Synthesis of novel benzopyran-connected pyrimidine and pyrazole derivatives via a green method using Cu(II)-tyrosinase enzyme catalyst as potential larvicidal, antifeedant activities

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A series of benzopyran-connected pyrimidine (1a–g) and benzopyran-connected pyrazole (2a–i) derivatives were synthesized via Biginelli reaction using a green chemistry approach. Cu(II)-tyrosinase was used as a catalyst in the synthesis of compounds 1a–g and 2a–i via the Biginelli reaction. The as-synthesized compounds were characterized by IR, 1H NMR, 13C NMR, mass spectroscopy, and elemental analysis. The as-synthesized compounds were screened for larvicidal and antifeedant activities. The larvicidal activity was evaluated using the mosquito species Culex quinquefasciatus, and the antifeedant activity was evaluated using the fishes of Oreochromis mossambicus. The compounds 2a–i demonstrated lethal effects, killing 50% of second instar mosquito larvae when their LD50 values were 44.17, 34.96, 45.29, 45.28, 75.96, and 28.99 μg mL⁻¹, respectively. Molecular docking studies were used for analysis based on the binding ability of an odorant binding protein (OBP) of Culex quinquefasciatus with compound 2h (binding energy = −6.12 kcal mol⁻¹) and compound 1g (binding energy = −5.79 kcal mol⁻¹). Therefore, the proposed target compounds were synthesized via a green method using Cu(II)-enzyme as a catalyst to give high yield (94%). In biological screening, benzopyran-connected pyrazole (2h) was highly active compared with benzopyran-connected pyrimidine (1a–g) series in terms of larvicidal activity.

Benzopyrans (coumarins) are an important group of naturally occurring compounds widely distributed in the plant kingdom and have been produced synthetically for many years for commercial uses. In addition, these core compounds are used as fragrant additives in food and cosmetics. The commercial applications of coumarins include dispersed fluorescent brightening agents and as dyes for tuning lasers. Some important biologically active natural benzopyran (coumarin) derivatives are shown in Fig. 1. Mosquitoes are the vectors for a large number of human pathogens compared to other groups of arthropods. Their uncontrollable breeding poses a serious threat to the modern humanity. Every year, more than 500 million people are severely affected by malaria. The mosquito larvicide is an insecticide that is specially targeted against the larval life stage of a mosquito. Particularly, the compound bergapten (Fig. 1), which shows the standard of larvicidal activity, is commercially available, and it was used as a control in this study for larvicidal screening. Moreover, the antifeedant screening defense mechanism makes it a potential candidate for the development of eco-friendly ichthyocides. Coumarin derivatives exhibit a remarkably broad spectrum of biological

Fig. 1 Biologically active natural benzopyran compound.
activities, including antibacterial,\textsuperscript{5,7} antifungal,\textsuperscript{8-10} anticoagulant,\textsuperscript{11} anti-inflammatory,\textsuperscript{12} antitumor,\textsuperscript{13,24} and anti-HIV.\textsuperscript{15}

Coumarin and its derivatives can be synthesized by various methods, which include the Perkin,\textsuperscript{16} Knoevenagel,\textsuperscript{17} Wittig,\textsuperscript{18} Pechmann,\textsuperscript{19} and Reformatsky reactions.

Among these reactions, the Pechmann reaction is the most widely used method for the preparation of substituted coumarins since it proceeds from very simple starting materials and gives good yields of variously substituted coumarins. For example, coumarins can be prepared by using various reagents, such as \(\text{H}_2\text{SO}_4\), \(\text{POCl}_3\), \(\text{AlCl}_3\), cation exchange resins, trifluoroacetic acid,\textsuperscript{23} montmorillonite clay,\textsuperscript{23} solid acid catalysts,\textsuperscript{24} \(\text{W/ZrO}_2\) solid acid catalyst,\textsuperscript{25} chloroaluminate ionic liquid,\textsuperscript{26} and Nafion-H catalyst.\textsuperscript{27}

Keeping the above literature observations, coumarin derivatives \(1\text{a} - \text{g}\) and \(2\text{a} - \text{i}\) are usually prepared with the conventional method involving \(\text{CuCl}_2 \cdot 2\text{H}_2\text{O}\) catalysis with using \(\text{HCl}\) additive. This reduces the yield and also increases the reaction time. To overcome this drawback, we used mushroom tyrosinase as a catalyst without any additive, a reaction condition not reported previously. The as-synthesized compounds were used for the biological screening of larvicidal and antifeedant activities (marine fish). In addition, in this study, we considered the molecular docking studies study based on previous studies for performing the binding ability of hydroxy-2-methyl-4H-pyran-4-one (the root extract of \textit{Senecio laetus} Edgew) with the odorant binding protein (OBP) of \textit{Culex quinquefasciatus}.\textsuperscript{28}

## Results and discussion

### Chemistry

The compounds \(1\text{a} - \text{g}\) and \(2\text{a} - \text{i}\) were prepared according to the synthesis sequences illustrated in Scheme 1. The proposed mechanism for the formation of the compounds \(1\text{a} - \text{g}\) and \(2\text{a} - \text{i}\) are shown in Schemes 2 and 3. The Cu-tyrosinase enzyme catalysis performed well to afford yields ranging from 82 to 98% within 2 to 4 min of reaction time compared with other catalysts. The catalyst optimization is shown in Table 1, and the optimization of reaction conditions is shown in Table 2.

