was evaluated in the marine fish *Oreochromis mossambicus*. Toxicity was defined as ratios of deaths (%) at 24h. Structure activity relationships showed that the final compounds contain 1,5-diphenylpent-4-en-1-one with different types of amines, thus producing larvicidal activity and toxicity based on the formation of chemical compositions. Compound **1i** showed more larvicidal activity relative to other compounds, with an LD₅₀ of 12.09 ± 0.23 µg/mL, which was better than that of the

controls temephos (LD_{50} of $17.74 \pm 0.01 \mu\text{g/mL}$)³³ and permethrin (LD_{50} of $21.40 \pm 0.02 \mu\text{g/mL}$). The compound **1a** induced 80% mortality at 100 $\mu\text{g/mL}$ and its LD_{50} value was $59.45 \pm 0.02 \mu\text{g/mL}$, whereas the antifeedant induced 100% mortality at 100 $\mu\text{g/mL}$ and had a LD_{50} value of 13.23 $\mu\text{g/mL}$; this is due to the compound presence of the hydrazine group, which showed full toxicity against *O. mossambicus* fingerlings within 15 min of screening.

Compounds **1b**, **1g**, and **1p** induced 40% mortality at 100 $\mu\text{g/mL}$, whereas the antifeedant induced 20% mortality at 100 $\mu\text{g/mL}$ due to the compound presence of the benzylidenehydrazine group, indicating that they are less toxic than compound **1a**. Moderate activity was observed from compound **1c**, which reached 40% mortality at 100 $\mu\text{g/mL}$, whereas the antifeedant reached 0% mortality at 100 $\mu\text{g/mL}$ due to the presence of the (3-phenylallylidene) hydrazine group; therefore it is less toxic than compounds **1b**, **1g**, **1p**, and **1c**. Compound **1d** and **1o** induced 0% mortality at 100 $\mu\text{g/mL}$ in both the larvicidal and antifeedant screening due to the presence of the 5-hydrazonopentanal and 1-benzylideneurea groups; thus they exhibited no active and no toxic behavior.

Compound **1k** also induced 0% mortality at 100 $\mu\text{g/mL}$ in larvicidal screening, and was highly toxic in antifeedant screening, inducing 100% mortality with a LD_{50} value of 13.78 $\mu\text{g/mL}$ due to the presence of the p-toluidine group. Compounds **1h** and **1e** induced 60% mortality at 100 $\mu\text{g/mL}$ in larvicidal screening, and it induced LD_{50} values of 65.85 and 66.25 $\mu\text{g/mL}$ due to the presence of the 1-benzylidenethiourea and phenylhydrazine groups. Compounds **1f** and **1j** induced a mortality rate of 80% with an LD_{50} values of 58.10 and 58.49 $\mu\text{g/mL}$ in larvicidal screening, whereas they induced 100% mortality in antifeedant screening due to the presence of aniline and naphthalen-2-amine, respectively. Compounds **1m** and **1n** induced a mortality rate of 80% with an LD_{50} values of 56.77 and 56.16 $\mu\text{g/mL}$ in larvicidal screening, whereas they induced 0% mortality in antifeedant screening due to the presence of benzamide and urea, respectively. Compounds **1q** and **1l** induced a mortality rate of 20% in larvicidal screening, whereas they induced 20% mortality in antifeedant screening due to the presence of methylamine and acetamide, respectively. Therefore, the above analysis indicates that the compound **li** was significantly active in larvicidal and displayed less toxicity in antifeedant screening. The percentage of mortality and LD_{50} values are presented in Table 3 and Table 4.

Table 3
Larvicidal activity of compounds (1a-1q).

Compounds	% of Mortality at 25 µg/mL	% of Mortality at 50 µg/mL	% of Mortality at 100 µg/mL	LD ₅₀ (µg/mL) ^a
1a	24.13 ± 0.23	43.19 ± 0.12	80.23 ± 0.23	59.45 ± 0.02
1b	11.18 ± 0.19	27.11 ± 0.16	40.12 ± 0.12	> 100
1c	19.29 ± 0.41	26.27 ± 0.41	40.25 ± 0.62	> 100
1d	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1e	33.31 ± 0.11	48.30 ± 0.18	60.36 ± 0.21	66.25 ± 0.27
1f	25.00 ± 0.20	44.12 ± 0.20	80.00 ± 0.20	58.1 ± 0.20
1g	22.10 ± 0.21	34.18 ± 0.19	40.12 ± 0.29	> 100
1h	34.50 ± 0.22	47.91 ± 0.28	60.13 ± 0.22	65.85 ± 0.2
1i	68.23 ± 0.38	88.21 ± 0.60	100 ± 0.00	12.09 ± 0.23
1j	26.1 ± 0.21	44.66 ± 0.21	80.36 ± 0.28	58.49 ± 0.23
1k	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1l	20.85 ± 0.14	20.85 ± 0.14	20.85 ± 0.14	> 100
1m	29.94 ± 0.27	42.32 ± 0.27	80.95 ± 0.27	56.77 ± 0.23
1n	29.90 ± 0.25	43.62 ± 0.25	80.98 ± 0.25	56.16 ± 0.03
1o	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1p	40.44 ± 0.09	40.44 ± 0.09	40.44 ± 0.09	> 100
1q	20.41 ± 0.16	20.41 ± 0.16	20.41 ± 0.16	> 100
Permethrin	51.12 ± 0.9	76.26 ± 0.10	100 ± 0.00	21.43 ± 0.02
Temephos	56.12 ± 0.23	79.26 ± 0.19	100 ± 0.00	17.74 ± 0.01
^a Values are mean ± SD (n = 3).				

Table 4
Antifeedant activity of compounds (**1a-1q**).

Compounds	% of Mortality at 10 µg/mL	% of Mortality at 25 µg/mL	% of Mortality at 50 µg/mL	% of Mortality at 100 µg/mL	LD ₅₀ (µg/mL) ^a
1a	33.32 ± 0.21	66.21 ± 0.00	88.25 ± 0.00	100 ± 0.00	13.23 ± 0.77
1b	20.23 ± 0.33	20.23 ± 0.33	20.23 ± 0.33	20.23 ± 0.33	> 100
1c	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1d	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1e	31.32 ± 0.00	66.1 ± 0.00	82.23 ± 0.00	100 ± 0.00	16.45 ± 0.05
1f	41.25 ± 0.00	51.32 ± 0.00	72.21 ± 0.00	100 ± 0.00	21.15 ± 0.48
1g	-	05.18 ± 0.13	10.31 ± 0.13	20.55 ± 0.21	> 100
1h	5.32 ± 0.13	20.18 ± 0.13	49.43 ± 0.13	60.41 ± 0.13	52.28 ± 0.80
1i	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1j	42.22 ± 0.41	59.25 ± 0.35	88.23 ± 0.00	100 ± 0.00	10.12 ± 0.24
1k	33.12 ± 0.00	67.11 ± 0.74	87.87 ± 0.00	100 ± 0.00	13.78 ± 0.63
1l	-	05.18 ± 0.13	10.31 ± 0.13	20.18 ± 0.13	> 100
1m	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1n	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1o	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1p	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	> 100
1q	-	05.18 ± 0.13	10.31 ± 0.13	20.18 ± 0.93	> 100
^a Values are mean ± SD (n = 3).					

Culex quinquefasciatus larval growth regulation

To explore the impact of 1,5-diphenylpent-4-en-1-one formulations on *C. quinquefasciatus* larvae growth, metamorphosis, and production, we exposed the larvae to compound **1i** for 72 hours. Table 5 summarizes the effects of compound **1i** impact on larval weight and growth inhibition. When subjected to 10 µg/mL of compound **1i**, the eclosion rate and time of the pupal and adult periods of administered *C. quinquefasciatus* is calculated, and the findings are seen in Table 6. Compound **1i** had a growth-inhibition score of 41.36 % and suppressed larval weight development. Furthermore, compound **1i** had little effect on the duration of the adult and pupal periods, but it did result in a 55 percent eclosion rate.

Compound **1i** hindered the production and growth of *C. quinquefasciatus* larvae, according to these findings.

Table 5
Compound **1i** on the growth of *Culex quinquefasciatus*

Compound	Weight (mg)		Weight gain (mg)	Inhibition (%)
	0 h	72 h		
1i ^a	100.28 ± 1.86	104.14 ± 0.23	3.86 ± 0.86	41.36 ± 2.80
Control ^b	100.06 ± 0.34	106.65 ± 1.54	6.58 ± 1.42	-
^a The concentration of 1i was 10 µg/mL				
^b Control is not containing the compounds				

Table 6
Compound **1i** on the development and growth of *Culex quinquefasciatus*

Compound	Duration of pupae (h)	Duration of adult (h)	Rate of eclosion (%)
1i ^a	68.1 ± 0.64	23.1 ± 1.36	55 ± 1.72
Control ^b	65.5 ± 1.21	24.2 ± 0.82	80 ± 0.96
^a The concentration of 1i was 10 µg/mL ^b Control is not containing the compounds			

Docked results

The Autodock Vina program was used to assess the docking behavior between compounds **1i**, **permethrin** and **temephos** with the 3OGN protein. Compound **1i** displayed more binding affinity (-10.0 kcal/mol) than other compounds and **permethrin** (-9.7kcal/mol) and **temephos** (-7.6 kcal/mol) with the 3OGN protein. Residues of the amino acids Leu19, Leu73, Leu76, His77, Ala78, Trp114, and Leu124 were tangled in hydrophobic connections. The interaction of compound **1i** with 3OGN is shown in Fig. 2. In the control **permethrin**, residues of the amino acids Leu15, Leu19, Phe59, Leu73, Leu76, His77, Leu80, Ala88, Met89, Gly92, His111, Trp114, Phe123, and Leu124 were tangled in hydrophobic connections.

The positive control **permethrin** connected in the 3OGN protein is shown in Fig. 3. The control **temephos** displayed three hydrogen bond interactions with the receptor 3OGN. The amino acid residue Ser79 showed two hydrogen bonds with **temephos**, with the bond lengths of 3.32 and 2.26 Å, and the amino acid residue Ala88 showed one hydrogen bond with **temephos**, with the bond length of 3.25 Å. Residues of the amino acids Leu19, Ala62, Leu76, Met91, Trp114, and Tyr122 were involved in hydrophobic contacts with the receptor. The interaction of the control **temephos** with the 3OGN protein is shown in

Fig. 4. The helix representation of inhibitor molecule docked into the receptor was shown in Fig. 2a, 3a, and 4a. The inhibitor molecule docked into the binding pocket of the receptor was shown in Fig. 2b, 3b, and 4b. The 3D representation of inhibitor molecule docked into the receptor was shown in Fig. 2c, 3c, and 4c. The 2D representation molecule docked with receptor was shown in Fig. 2d, 3d and 4d. The results show that compound **1i** possesses comparable inhibition abilities relative to the controls **permethrin** and **temephos**. The results are listed in Table 7.

Table 7
Molecular docking interaction of compounds (**1a-1q**) and control **Temephos**,
Permethrin.