All the as-synthesized compounds were characterized by IR, \(\textsuperscript{1}H\) NMR, and \(\textsuperscript{13}C\) NMR analyses. Important assignments of the compounds \(1\text{a} - \text{g}\) using the IR spectroscopy include absorption bands at \(3174.5, 3034, 2596.13\) cm\(^{-1}\), corresponding to the \(\text{NH}, \text{C} - \text{N}\), and \(\text{C} = \text{O}\). The \(\textsuperscript{1}H\) NMR spectrum shows signals at \(\delta 9.19 - 9.27, 3.97 - 4.17\), and \(1.21 - 1.29\) ppm, corresponding to the \(\text{NH}, \text{4CH}\) protons, and \(\text{CH}_3\). The \(\textsuperscript{13}C\) NMR spectrum shows peaks at \(\delta 160.4, 160.4,\) and \(15.0 - 15.7\) ppm, corresponding to the \(\text{C} = \text{O}, \text{C} - \text{N}\), and \(\text{CH}_3\) carbon atoms. The important assignments of the compounds \(2\text{a} - \text{i}\) using the IR spectroscopy include absorption bands at \(3174.54, 3034,\) and \(2596.13\) cm\(^{-1}\), corresponding to the \(\text{NH}, \text{Ar}-\text{H}\) and \(-\text{C}=\text{N}\). The \(\textsuperscript{1}H\) NMR spectrum shows signals at \(\delta 8.70, 3.99 - 4.59\), and \(2.42\) ppm, corresponding to the \(\text{NH}, \text{4CH}\) protons, and \(\text{CH}_3\). The \(\textsuperscript{13}C\) NMR spectrum shows peaks at \(\delta 150.6, 122.3 - 123.3,\) and \(15.0 - 15.7\) ppm, corresponding to the \(\text{C} = \text{N}, \text{C} = \text{C}\), and \(\text{CH}_3\) carbon atoms. All compounds were confirmed via mass spectroscopy and elemental analysis supported the expected target compounds.

### Larvicidal activity

Compounds \(1\text{a} - \text{g}\) and \(2\text{a} - \text{i}\) were screened for larvicidal activity. Compounds \(1\text{a} - \text{g}\) had low activity compared with compounds \(2\text{a} - \text{i}\). The experiments were performed at room temperature. The compound \(2\text{h}\) produced \(100\%\) mortality at \(100\ \mu\text{g mL}^{-1}\). The compound \(1\text{g}\) was the only highly active compound with the \(\text{LD}_{50}\) value of \(46.08\ \mu\text{g mL}^{-1}\) in the \(1\text{a} - \text{g}\) series. Overall, compounds \(1\text{g}, 2\text{a}, 2\text{b}, 2\text{d}, 2\text{e}\) and \(2\text{h}\) were highly active against \textit{Culex quinquefasciatus} with the \(\text{LD}_{50}\) values of \(46.08, 44.17, 34.96, 45.29, 45.28,\) and \(28.99\ \mu\text{g mL}^{-1}\), respectively, than the control bergapten with the \(\text{LD}_{50}\) value of \(73.68\ \mu\text{g mL}^{-1}\). Among the as-synthesized compounds \(1\text{a} - \text{g}\) and \(2\text{a} - \text{i}\), compound \(2\text{h}\) was highly active against \textit{Culex quinquefasciatus} with the \(\text{LD}_{50}\) value of \(28.99\ \mu\text{g mL}^{-1}\) compared with the control bergapten (the \(\text{LD}_{50}\) value of \(73.68\ \mu\text{g mL}^{-1}\)). The compounds \(1\text{g}, 2\text{a}, 2\text{d},\) and \(2\text{e}\) were equipotently active (range from \(44.17\) to \(46.08\ \mu\text{g mL}^{-1}\)) due to the nature of chemical performance; however, some important functional groups displayed the biological activity in different ways. For example, compound \(1\text{g}\) containing pyrimidine with furfuryl gave equipotent activity compared with compound \(2\text{a}\) (electron donating group phenyl with pyrazole), compound \(2\text{d}\) (electron donating group 4-HO-phenyl with

![Scheme 1](image1.png)  Synthesis of coumarin derivatives \(1\text{a} - \text{g}\) and \(2\text{a} - \text{i}\).

![Scheme 2](image2.png)  Proposed mechanism for the formation of \(1\text{a} - \text{g}\).

![Scheme 3](image3.png)  Proposed mechanism for the formation of \(2\text{a} - \text{i}\).
Table 1  Optimization of the catalyst for compounds (1a)  

| Entry | Catalyst | Additive | Yield (%) |
|-------|----------|----------|-----------|
| 1     | ZnCl₂    | HCl      | 47        |
| 2     | SnCl₂·2H₂O| HCl      | 53        |
| 3     | ZrOCl₂   | HCl      | 68        |
| 4     | AlCl₃    | HCl      | 76        |
| 5     | CuCl₂·2H₂O| HCl      | 87        |
| 6     | Cu-tyrosinase enzyme catalysis | Without additive | 94 |