Compounds	Mosquito odorant-binding protein 30GN		
	Binding affinity (kcal/mol)	No. of H-bonds	H-bonding residues
1a	-9.0	2	His121, Phe123
1b	-9.7	0	-
1c	-9.0	0	-
1d	-8.8	0	-
1e	-9.7	1	Phe123
1f	-9.6	0	-
1g	-8.3	0	-
1h	-9.3	0	-
1i	-10.0	0	-
1j	-9.8	0	-
1k	-9.8	0	-
1l	-8.9	0	-
1m	-9.8	0	-
1n	-8.8	0	-
1o	-9.5	0	-
1p	-9.2	0	-
1q	-8.3	0	-
Temephos	-7.6	3	Ser79, Ala88
Permethrin	-9.7	0	-

Catalyst recovery studies

The recovered catalyst was recycled for at least 10 run times with a small defeat in catalytic action (Fig. 5). The decrease in catalytic action perceived through the reinforced catalyst on recycling might be owing to limited loss of basic locates or loss of catalyst surface area during regeneration/reaction. The values are displayed in Table 8.

Table 8
Catalyst recyclability

Entry	Catalyst	Yield (%)
1	1st use	92
2	2nd use	92
3	3rd use	90
4	4th use	90
5	5th use	88
6	6th use	87
7	7th use	87
8	8th use	86
9	9th use	86
10	10th use	85

Materials And Methods

Chemistry

Thermo scientific Nicolet iS5 FTIR ($4000\text{--}400\text{ cm}^{-1}$) was used for analysis of all compounds. Bruker DRX-300 MHz, 75 MHz was used for the analysis of ^1H and ^{13}C NMR spectra. An elemental analyzer (model Vario EL III) was used to analyze elements (C, H, N, and S) percentage (%). Mass spectra were recorded by Perkin Elmer GCMS model Clarus SQ8 (EI).

General Procedure For The Synthesis Of Compound 1a

A reaction mixture made up of cinnamaldehyde (0.01 mol, 1.32 mL), acetophenone (0.01 mol, 1.20 mL), substituted amine (0.01 mol) and tyrosinase enzyme (0.5g) was mixed in a mortar and ground at RT. Then 2mL of 50mM potassium phosphate buffer (pH 6.0) was added and filtered to recover the catalyst. The final filtered solid material was separated using column chromatography (Ethyl acetate4:hexane6). The same method was followed when mixing compounds **1b–1q**.

3-hydrazinyl-1,5-diphenylpent-4-en-1-one (1a)

White solid; mp 110–112°C; IR(KBr) ν : 3171.48, 3065.51, 3041.02, 1715.02, 1624.53 cm^{-1} ; ^1H NMR (300 MHz): δ 9.20 (s, 1H), 8.84 (s, 2H, NH_2), 7.97–7.96 (dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 2H, Ar-ring), 7.63–7.60 (d, $J = 6.21\text{Hz}$, 1H, Ar-ring), 7.53–7.51 (dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H, Ar-ring), 7.41–7.37 (dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 2H, Ar-ring), 7.33 (d, $J = 6.21\text{Hz}$, 1H, Ar-ring), 7.21 (dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H, Ar-ring), 6.56–6.51 (d, $J = 6.21\text{Hz}$, 1H, CH), 6.19–6.14 (d, $J = 6.21\text{Hz}$, 1H), 3.84–3.80 (m, 1H), 2.94–2.91 (d, $J = 6.21\text{Hz}$, 2H); ^{13}C NMR (75 MHz): 197.4 (1C), 136.7, 133.1, 128.8, 128.6 (6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 133.4 (1C), 128.4 (1C), 59.2 (1C), 48.0 (1C); EIMS (m/z): 267.15 (M^+ , 18%); Anal. Calcd. for $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}$: C, 76.66; H, 6.81; N, 10.52%; found: C, 76.68; H, 6.80; N, 10.51%.

3-(2-benzylidenehydrazinyl)-1,5-diphenylpent-4-en-1-one (1b)

Greenish solid; mp 145–148°C; IR(KBr) ν : 3176.51 (NH), 3072.50, 3032.32, 2596.43, 1716.08, 1623.43; ^1H NMR(300MHz): δ 9.21(s,1H), 8.36(s,1H,CH), 7.97–9.94(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$), 7.86–7.81 (dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$), 7.63–7.60(d, $J = 6.21\text{Hz}$, 1H, Ph), 7.55–7.53(dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H), 7.50–7.47(m, 3H, Ar ring), 7.40–7.38(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 2H, Ar ring), 7.34–7.31(d, $J = 6.21\text{Hz}$, 1H, Ar ring), 7.20–7.17(dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H, Ar ring), 6.58–6.54 (d, 1H, $J = 6.21\text{Hz}$, CH), 6.18–6.14(d, $J = 6.21\text{Hz}$, 1H, CH), 3.80–3.76(m, 1H, CH), 2.95–2.92(d, $J = 6.21\text{Hz}$, 2H, CH_2); ^{13}C NMR (75MHz): 197.6 (1C), 143.3 (1C), 136.6, 133.0, 128.7, 128.5(6C, Ph ring), 136.5, 128.7, 128.6, 128.0(6C, Ar ring), 134.4(1C), 133.7, 131.0, 129.2, 128.8 (6C, Ph ring), 128.5(1C), 55.1(1C), 48.5(1C); EIMS(m/z) 355.18 (M^+ , 26%); Anal. Calcd. for $\text{C}_{24}\text{H}_{22}\text{N}_2\text{O}$: C, 81.33; H, 6.26; N, 7.90 %; found: C, 81.31; H, 6.27; N, 7.91 %.

1,5-diphenyl-3-(2-(3-phenylallylidene)hydrazinyl)pent-4-en-1-one (1c)

Light green powder; mp 148–150°C; IR(KBr) ν 3176.50, 3073.51, 3031.30, 2595.45, 1714.08, 1624.40 cm^{-1} ; ^1H NMR(300MHz): δ 9.26(s, 1H, NH), 7.95–7.91(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$), 7.63–7.60–7.58(d, $J = 6.21\text{Hz}$, 1H), 7.57–7.54(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 2H), 7.53–7.50(dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H, Ph), 7.50(s, 1H, CH), 7.40–7.37(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 4H, Ar ring), 7.36–7.33 (d, $J = 6.21\text{Hz}$, 2H, Ar-ring), 7.24–7.21(dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H, Ar ring), 6.54–6.52(d, $J = 6.21\text{Hz}$, 2H, CH), 6.17–6.12(d, $J = 6.21\text{Hz}$, 2H, CH), 3.78–3.75(m, 1H), 2.91–2.89 (d, $J = 6.21\text{Hz}$, 2H); ^{13}C NMR (75 MHz): 197.2 (1C), 137.2 (1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.8 (6C, Ar ring), 135.2, 128.6, 128.5, 127.9(6C, Ph ring), 133.9, 133.7, 128.2, 125.3, 56.2, 48.5; EIMS(m/z): 381.19(M^+ , 28%); Anal. Calcd. for $\text{C}_{26}\text{H}_{24}\text{N}_2\text{O}$: C, 82.07; H, 6.36; N, 7.36 %; found: C, 82.05; H, 6.37; N, 7.37 %.

5-(2-(5-oxo-1,5-diphenylpent-1-en-3-yl)hydrazono)pentanal (1d)

White powder; mp 126–129°C; IR(KBr) ν : 3176.54, 3073.50, 3031.32, 2595.48, 1714.18, 1624.45 ; ^1H NMR (300MHz): δ 9.70(s, 1H, CH), 9.24(s, 1H), 7.97–7.95(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 2H), 7.60–7.57(d, $J = 6.21\text{Hz}$, 1H), 7.53–7.50(dd, $J = 7.31\text{Hz}$, $J = 7.33\text{Hz}$, 2H), 7.42–7.37(dd, $J = 7.33\text{Hz}$, $J = 7.37\text{Hz}$, 2H, Ar ring), 7.34–7.31(d, $J = 6.21\text{Hz}$, 1H, Ar ring), 7.21(dd, $J = 7.31\text{Hz}$, $J = 7.35\text{Hz}$, 2H, Ar ring), 6.97(s, 1H, CH), 6.56–

6.51(d, J = 6.21Hz, 1H), 6.16–6.13(1H, d, J = 6.21Hz, CH), 3.83–3.83-2(m, 1H, CH), 2.93–2.88 (d, J = 6.21Hz, 2H), 2.42–2.36(m, 2H), 1.82–1.76(m, 2H), 1.53–1.49 (m, 2H); ^{13}C NMR(75MHz): 202.2, 197.4, 158.3, 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 28.5, 127.9(6C, Ar ring), 134.7, 134.1, 127.9, 56.1, 48.5, 43.3, 25.9(1C); EIMS(m/z): 349.19(M^+ , 24%); Anal.Calcd.for $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_2$: C, 75.83; H, 6.94; N, 8.04 %; found: C, 75.80; H, 6.96; N, 8.06 %.

1,5-diphenyl-3-(2-phenylhydrazinyl)pent-4-en-1-one (1e)

White powder; mp 143–145°C; IR(KBr) ν : 3176.52, 3073.50, 3031.28, 1714.10, 1624.38 cm^{-1} ; ^1H NMR(300MHz): δ 9.25 (s, 1H), 9.18(s, 1H), 7.97(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ph), 7.65(d, J = 6.21Hz, 1H), 7.55–7.53(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.38–7.34(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar ring), 7.35–7.32(dd, J = 7.31Hz, J = 7.35Hz, Ph), 7.32–7.30(d, J = 6.21Hz, 1H, Ar-ring), 7.21–7.19(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar-ring), 7.02–6.98(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ph), 6.88–6.86 (d, J = 6.21Hz, 1H, Ar-ring), 6.56–6.54(d, J = 6.22Hz, 1H), 6.17–6.15(d, J = 6.21Hz, 1H), 3.84–3.81(m, 1H), 2.95–2.92(d, J = 6.21Hz, 2H); ^{13}C NMR (75 MHz): 197.4(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.8 (6C, Ar ring), 151.0, 129.2, 122.8, 113.2 (6C, Ph ring), 134.2, 127.9, 56.6, 48.3; EIMS(m/z): 343.18 (M^+ , 25%); Anal. Calcd. for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}$: C, 80.67; H, 6.48; N, 8.18 %; found: C, 80.65; H, 6.47; N, 8.19 %.