Table 2  Optimization of the reaction conditions for compounds 1a–g and 2a–i  

| Compd no. | Ar            | CuCl₂ | Cu-tyrosinase enzyme catalysis | Catalysis activity, time (min)/yield (%) |
|-----------|---------------|-------|-------------------------------|----------------------------------------|
| 1b        | -CH==CH-Ph    | 5/87  | 2/94                          | 2/89                                   |
| 1c        | -4-ClC₆H₄    | 5/83  | 4/87                          |                                        |
| 1d        | -4-OH₆C₆H₄  | 5/87  | 2/89                          |                                        |
| 1e        | -3-N₂O₆C₆H₄ | 5/79  | 2/89                          |                                        |
| 1f        | 4-OCH₂C₆H₄  | 5/80  | 2/89                          |                                        |
| 1g        | Furfuryl     | 5/83  | 2/89                          |                                        |
| 2a        | -Ph          | 5/72  | 2/82                          |                                        |
| 2b        | -CH==CH-Ph   | 5/87  | 2/89                          |                                        |
| 2c        | -4-ClC₆H₄   | 5/89  | 2/91                          |                                        |
| 2d        | -4-OH₆C₆H₄ | 5/82  | 2/92                          |                                        |
| 2e        | -3-N₂O₆C₆H₄ | 5/83  | 2/95                          |                                        |
| 2f        | -N(CH₃)₂C₆H₄| 5/89  | 2/96                          |                                        |
| 2g        | 4-OCH₂C₆H₄  | 5/71  | 2/98                          |                                        |
| 2h        | Furfuryl     | 5/83  | 3/89                          |                                        |
| 2i        | Citral       | 5/79  | 4/86                          |                                        |

Antifeedant activity (ichthyotoxicity activity)  
The as-synthesized compounds 1a–g and 2a–i were screened for the antifeedant activity with many of the compounds showing high toxicity. All the as-synthesized compounds had 100% mortality at 100 μg mL⁻¹ except compounds 1a, 1b, and 2a. Among the as-synthesized compounds 1a–g and 2a–i, the compound 1a was the least toxic with the mortality of 0% at 100 μg mL⁻¹. The compound 1e was highly active with the LD₅₀ value of 18.52 μg mL⁻¹ compared with compounds 1a–g and 2a–i. The compounds 1f, 2c, and 2e (range from 35.07 to 35.98 μg mL⁻¹) had equipotent activity due to the presence of different functional groups, such as electron donating groups at 4-CH₃O-Ph with pyrimidine in compound 1f, electron donating groups at 4-Cl-Ph with pyrazole in compound 2c, and electron withdrawing group 3-NO₂-Ph with pyrazole in compound 2e. The values are summarized in Table 3.

Mosquito larval growth inhibition activities  
Considering the regulation of coumarin analogues on growth and metamorphosis, the effect of the compound 2h after a 72 h treatment on the weight gain of larvae and rate of inhibition (Table 5), the duration of the pupal and adult stages and the eclosion rate of the treated Culex quinquefasciatus were evaluated at 10 μg mL⁻¹ (Table 6). The weight gain of larvae were controlled by the compound 2h, which gave an inhibitory rate of 55.06%. The effect of the compound 2h on the duration of the pupal and adult stages was evident, and the rate of eclosion was only 40% after treatment with the compound 2h. The results show that the compound 2h exhibited potent inhibitory activity against the growth and development of Culex quinquefasciatus.

Table 3  Larvicidal activity of title compounds 1a–g and 2a–i  

| Compounds | Concentration (μg mL⁻¹)/mortality* (%) | 10 | 25 | 50 | 100 |
|-----------|---------------------------------------|----|----|----|-----|
| 1a        | —                                     | 0 ± 0.00 | 20 ± 0.07 | 43 ± 0.87 | >100 |
| 1b        | —                                     | 0 ± 0.00 | 14 ± 0.96 | 33 ± 1.65 | >100 |
| 1c        | —                                     | —    | —    | —    | —   |
| 1d        | —                                     | —    | 0 ± 0.00 | 20 ± 1.61 | >100 |
| 1e        | —                                     | —    | 0 ± 0.00 | 20 ± 0.88 | >100 |
| 1f        | —                                     | —    | —    | —    | —   |
| 1g        | 18 ± 1.87                            | 40 ± 1.21 | 60 ± 0.56 | 80 ± 1.54 | 46.08 |
| 2a        | 18 ± 1.20                            | 40 ± 1.87 | 60 ± 0.67 | 84 ± 0.00 | 44.17 |
| 2b        | 16 ± 1.87                            | 40 ± 1.61 | 60 ± 1.67 | 80 ± 0.09 | 34.96 |
| 2c        | —                                     | —    | 0 ± 0.00 | 20 ± 1.14 | >100 |
| 2d        | 18 ± 0.95                            | 42 ± 1.38 | 60 ± 1.42 | 80 ± 1.23 | 45.29 |
| 2e        | 20 ± 1.76                            | 40 ± 1.23 | 62 ± 1.94 | 80 ± 1.11 | 45.28 |
| 2f        | 0 ± 0.00                             | 20 ± 0.67 | 44 ± 1.47 | 60 ± 2.12 | 75.96 |
| 2g        | —                                     | 0 ± 0.00 | 8 ± 1.63 | 20 ± 1.42 | >100 |
| 2h        | 46 ± 1.33                            | 62 ± 1.34 | 84 ± 1.35 | 100 ± 0.00 | 28.99 |
| 2i        | —                                     | 0 ± 0.00 | 10 ± 1.34 | 20 ± 1.34 | >100 |
| Bergapten | 11 ± 2.05                            | 25 ± 1.90 | 41 ± 1.65 | 63 ± 0.58 | 73.68 |

* Values are the means of three replicates ± SD.
shown in Fig. 4b. The best-selected pose of the 3OGN-complex (binding energy <100 \text{ kcal mol}^{-1}) with binding site residues is shown in Fig. 4c. The hydrogen bonds and the types of contacts involved in the 3OGN-2h docked complex are shown in Fig. 4d. It was observed that ASP-70 and LYS-106 are involved in hydrogen bond interactions and the residues VAL-71, TYR-97, TYR-97 and PRO-98 are involved in van der Waals interactions. Then, the selected ligand 1g was docked against the 3OGN. The ball and stick representation of 3OGN-1g docked complex is shown in Fig. 5a. The molecular surface representation of the 3OGN-1g docked complex is shown in Fig. 5b. The best-selected pose of the 3OGN-1g docked complex (binding energy =−5.79 \text{ kcal mol}^{-1}) with binding site residues is shown in Fig. 5c.