1,5-diphenyl-3-(phenylamino)pent-4-en-1-one (1f)

Yellow powder; mp 101–103°C; IR(KBr) ν : 3176.53, 3072.50, 3030.28, 1715.10, 1623.38; ^1H NMR (300MHz): δ 9.26(s, 1H, NH), 7.97–7.95(dd, J = 7.33Hz, J = 7.37Hz, 2H), 7.66–7.64(d, J = 6.21Hz, 1H), 7.53–7.51(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.44–7.41(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar ring), 7.35–7.31 (d, J = 6.21Hz, 1H, Ar-ring), 7.28–7.23(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar-ring), 7.23–7.20(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ph), 6.83–6.80(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ph), 6.74–6.71(d, J = 6.21Hz, 1H, Ar ring), 6.56–6.54 (d, J = 6.20Hz, 1H, CH), 6.19–6.17(d, J = 6.21Hz, 1H), 3.84–3.80(m, 1H, -CH), 2.90–2.87(d, J = 6.21Hz); ^{13}C NMR(75 MHz): 197.4(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 147.6, 129.5, 120.8, 119.7 (6C, Ph ring), 133.1, 127.7, 57.2, 50.5; EIMS(m/z): 328.17 (M^+ , 25%); Anal. Calcd. for $\text{C}_{23}\text{H}_{21}\text{NO}$: C, 84.37; H, 6.46; N, 4.28 %; found: C, 84.30; H, 6.49; N, 4.30 %.

1-(5-oxo-1,5-diphenylpent-1-en-3-yl)thiourea (1g)

Green solid; mp 139–141°C; IR(KBr) ν : 3176.51, 3072.74, 3029.32, 1712.18, 1625.45; ^1H NMR (300MHz), δ 9.22(1H, s, NH), 8.52(s, 2H, NH_2), 7.97–7.94(dd, J = 7.33Hz, J = 7.37Hz, 2H), 7.63–7.61(d, J = 6.21Hz, 1H, Ph), 7.55–7.50(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ph), 7.40–7.36(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar ring), 7.33–7.30(1H, d, J = 6.21Hz, Ar ring), 7.23–7.19(dd, 2H, J = 7.31Hz, J = 7.35Hz, Ar ring), 6.56–6.54(d, J = 6.21Hz, 1H), 6.19–6.17(d, J = 6.21Hz, 1H), 3.82–3.79(m, 1H), 2.99–2.97 (d, J = 6.21Hz, 2H); ^{13}C NMR(75MHz): 197.4(1C), 182.0(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 28.5, 127.9 (6C, Ar ring), 134.2, 128.2, 55.6, 50.6; EI-MS(m/z) 311.12 (M^+ , 19%); Anal. Calcd. for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{OS}$: C, 69.65; H, 5.84; N, 9.02 %; found: C, 69.68; H, 5.85; N, 9.06 %.

1-benzylidene-3-(5-oxo-1,5-diphenylpent-1-en-3-yl)thiourea (1h)

Brown powder; mp 111–114°C; IR(KBr) ν : 3175.53, 3070.50, 3032.28, 2597.48, 1714.10, 1624.38; ^1H NMR(300MHz) δ 9.47(s, 1H), 9.26(s, 1H), 7.97–7.94(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar-ring), 7.86–7.84(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.63–7.59(d, 1H, J = 6.21Hz, Ar-ring), 7.53–7.51(2H, dd, J = 7.31Hz, J = 7.35Hz Ph), 7.50–7.44(3H, m, Ar-ring), 7.40–7.37(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar ring), 7.34–7.31(d, J = 6.21Hz, 1H, Ar ring), 7.26–7.24(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar ring), 6.56–6.54(d, J = 6.20Hz, 1H), 6.19–6.17(d, J = 6.21Hz, 1H), 3.84–3.81(m, 1H), 2.94–2.92(d, J = 6.21Hz, 2H); ^{13}C NMR (75MHz): 197.4(1C), 182.0(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9(6C, Ar ring), 135.2, 134.4, 116.1, 20.6 (6C, Ph ring), 134.6, 128.1, 55.6, 50.1, 14.4; EIMS(m/z): 399.15(M^+ , 27%); Anal. Calcd. for $\text{C}_{25}\text{H}_{22}\text{N}_2\text{OS}$: C, 75.35; H, 5.56; N, 7.03 %; found: C, 75.30; H, 5.60; N, 7.04 %.

1-(5-oxo-1,5-diphenylpent-1-en-3-yl)-3-(3-phenylallylidene)thiourea (1i)

Light yellow powder; mp 276–279°C; IR(KBr) ν : 3174.23, 3069.30, 3031.68, 2598.98, 1715.70, 1626.38; ^1H NMR (300MHz): δ 9.24(s, 1H), 7.52(s, 1H), 7.97–9.96(dd, J = 7.33Hz, J = 7.37Hz, 2H), 7.65–7.63 (d, J = 6.21Hz, 1H), 7.62–7.59(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.53–7.50(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.42–7.39 (dd, J = 7.33Hz, J = 7.37Hz, 4H, Ar ring), 7.31–7.27(d, J = 6.21Hz, 2H, Ar ring), 7.27–7.25(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar ring), 7.22–7.18(d, J = 6.21Hz, 1H, CH), 6.81–6.79(d, J = 6.21Hz, 1H), 6.56–6.52(d, J = 6.21Hz, 1H), 6.19–6.17(d, J = 6.21Hz, 1H, CH), 3.82–3.79(m, 1H), 2.94–6.92(d, J = 6.21Hz, 2H); ^{13}C NMR(75MHz): 197.4 (1C), 189.3(1C), 163.7, 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.9(6C, Ar ring), 135.2, 134.4, 116.1, 20.6(6C, Ph ring), 134.6, 132.9, 128.3, 119.9, 55.9, 50.6; EIMS(m/z) 425.16 (M^+ , 30%); Anal. Calcd. for $\text{C}_{27}\text{H}_{24}\text{N}_2\text{OS}$: C, 76.38; H, 5.70; N, 6.60 %; found: C, 76.30; H, 5.74; N, 6.62 %.

3-(naphthalen-2-ylamino)-1,5-diphenylpent-4-en-1-one (1j)

Dark yellow colour; mp 101–104°C; IR(KBr) ν : 3174.63, 3069.70, 3031.48, 1715.50, 1626.48 cm^{-1} ; ^1H NMR (300MHz): δ 9.26(s, 1H, NH), 7.97–7.94 (dd, J = 7.33Hz, J = 7.37Hz, 2H, Ph), 7.88–7.84 (d, J = 6.21Hz, 1H, Naphthyl), 7.83–7.81(d, J = 6.21Hz, 1H, Naphthyl), 7.77–7.74(d, J = 6.21Hz, 1H, Naphthyl), 7.49–7.47 (d, J = 6.21Hz, 1H, Naphthyl), 7.45–7.41 (d, J = 6.21Hz, 1H, Naphthyl), 7.50–7.48(dd, J = 7.31Hz, J = 7.35Hz, 2H, Naphthyl), 7.63–7.60(d, J = 6.21Hz, 1H, Ph), 7.53–7.50(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar-ring), 7.42–7.40 (dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar ring), 7.35–7.33(d, J = 6.21Hz, 1H, Ar-ring), 7.25–7.21(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar, ring), 6.56–6.54(d, J = 6.21Hz, 1H, CH), 6.19–6.17(d, J = 6.21Hz, 1H), 3.84–3.81(m, 1H), 2.90–2.87(d, J = 6.21Hz, 2H); ^{13}C NMR (75MHz): 197.4(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9(6C, Ar ring), 146.0, 133.7, 129.0, 126.8, 126.5, 125.3, 124.6, 121.4, 118.1, 104.5(10C, Naphthyl ring), 134.4, 128.1, 57.2, 50.5; EI-MS(m/z) 378.18 (M^+ , 29%); Anal. Calcd. for $\text{C}_{27}\text{H}_{23}\text{NO}$: C, 85.91; H, 6.14; N, 3.71 %; found: C, 85.90; H, 6.10; N, 3.76 %.

1,5-diphenyl-3-(p-tolylamino)pent-4-en-1-one (1k)

White powder; mp 72–74°C; IR(KBr) ν : 3173.23, 3068.30, 3030.68, 1714.70, 1625.38; ^1H NMR (300MHz): δ 9.28(s, 1H), 7.50(s, 1H, -CH), 7.97–7.96(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ph), 7.64(d, J = 6.21Hz, 1H), 7.53(dd, J = 7.31Hz, J = 7.34Hz, 2H), 7.39(dd, J = 7.33Hz, J = 7.37Hz, 4H, Ar-ring), 7.33–7.31(d, J = 6.21Hz, 2H, Ar-ring), 7.25–7.22(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar-ring), 7.22–7.18(d, J = 6.21Hz, 1H), 7.01–6.98(dd, J = 7.31Hz, J = 7.35Hz, 1H, Ph), 6.85–6.84(1H, d, J = 6.21Hz), 2.90–2.87 (d, J = 6.21Hz, 2H), 2.34(s, 3H); ^{13}C NMR (75MHz): 197.4(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 144.6, 129.8, 129.6, 113.4(6C, 4-CH₃-Ph ring), 134.5, 128.6, 55.2, 50.6, 21.3; EIMS(m/z) 342.18(M^+ , 26%); Anal. Calcd. for C₂₄H₂₃NO: C, 84.42; H, 6.79; N, 4.10 %; found: C, 84.30; H, 6.89; N, 4.12 %.

N-(5-oxo-1,5-diphenylpent-1-en-3-yl)acetamide (1l)

Pale yellow powder; mp 172–174°C; IR(KBr) ν : 3170.23, 3065.30, 3027.68, 1711.70, 1622.38; ^1H NMR (300MHz): δ 8.05(s, 1H, NH), 7.95–7.92 (dd, J = 7.33Hz, J = 7.37Hz, 2H, Ph), 7.65–7.66(d, J = 6.21Hz, 1H), 7.54–7.50(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar-ring), 7.38–7.34(dd, J = 7.33Hz, J = 7.37Hz, 1H, Ar ring), 7.31–7.28(d, J = 6.21Hz, 2H, Ar-ring), 7.25–7.21(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar ring), 6.56–6.53(d, J = 6.21Hz, 1H, CH), 6.17–6.15(d, J = 6.21Hz, 1H), 4.81–4.78 (m, 1H), 2.94–2.91(d, J = 6.21Hz, 2H), 1.84 (s, 3H); ^{13}C NMR (75MHz): 197.4(1C), 170.7(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9(6C, Ar ring), 134.1, 127.9, 48.4, 50.4, 23.7; EIMS(m/z) 294.14 (M^+ , 20%); Anal. Calcd. for C₁₉H₁₉NO₂: C, 77.79; H, 6.53; N, 4.77 %; found: C, 77.80; H, 6.51; N, 4.75 %.

N-(5-oxo-1,5-diphenylpent-1-en-3-yl)benzamide (1m)

Brown powder; mp 205–208°C; IR(KBr) ν : 3172.21, 3063.28, 3025.66, 1710.68, 1620.36; ^1H NMR (300MHz): δ 8.41(s, 1H, NH), 8.03–7.96(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar-ring), 7.97(dd, J = 7.33Hz, J = 7.37Hz, 2H), 7.70–7.67(1H, d, J = 6.21Hz, Ar ring), 7.63–7.60(3H, m, Phenyl), 7.53–7.50(dd, J = 7.31Hz, J = 7.33Hz, 2H), 7.42–7.38 (dd, J = 7.33Hz, J = 7.37Hz, 4H, Ar ring), 7.33–7.30 (1H, d, J = 6.21Hz, Ph), 6.51–6.49(d, J = 6.21Hz, 1H), 6.19–6.17(d, J = 6.21Hz, 1H), 4.81–4.78(1H, m, -CH), 2.98–2.95(d, J = 6.21Hz, 2H); ^{13}C NMR (75MHz): 197.4(1C), 167.5(1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 134.2, 132.1, 128.8, 127.5 (6C, Ph ring), 135.1, 127.9, 49.2, 50.4; EIMS(m/z): 356.16 (M^+ , 26%); Anal. Calcd. for C₂₄H₂₁NO₂: C, 81.10; H, 5.96; N, 3.94 %; found: C, 80.10; H, 6.86; N, 4.04 %.