### Table 4 Antifeedant activity of title compounds 1a–g and 2a–i

| Compounds | Concentration (µg mL\(^{-1}\))/mortality\(^a\) (%) |
|-----------|------------------------------------------|
|           | 10 | 25 | 50 | 100 | LD\(_{50}\) (µg mL\(^{-1}\)) |
| 1a | — | — | — | — | >100 |
| 1b | 0 ± 0.00 | 10 ± 1.23 | 24 ± 0.96 | 40 ± 1.65 | >100 |
| 1c | 34 ± 1.23 | 68 ± 1.54 | 80 ± 1.42 | 100 ± 0.00 | 18.52 |
| 1d | 14 ± 1.35 | 38 ± 1.42 | 60 ± 1.67 | 100 ± 0.00 | 32.63 |
| 1e | 24 ± 1.56 | 46 ± 1.23 | 80 ± 1.78 | 100 ± 0.00 | 30.28 |
| 1f | 18 ± 1.12 | 40 ± 1.32 | 60 ± 1.42 | 100 ± 0.00 | 35.07 |
| 1g | 20 ± 1.87 | 38 ± 1.21 | 62 ± 0.56 | 100 ± 0.00 | 36.54 |
| 2a | 0 ± 0.00 | 20 ± 1.87 | 42 ± 0.67 | 80 ± 1.78 | 59.97 |
| 2b | 10 ± 1.87 | 32 ± 1.61 | 60 ± 1.67 | 100 ± 0.00 | 41.87 |
| 2c | 20 ± 1.20 | 42 ± 1.42 | 68 ± 1.65 | 100 ± 0.00 | 35.33 |
| 2d | 24 ± 0.95 | 60 ± 1.38 | 80 ± 1.42 | 100 ± 0.00 | 25.58 |
| 2e | 14 ± 1.76 | 32 ± 1.23 | 64 ± 1.94 | 100 ± 0.00 | 35.98 |
| 2f | 28 ± 1.20 | 46 ± 0.67 | 68 ± 1.47 | 100 ± 0.00 | 33.72 |
| 2g | 12 ± 1.42 | 38 ± 1.87 | 70 ± 1.63 | 100 ± 0.00 | 31.56 |
| 2h | 24 ± 1.33 | 40 ± 1.34 | 74 ± 1.35 | 100 ± 0.00 | 33.75 |
| 2i | 20 ± 1.42 | 42 ± 1.23 | 60 ± 1.34 | 100 ± 0.00 | 34.52 |

\(^a\) Values are the means of three replicates ± SD.

### Table 6 The effect of the title compound 2h on the growth and development of mosquito larvae

| Compound | Culex quinquefasciatus\(^a\) |
|----------|---------------------------------|
|          | Duration of pupae (h) | Duration of adult (h) | Rate of eclosion (%) |
| 2h\(^b\) | 70.1 ± 1.32 | 25.1 ± 2.34 | 40 ± 3.10 |
| Control\(^c\) | 65.5 ± 1.28 | 24.2 ± 1.98 | 90 ± 2.80 |

\(^a\) Values are the means of three replicates ± SD. \(^b\) The concentration of 2h was 10 \text{ µg mL}^{-1}. \(^c\) Control does not contain the compounds.

### Table 5 The effect of the title compound 2h on the growth of mosquito larvae

| Compound | Weight (mg) |
|----------|-------------|
|          | Weight gain (mg) | Inhibition (%) |
|          | 0 h | 72 h | 0 h | 72 h |
| 2h\(^b\) | 100.28 ± 1.20 | 102.14 ± 1.33 | 3.86 ± 1.14 | 55.06 ± 1.42 |
| Control\(^c\) | 100.06 ± 1.21 | 108.65 ± 0.67 | 8.59 ± 1.11 | — |

\(^a\) Values are the means of three replicates ± SD. \(^b\) The concentration of 2h was 10 \text{ µg mL}^{-1}. \(^c\) Control does not contain the compounds.

**Molecular docking**

The docking process involves two basic steps: the prediction of the ligand conformation and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes respectively. Docking studies were accomplished for compounds 1g and 2h in order to calculate their binding affinities against 3OGN protein using the AutoDock4 (version 4.2.6) software. The 3D structure of the ligand 2h is shown in Fig. 2. The 3D structure of the 3OGN protein is shown in Fig. 3. The results were examined based on the binding energies of the docked complexes. AutoDock4 generated 10 conformers for each ligand. The selection of the best conformer was based on the smallest binding energy between the ligand and the protein. After docking, the ligands were ranked according to their binding energies.