1-(5-oxo-1,5-diphenylpent-1-en-3-yl)urea (1n)

Pale green powder; mp 260–262°C; IR (KBr) ν : 3173.21, 3064.28, 3026.66, 1711.68, 1621.36; ^1H NMR (300MHz): δ 9.22(s, 1H, NH), 8.83(s, 2H, NH₂), 7.97–7.94 (dd, J = 7.33Hz, J = 7.37Hz, 2H), 7.63–7.60 (m, 1H, Phenyl), 7.55–7.53(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.40–7.37(dd, J = 7.33Hz, J = 7.37Hz, 1H, Ar ring), 7.34–7.31(d, J = 6.21Hz, 2H, Ar ring), 7.22–7.19(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar ring), 6.56–6.54(d, J = 6.20Hz, 1H), 6.17–6.15(d, J = 6.21Hz, 1H), 4.81–4.78 (m, 1H), 2.94–2.91 (d, J = 6.21Hz, 2H); ^{13}C NMR(75MHz): 197.4 (1C), 162.7 (1C), 136.7, 133.1, 128.8, 128.6, (6C, Ph ring), 136.4, 128.6, 128.5,

127.9(6C, Ar ring), 133.9, 128.9, 50.5, 49.9; EIMS(*m/z*): 295.14 (M^+ , 19%); Anal. Calcd. for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52 %; found: C, 73.40; H, 6.17; N, 9.54 %.

1-benzylidene-3-(5-oxo-1,5-diphenylpent-1-en-3-yl)urea (1o)

Green solid; mp 132–135°C; IR(KBr) ν : 3174.23, 3069.30, 3031.68, 2598.98, 1715.70, 1626.38; 1H NMR (300MHz) δ 9.48(s, 1H), 8.06 (s, 1H), 7.97(dd, J = 7.33Hz, J = 7.37Hz, 2H), 7.85–7.53(dd, J = 7.31Hz, J = 7.35Hz, 2H, Phenyl), 7.60–7.57 (dd, J = 7.31Hz, J = 7.35Hz, 1H), 7.63–7.60 (d, J = 6.21Hz, 1H, Phenyl), 7.55–7.52(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.52 (m, 2H, Ph), 7.40–7.37(dd, J = 7.33Hz, J = 7.37Hz, 1H, Ar ring), 7.35–7.31(d, J = 6.21Hz, 2H, Ar ring), 7.27–7.23(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar ring), 6.56–6.54 (d, J = 6.21Hz, 1H), 6.19–6.16(d, J = 6.21Hz, 1H), 4.81–4.79 (m, 1H), 2.94 (d, J = 6.21Hz, 2H); ^{13}C NMR (75MHz): 197.4(1C), 164.5 (1C), 163.7 (1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9 (6C, Ar ring), 133.7, 131.0, 129.2, 128.8(6C, Ph ring), 133.8, 127.7, 50.8, 49.9; EIMS(*m/z*): 383.17 (M^+ , 28%); Anal. Calcd. for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32 %; found: C, 78.50; H, 5.82; N, 7.31 %.

1-(5-oxo-1,5-diphenylpent-1-en-3-yl)-3-(3-phenylallylidene)urea (1p)

White greenish powder; mp 145–148°C; IR(KBr) ν : 3175.23, 3070.30, 3032.68, 2599.98, 1716.70, 1627.38; 1H NMR (300MHz): δ 8.04(s, 1H), 7.50(s, 1H), 7.97–7.95(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar-ring), 7.60–7.54(dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar-ring), 7.64–7.60(d, J = 6.21 Hz, 1H, Ph), 7.54–7.51(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.42–7.39(dd, J = 7.33Hz, J = 7.37Hz, 4H, Ar-ring), 7.32–7.28(d, J = 6.21Hz, 2H, Ar ring), 7.28–7.25 (dd, J = 7.31Hz, J = 7.35Hz, 2H, Ar ring), 7.24–7.21(d, J = 6.21Hz, 1H, CH), 6.85–6.83 (d, J = 6.21Hz, 1H), 6.54–6.51 (d, J = 6.21Hz, 1H, CH), 6.17–6.13(d, J = 6.21Hz, 1H, CH), 4.81–4.78 (m, 1H), 2.94–2.92 (d, J = 6.21Hz, 2H); ^{13}C NMR (75MHz): 197.4(1C), 164.5(1C), 163.7 (1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9(6C, Ar ring), 135.2, 134.4, 116.1, 20.6(6C, Ph ring), 134.1, 133.5, 128.5, 119.9, 50.8, 49.9; EI-MS: 409.19 (M^+ , 29%); Elemental analysis: Anal. Calcd. for $C_{27}H_{24}N_2O_2$: C, 79.39; H, 5.92; N, 6.86 %; found: C, 79.30; H, 5.96; N, 6.91 %.

3-(methylamino)-1,5-diphenylpent-4-en-1-one (1q)

Light yellow powder; mp 84–88°C; IR(KBr) ν : 3173.21, 3064.28, 3026.66, 1711.68, 1621.36; 1H NMR(300MHz) δ 9.28 (s, 1H, NH), 7.97–7.94(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ph), 7.67–7.63 (m, 1H, Ar-ring), 7.51–7.48(dd, J = 7.31Hz, J = 7.35Hz, 2H), 7.41–7.37(dd, J = 7.33Hz, J = 7.37Hz, 2H, Ar-ring), 7.31–7.29(d, J = 6.21Hz, 2H, Ar ring), 7.24–7.20(dd, J = 7.31Hz, J = 7.35Hz, 1H, Ar ring), 6.56–6.54 (d, J = 6.21Hz, 1H, CH), 6.19–6.17(d, J = 6.20Hz, 1H), 3.84–3.81 (m, 1H, -CH), 3.36(s, 3H), 2.79–2.77(d, J = 6.20Hz, 2H); ^{13}C NMR (75MHz): 197.4(1C), 136.7, 133.1, 128.8, 128.6(6C, Ph ring), 136.4, 128.6, 128.5, 127.9(6C, Ar-ring), 134.6 (1C), 127.5, 57.1, 50.3, 23.1; EIMS(*m/z*): 266.15 (M^+ , 19%); Anal. Calcd. for $C_{18}H_{19}NO$: C, 81.47; H, 7.22; N, 5.28 %; found: C, 81.40; H, 7.25; N, 5.32 %.

Biological activities

Larvicidal Activity

Test compounds were deviated in various concentrations of 10, 25, 50 and 100 µg/mL according to a method described previously⁸. Mortality caused by the compounds was evaluated as ratios (%) of the numbers of dead vs. live larvae. The 50% lethal doses (LD₅₀) values were calculated using probit analysis.

Antifeedant Activity

The antifeedant activity was screened via 10, 25, 50 and 100 µg/mL concentrations of the tested samples and evaluated for marine fingerlings (*O. mossambicus*). Mortality caused by the compounds was evaluated as ratios (%) of the numbers of dead vs. live fingerlings. Table 2 summarizes the results. The method followed was described previously⁸.

Larval growth inhibition and regulation

The regulation and inhibition of larval growth in *C. quinquefasciatus* by compound **1i** (10 µg/mL) were analysed via the water-immersion method³⁴.

Molecular Docking

Preparation of ligands

The ligand molecules (**1a-1q**) were drawn via Chemdraw 12.0 and energy was minimized by using the MM2 force field in Chem3Dpro software. The ligand molecules were then saved in Protein Data Bank (PDB) format and further used for molecular docking studies.

Preparation Of Receptor

The 3D crystal structure of mosquito odorant binding protein (PDB ID: 3OGN) was downloaded from Protein Data Bank. The water molecules and inbound co-crystallized ligands were removed from the receptor using the Discovery Studio 2019 program. The receptor was energy minimized via the SWISS PDB Viewer program. The receptor was then used for molecular docking evaluation.

Identification Of Binding Pocket

The binding pocket of the target protein was recognized by using inbound co-crystallized ligands via the Discovery Studio 2019 Program. Residues of the amino acids Tyr10, Leu15, Leu19, Leu73, Leu80, Met84, Ile87, Ala88, Met91, His111, Trp114, His121, and Phe123 were situated in the binding pocket.

Docking

The interaction of binding modes between compounds **1a-1q**, **permethrin**, **temephos** and the mosquito odorant binding protein was assessed using molecular docking studies via Autodock vina 1.1.2. software³⁵. The selection of docking grid box was based on the active amino acid residues situated on

the binding pocket. The search grid of the 3OGN protein was stable with the dimensions sizes x: 22, y: 20, and z: 22 with center_x: 18.681, y: 49.66, and z: 11.409, with a spacing of 1.0 Å³⁶. The value of exhaustiveness was set to 8 and the interactions were visually examined using the Pymol and Discovery studio 2019 programs.

Statistical analysis

The mean of the results (LD₅₀ values) was calculated based on at least three independent evaluations and the standard deviations (SD) were calculated using Microsoft Excel.

Conclusions

In this study, we identified the most effective and easily prepared larvicidal active Mannich base derivatives synthesis by way of the grindstone method by using Tyrosinase enzyme as a catalyst; this method is economical and produces good coating and high yield. These compounds were investigated using larvicides against *Culex quinquefasciatus* and toxicity screening against non-target aquatic species of ichthyotoxicity activity. A total of 17 compounds were screened, and compound **1i** was found to be the most active among them (LD₅₀ = 12.09 µg/mL) against *Culex quinquefasciatus* compared with permethrin, and also induced 0% mortality within 24 h against *Oreochromis mossambicus* in an antifeedant screening. Molecular docking was carried out with all compounds **1a–1q** and the controls temephos and permethrin against the 3OGN protein, and the docking score was the best for compound **1i**. Therefore, our results indicate that compound **1i** was the best insecticide, and these compounds may serve as a prospective foundation for emerging ecologically important bioactive compounds, as well as eco-friendly pesticides and biopharmaceuticals.

Declarations

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

C.S synthesis of compounds and docking result analysis; D.A and S.A methodology of biological activity analysis; preparation; R.S chemical data analysis, A.I investigation total work chemistry and Biology. All authors were contributions preparation of through writing—original draft.

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Figures

Figure 1

Target molecules and commercial insecticide.

Figure 2

Molecular docking representation of ligand 1i within the binding site of 30GN protein.

Figure 3

Molecular docking representation of ligand permethrin within the binding site of 30GN.

Figure 4

Molecular docking representation of ligand temephos within the binding site of 3OGN.

Figure 5

Recyclability of Tyrosinase enzyme catalyst

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