**Docked results using the AutoDock4 software**

The selected ligand 2h was docked against 3OGN protein using the AutoDock4 software. The ball and stick representation of the 3OGN-2h docked complex is shown in Fig. 4a. The molecular surface representation of the 3OGN-2h docked complex is shown in Fig. 4b. The best-selected pose of the 3OGN-2h docked complex (binding energy =−6.12 \text{ kcal mol}^{-1}) with binding site residues is shown in Fig. 4c. The hydrogen bonds and the types of contacts involved in the 3OGN-2h docked complex are shown in Fig. 4d. It was observed that ASP-70 and LYS-106 are involved in hydrogen bond interactions and the residues VAL-71, TYR-97, TYR-97 and PRO-98 are involved in van der Waals interactions.

Then, the selected ligand 1g was docked against the 3OGN. The ball and stick representation of 3OGN-1g docked complex is shown in Fig. 5a. The molecular surface representation of the 3OGN-1g docked complex is shown in Fig. 5b. The best-selected pose of the 3OGN-1g docked complex (binding energy =−5.79 \text{ kcal mol}^{-1}) with binding site residues is shown in Fig. 5c.
The hydrogen bonds and the types of contacts involved in the 3OGN-1g docked complex are shown in Fig. 5d. It was observed that ASP-78 and LEU-15 are involved in hydrogen bond interactions and the residues GLU-14, PRO-81 and ASN-82 are involved in van der Waals interactions. The compound 2h has the highest binding affinity with the mosquito odorant binding protein 3OGN compared with compound 1g. The molecular docking characterizations for compound 2h are listed in Table 7, while those for 1g are listed in Table 8.

Structure–activity relationship

The as-synthesized compounds are compared for the structure and activity relationship, as shown in Fig. 6. The larvicidal activities of the as-synthesized compounds are compared with those of the control bergapten. The larvicidal activity in the presence of pyrazole moiety at the five-membered ring in compound 2h is highly active with the LD_{50} value of 28.99 μg mL\(^{-1}\) than the five-membered ring at control bergapten with the LD_{50} value of 73.68 μg mL\(^{-1}\). The presence of natural aldehyde scaffolds in the title compound shows high larvicidal and antifeedant activities. The presence of the furfuraldehyde scaffold in 2h shows high larvicidal activity with the LD_{50} value of 28.99 μg mL\(^{-1}\) than that in the compounds 1a–g and 2a–i. The presence of 4-Cl-phenyl scaffold in 1c shows high antifeedant activity with the LD_{50} value of 18.52 μg mL\(^{-1}\) than that in the compounds 1a–g and 2a–i.

Experimental

General

The chemicals 7-hydroxy-4-methylcoumarin, tyrosinase, and bergapten were purchased from Sigma-Aldrich and used without further purification. Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra (KBr) were recorded in KBr on a Shimadzu 8201 pc (4000–400 cm\(^{-1}\)). The \(^1\)H NMR and \(^13\)C NMR spectra were recorded on a Bruker DRX-300 MHz. Elemental analysis (C, H, and N) was performed using an Elemental analyzer model (Varian EL III). The purity of the compounds was checked via thin layer chromatography (TLC) with silica gel plates.

General method for the preparation of 8-hydroxy-5-methyl-4-phenyl-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (1a)

The mixture of 7-hydroxy-4-methyl-2H-chromen-2-one (0.001 mol, 0.17 g), substituted aldehyde (0.003 mol), urea (0.003 mol, 0.18 g), and Cu(II)-tyrosinase enzyme (0.5 g) were mixed in a mortar. Then, 2 mL of 50 mM potassium phosphate buffer (pH 6.0) was added and filtered. The insoluble product was washed with excess ice-cold water, and then filtered and dried. The product was confirmed via TLC. The product was washed in ethanol to get pure product. The same method was followed for the synthesis of compounds 1b–g.

White solid; mw: 306.32; mp 164 °C; IR (cm\(^{-1}\)): 3174.54 (NH), 3074.57 (Ph–CH\(_{str}\)), 3034 (Ar–O); 1H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 10.5 (1H, s, OH), 8.70 (1H, s, NH), 7.33 (2H, dd, \(J = 7.31\) Hz, \(J = 7.35\) Hz, phenyl), 7.26 (1H, d, \(J = 6.21\) Hz, phenyl), 7.23 (2H, dd, \(J = 7.31\) Hz, \(J = 7.35\) Hz, phenyl), 7.04 (1H, d, \(J = 6.21\) Hz, phenyl), 6.45 (1H, s, phenyl), 6.21 (1H, d, \(J = 6.21\) Hz, phenyl), 5.13 (1H, d, \(J = 6.21\) Hz, –CH), 2.35 (3H, s, –CH\(_3\)); \(^13\)C NMR (300 MHz, DMSO-d\(_6\)): 160.4 (1C, C=O), 160.4 (1C, C=O), 159.1 (1C, –C=OH), 155.7 (1C, –C=O–), 143.3, 128.5, 127.2, 126.9, 126.7, 108.4, 107.9, 99.5 (10C, Ar ring), 136.4 (1C, –C=CH\(_3\)), 112.8 (1C, –C=C=CH\(_3\)), 42.2 (1C, –C=NH), 15.6 (1C, –CH\(_3\)); EI-MS: 307.10 (M\(^+\), 20%); elemental analysis (C\(_{18}\)H\(_{14}\)N\(_2\)O\(_3\)) : calculated: C, 70.58; H, 4.61; N, 9.15%; found: C, 70.56; H, 4.62; N, 9.16%.

8-Hydroxy-5-methyl-4-styryl-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (1b)

Light yellow solid; mw: 332.35; mp 146 °C; IR (cm\(^{-1}\)): 3174.54 (NH), 3074.57 (Ph–CH\(_{str}\)), 3034 (Ar–H), 2596.43 (C=N), 162.5 (C=O); 1H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 10.5 (1H, s, OH), 8.70 (1H, s, NH), 7.40 (2H, dd, \(J = 7.33\) Hz, \(J = 7.37\) Hz, phenyl), 7.33 (1H, d, \(J = 6.21\) Hz, phenyl), 7.24 (2H, dd, \(J = 7.31\) Hz, \(J = 7.35\) Hz, phenyl), 7.04 (1H, d, \(J = 6.21\) Hz, phenyl), 6.56 (1H, d, \(J = 7.35\) Hz, phenyl), 7.04 (1H, d, \(J = 6.21\) Hz, phenyl), 6.45 (1H, s, phenyl), 6.21 (1H, d, \(J = 6.21\) Hz, phenyl), 5.13 (1H, d, \(J = 6.21\) Hz, –CH), 2.35 (3H, s, –CH\(_3\)); \(^13\)C NMR (300 MHz, DMSO-d\(_6\)): 160.4 (1C, C=O), 160.4 (1C, C=O), 159.1 (1C, –C=OH), 155.7 (1C, –C=O–), 143.3, 128.5, 127.2, 126.9, 126.7, 108.4, 107.9, 99.5 (10C, Ar ring), 136.4 (1C, –C=CH\(_3\)), 112.8 (1C, –C=C=CH\(_3\)), 42.2 (1C, –C=NH), 15.6 (1C, –CH\(_3\)); EI-MS: 307.10 (M\(^+\), 20%); elemental analysis (C\(_{18}\)H\(_{14}\)N\(_2\)O\(_3\)) : calculated: C, 70.58; H, 4.61; N, 9.15%; found: C, 70.56; H, 4.62; N, 9.16%.
¼ 
6.21 Hz, –CH), 6.45 (1H, s, phenyl), 6.21 (1H, d, \( J = 6.21 \) Hz, phenyl), 6.19 (1H, d, \( J = 6.21 \) Hz, –CH), 4.96 (1H, d, \( J = 6.21 \) Hz, –CH), 2.42 (3H, s, –CH₃); 13C NMR (300 MHz, DMSO-d₆): 160.4 (1C, C=O), 160.4 (1C, C=N), 159.1 (1C, –C=O–O), 155.7 (1C, –C–O–), 136.4 (1C, –C–CH₃), 136.4, 128.6, 128.5, 127.9, 127.4, 108.4, 107.9, 99.5 (10C, Ar ring), 129.5 (1C, –C=C), 123.3 (1C, –C=C), 112.8 (1C, –C=C–CH₃), 41.6 (1C, –C–NH), 15.7 (1C, –CH₃); EI-MS: 333.12 (M⁺, 22%); elemental analysis (C₂₀H₁₆N₂O₃): calculated: C, 72.28; H, 4.85; N, 8.43%; found: C, 72.27; H, 4.86; N, 8.45%.

4-(4-chlorophenyl)-8-hydroxy-5-methyl-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (1c)
Brown solid; mw: 340.76; mp 178 °C; IR (cm⁻¹): 3174.54 (NH), 3074.57 (Ph-CHstr), 3034 (Ar–H), 2596.43 (C==N), 1612.5 (C==O); ¹H NMR (300 MHz, DMSO-d₆): \( \delta \) 10.5 (1H, s, OH), 8.70 (1H, s, 333.12 (M⁺, 22%); elemental analysis (C₂₀H₁₆N₂O₃): calculated: C, 72.28; H, 4.85; N, 8.43%; found: C, 72.27; H, 4.86; N, 8.45%.
NH), 7.37 (2H, dd, J = 7.33 Hz, J = 7.37 Hz, phenyl), 7.34 (2H, dd, J = 7.31 Hz, J = 7.35 Hz, phenyl), 7.04 (1H, d, J = 6.21 Hz, phenyl), 6.45 (1H, s, phenyl), 6.21 (1H, d, J = 6.21 Hz, phenyl), 5.13 (1H, d, J = 6.21 Hz, –CH), 2.42 (3H, s, –CH3); 13C NMR (300 MHz, DMSO-d6): 160.4 (1C, C=O), 160.4 (1C, C==N), 159.1 (1C, –C–OH), 155.7 (1C, –C–O–), 141.4, 132.3, 128.6, 127.2, 126.1, 108.4, 107.9, 99.5 (10C, Ar ring), 136.4 (1C, –C–CH3), 112.8 (1C, –C==C–CH3), 42.2 (1C, –C–NH), 15.6 (1C, –CH3); EI-MS: 342.06 (M+, 20%); elemental analysis (C18H14N2O4): calculated: C, 67.07; H, 4.38; N, 8.69%; found: C, 67.06; H, 4.40; N, 8.68%.

8-Hydroxy-4-(4-hydroxyphenyl)-5-methyl-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (1d)

Brown solid; mw: 322.31; mp 172 °C; IR (cm⁻¹): 3174.54 (NH), 3074.57 (Ph-CHstr), 3034 (Ar–H), 2956.43 (C==N), 1612.5 (C=O); 1H NMR (300 MHz, DMSO-d6): δ 10.5 (2H, s, OH), 8.70 (1H, s, NH), 7.06 (2H, dd, J = 7.33 Hz, J = 7.37 Hz, phenyl), 7.04 (1H, d, J = 6.21 Hz, phenyl), 6.63 (2H, dd, J = 7.31 Hz, J = 7.35 Hz, phenyl), 6.45 (1H, s, phenyl), 6.21 (1H, d, J = 6.21 Hz, phenyl), 5.13 (1H, d, J = 6.21 Hz, –CH), 2.42 (3H, s, –CH3); 13C NMR (300 MHz, DMSO-d6): 160.4 (1C, C=O), 160.4 (1C, C==N), 159.1 (1C, –C–OH), 155.7 (1C, –C–O–), 156.5, 135.9, 126.1, 127.2, 115.7, 108.4, 107.9, 99.5 (10C, Ar ring), 136.4 (1C, –C–CH3), 112.8 (1C, –C==C–CH3), 42.2 (1C, –C–NH), 15.6 (1C, –CH3); EI-MS: 323.10 (M+, 20%); elemental analysis (C19H14N2O4): calculated: C, 67.07; H, 4.38; N, 8.69%; found: C, 67.06; H, 4.40; N, 8.68%.

Table 7 Molecular docking for highly active compound 2h

| Conformer | Binding energy (kcal mol⁻¹) | Inhibition constant (K(i) (µM) | Intermolecular energy (kcal mol⁻¹) | Internal energy (kcal mol⁻¹) |
|-----------|----------------------------|-------------------------------|----------------------------------|----------------------------|
| 1         | -5.79                      | 57.40                         | -6.38                           | -0.20                      |
| 2         | -5.76                      | 60.25                         | -6.36                           | -0.20                      |
| 3         | -5.73                      | 63.54                         | -6.32                           | -0.20                      |
| 4         | -5.71                      | 65.63                         | -6.30                           | -0.07                      |
| 5         | -5.69                      | 69.10                         | -6.28                           | -0.20                      |
| 6         | -5.66                      | 71.39                         | -6.25                           | -0.20                      |
| 7         | -5.61                      | 77.81                         | -6.20                           | -0.20                      |
| 8         | -5.34                      | 121.82                        | -5.94                           | -0.20                      |
| 9         | -4.99                      | 220.99                        | -5.58                           | -0.20                      |
| 10        | -4.86                      | 272.58                        | -5.46                           | -0.20                      |

8-Hydroxy-4-(4-hydroxyphenyl)-5-methyl-3,4-dihydro-2H-chromeno[2,3-d]pyrimidin-2-one (1e)

White solid; mw: 351.31; mp 108 °C; IR (cm⁻¹): 3174.54 (NH), 3074.57 (Ph-CHstr), 3034 (Ar–H), 2956.43 (C==N), 1612.5 (C=O); 1H NMR (300 MHz, DMSO-d6): δ 10.5 (1H, s, OH), 8.70 (1H, s, NH), 8.12 (1H, s, phenyl), 7.59 (1H, d, J = 7.31 Hz, J = 7.35 Hz, phenyl), 7.04 (1H, d, J = 6.21 Hz, phenyl), 6.45 (1H, s, phenyl), 6.15 (1H, d, J = 6.21 Hz, phenyl), 5.61 (1H, d, J = 6.21 Hz, –CH), 2.42 (3H, s, –CH3); 13C NMR (300 MHz, DMSO-d6): δ 10.5 (1H, s, OH), 8.70 (1H, s, NH), 8.12 (1H, s, phenyl), 7.59 (1H, d, J = 7.31 Hz, J = 7.35 Hz, phenyl), 7.04 (1H, d, J = 6.21 Hz, phenyl), 6.45 (1H, s,
phenyl), 6.21 (1H, d, \(J = 6.21 \text{ Hz, phenyl}\)), 5.13 (1H, d, \(J = 6.21 \text{ Hz, } \text{CH} - \text{phenyl}\)), 2.42 (3H, s, \(-\text{CH}_3\)); \(^{13}\text{C} \text{ NMR} (300 \text{ MHz, DMSO-}d_6): 157.0 (1\text{C, }-\text{C} = \text{O}), 152.5, 142.1, 127.2, 110.6, 108.4, 107.9, 106.7, 99.5 (8\text{C, Ar ring}), 136.4 (1\text{C, }-\text{C} = \text{CH}_2\), 112.8 (1\text{C, }-\text{C} = \text{CH}_3\), 43.4 (1\text{C, }-\text{C} = \text{NH})), 15.0 (1\text{C, }-\text{CH}_2\); EM-MS: 297.08 (M\(^+\), 18%); elemental analysis (C\(_{18}\)H\(_{15}\)N\(_2\)O\(_2\)): calculated: C, 64.86; H, 4.08; N, 9.46%; found: C, 64.87; H, 4.10; N, 9.43%.

General method for preparation of 4-methyl-3-phenyl-2,3-dihydrochromeno[2,3-c]pyrazol-7-ol (2a)

The mixture of 7-hydroxy-4-methyl-2H-chromen-2-one (0.001 mol, 0.17 g), substituted aldehyde (0.003 mol, 0.3 mL), hydrazine hydrate (0.003 mol, 0.1 mL), and Cu(i)-lysinoaspe enzyme (0.5 mL) is mixed together in a mortar. Then 2 mL of 50 mM potassium phosphate buffer (pH 6.0) was added and filtered. The insoluble product was washed with excess of ice-cold water filtered and dried. The product was confirmed by TLC. The product was washed in ethanol to get pure product. The same method was followed to the synthesis of other compounds 2b-i.

Black solid; mw: 278.31; mp 112 \(^\circ\)C; IR (cm\(^{-1}\)) : 3174.54 (NH), 3074.57 (Ph-CH\(_3\)), 3034 (Ar-H), 2596.43 (C=\(\cdot\)N); \(^1\text{H} \text{ NMR} (300 \text{ MHz, DMSO-}d_6): \delta 10.5 (1\text{H, s, OH}), 8.70 (1\text{H, s, NH}), 7.33 (2\text{H, dd, } J = 7.33 \text{ Hz, phenyl}), 7.26 (1\text{H, d, } J = 6.21 \text{ Hz, phenyl}), 7.23 (2\text{H, dd, } J = 7.31 \text{ Hz, phenyl}), 7.04 (1\text{H, d, } J = 6.21 \text{ Hz, phenyl}), 6.45 (1\text{H, s, phenyl}), 6.21 (1\text{H, d, } J = 6.21 \text{ Hz, phenyl}), 4.59 (1\text{H, d, } J = 6.21 \text{ Hz, CH} - \text{phenyl}), 2.42 (3\text{H, s, } -\text{CH}_3\); \(^{13}\text{C} \text{ NMR} (300 \text{ MHz, DMSO-}d_6): 159.1 (1\text{C, }-\text{C} = \text{OH}), 155.7 (1\text{C, }-\text{C} = \text{O}), 142.1, 127.2, 126.9, 126.7, 108.4, 107.9, 99.5 (10\text{C, Ar ring}), 137.2 (1\text{C, }-\text{C} = \text{CH}_2\), 122.3 (1\text{C, }-\text{C} = \text{CH}_2\), 45.8 (1\text{C, }-\text{C} = \text{NH})), 15.6 (1\text{C, }-\text{CH}_2\); EM-MS: 314.06 (M\(^+\), 32%); elemental analysis (C\(_{18}\)H\(_{16}\)Cl\(_{2}\)N\(_2\)O\(_2\)): calculated: C, 65.27; H, 4.20; N, 8.97%; found: C, 65.27; H, 4.10; N, 8.96%;
**4-Methyl-3-(3-nitrophenyl)-2,3-dihydrochromeno[2,3-c]pyrazol-7-ol (2e)**

Yellow solid; mw: 324.42; mp 68 °C; IR (cm⁻¹): 3174.54 (NH), 3074.57 (Ph–C=Hstr), 3034 (Ar–H), 2964.43 (C–CH3); 1H NMR (300 MHz, DMSO-d₆): δ 10.5 (1H, s, OH), 8.70 (1H, s, NH), 7.04 (2H, d, J = 6.21 Hz, phenyl), 6.45 (1H, s, phenyl), 2.42 (3H, s, –CH3); EI-MS: 324.09 (M +, 20%); elemental analysis (C₁₈H₁₆N₂O₃): calculated: C, 67.16; H, 5.51; N, 10.44%; found: C, 67.17; H, 5.50; N, 10.45%.

**Fingerlings (1.5–2.0 cm) of marine-acclimated Oreochromis mossambicus were used for evaluating ichthyotoxic potential.**

**Antifeedant activity**

**Protein preparation for docking**

The mosquito odorant binding protein 3OGN was obtained from the protein data bank (PDB). For docking, all water molecules were removed from protein, and hydrogen atoms were added to the refined model using AutoDock Tools (ADT), and finally merging the non-polar hydrogens. The as-prepared...
protein was saved in PDB, partial charge (Q), and atom type (T) (PDBQT) formats.

Ligand preparation for docking
The ligand was drawn in the Chem 3D pro software and converted into a protein data bank (PDB) file format using the Open Babel software. The ligands were prepared using AutoDock Tools (ADT). Gasteiger charges were assigned to the ligands. The as-prepared ligands were saved in a PDBQT format. Fig. 2 shows the chemical structure of the ligand 2h.

Molecular docking
AutoDock4 (version 4.2.6) was used for the molecular docking studies. AutoGrid program supplied with AutoDock4 was used for the preparation of grid maps. The grid box size was set at 76, 94, and 76 Å for x, y, and z respectively. The spacing between the grid points was 1.0 Å. The grid center was set at 8.639, 35.906, and 8.905 Å for x, y, and z, respectively. The Lamarckian genetic algorithm (LGA) was chosen from the ADT tool to search for the best conformers. During the docking process, a maximum of 10 conformers was considered for the ligand. The docking processes were performed with the default parameters of AutoDock 4. Population size was set to 150, maximum number of evaluations 2,500,000, the maximum number of generations 27,000, the maximum number of a top individual that automatically survived 1, gene mutation rate 0.02 and crossover rate 0.8. AutoDock4 was produced and run under the windows 7 operating system. All figures with structure representations were generated using a python molecule viewer.

Conclusions
From this present study, as-synthesized compounds showed significant activity in the larvicidal and antifeedent screenings. Cu(u)-tyrosinase enzyme was used as a catalyst for Biginelli reaction to give good yield. Compound 2h showed significant activity against mosquito larvae and low toxic with eco-friendly 1,2-3,4,6-tetrahydrophthalazine-1,2-dione and 1,2-3,4,6-tetrahydrophthalazine-1,2-dione with good binding ability (OBP) of Culex quinquefasciatus. Therefore, these compounds might be a potential source for developing ecologically significant bioactive compounds, including biodegradable pesticides and biopharmaceuticals.

Conflicts of interest
There are no conflicts to declare.

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