Sümeyye Yücetepe, Bedia Koçyiğit-Kaymakçıoğlu, Xiangbing Yang, Nurhayat Tabanca*
and Fatih Tok*

Insecticidal effect of new synthesized chalcone derivatives on Caribbean fruit fly, *Anastrepha suspensa*

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Abstract: In this present study, new chalcone derivatives were synthesized from 4-aminoacetophenone, which were confirmed by spectroscopic methods. The toxic risks of chalcones to humans and the environment were investigated by a web-based platform called ADMETlab. With this program, the possible toxic effects of the compounds on liver, respiratory system, and eyes were evaluated. For the topical insecticidal activity, adult female Caribbean fruit fly, *Anastrepha suspensa*, was targeted. Results of the toxicity tests showed that chalcone derivatives are effective against female *A. suspensa*. Among the synthesized chalcones, 1-(4-cinnamoylphenyl)-3-(*p*-tolyl)urea (**2**) exhibited the greatest insecticidal activity, resulting in 73 % mortality at 100 µg/fly after 24 h, whereas other derivatives showed less than 30 % mortality. Our results demonstrate that insecticidal activity may be modulated by the presence of a certain phenyl ring in the structure of derivative **2** and, therefore, has potential for design of efficient chemicals for tephritid fruit fly management.

Keywords: urea; chalcone; fruit fly; tephritidae; mortality

1 Introduction

Urea and its derivatives have an important place in synthetic chemistry due to possessing a broad range of pharmacological activity [1]. Suramin was the first urea structure-bearing molecule to be introduced to clinics due to its trypanocidal activity [2]. Research over the years has revealed many urea derivatives as candidates for medical intervention or approved drugs due to their multiple stable hydrogen binding capabilities with protein and receptor targets [3, 4]. Urea-containing substances are increasingly utilized for a broad range of applications, including antibacterial, antifungal, antileishmanial, pesticidal, and insecticidal activity [5].

Chalcones represent flavonoid intermediates with the general structure of 1,3-diphenyl-2-propen-1-one [6]. These compounds occur in natural products such as in vegetables, fruits, and spices [7]. Their structural characteristics and potential biological activities provide new synthetic derivatives. However, synthesis studies and structure–activity studies on chalcones are promising for discovering new derivatives that show no toxicity against Vero cells [8]. Chalcone is one of the most prominent classes of metabolites in the plant kingdom, Fabaceae, Moraceae, Zingiberaceae, and Cannabaceae, which plays critical roles in various physiological processes [9]. It is an important class of precursor compounds due to its few side effects and simple synthesis properties [10, 11]. Chen et al. reported that the compounds they designed in chalcone structure showed higher antifungal activity than standard azoxystrobin against plant pathogenic fungi such as *Botrytis cinerea* [12]. Ali et al. determined that the chalcone compounds they synthesized demonstrate insecticidal activity against *Spodoptera frugiperda*, an economically important insect pest of corn crops [13]. Yungkun et al. found that the chalcone derivatives they synthesized showed insecticidal activity close to the reference imidacloprid against *Aphis craccivora* [14]. Gad et al. introduced the structures of urea derivatives that showed excellent insecticidal activity against *Spodoptera littoralis* damaging cotton crops [15]. Taken together, chalcone and urea derivatives demonstrate potential as insecticidal

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***Corresponding authors: Fatih Tok**, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, 34854, Istanbul, Türkiye, E-mail: fatih.tok@marmara.edu.tr. https://orcid.org/0000-0002-4569-008X (F. Tok); and **Nurhayat Tabanca**, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Subtropical Horticulture Research Station (SHRS), 13601 Old Cutler Rd., Miami, FL 33158, USA, E-mail: nurhayat.tabanca@usda.gov
Sümeyye Yücetepe, Institute of Health Sciences, Marmara University, 34865, Istanbul, Türkiye
Bedia Koçyiğit-Kaymakçıoğlu, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Biruni University, 34015, Istanbul, Türkiye
Xiangbing Yang, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Subtropical Horticulture Research Station (SHRS), 13601 Old Cutler Rd., Miami, FL 33158, USA

compounds against insect pests and could be used as sustainable alternatives for pest management [13, 15, 16].

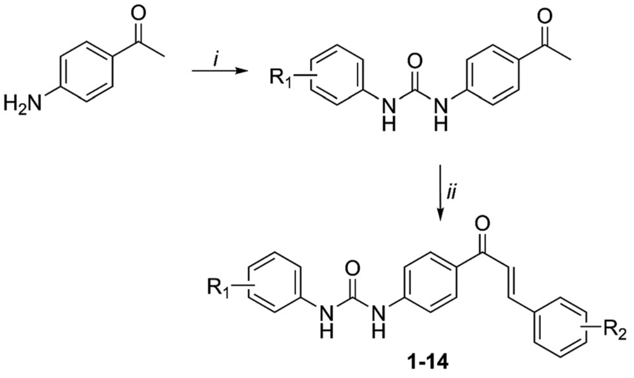
Tephritid fruit flies are critical insect pests for fruit production worldwide. In the USA, environmentally sound management strategies for tephritid fruit flies are conducive to subtropical and tropical fruit production in southern states, particularly in Florida. The Caribbean fruit fly (CFF) or Caribfly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), is one of the most serious economic pests of fruit crops in the tropical and subtropical areas of America. Female CFFs lay eggs under the skin of the fruits, resulting in reduced yield and fruit quality [17]. The predominant method for the management of fruit flies relies on conventional insecticide bait sprays in commercial groves. However, environmental contamination by conventional insecticides can expose not only the non-target beneficial insects but also the aquatic organisms that are more sensitive to surface-active substances. The continued use of these conventional insecticides also leads to undesirable effects on human health and the environment [18–21]. Reducing insecticide use in agriculture is necessary to avoid non-target beneficial insects as well as the natural enemies of insect pests.

Thus, in this study, 14 chalcone derivatives were designed and synthesized, and the structures were spectroscopically characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. The toxicity of these 14 derivatives against female CFF was also evaluated to assess their potential as alternatives to conventional pesticides for the management of CFFs. In addition, in order to evaluate the compounds as insecticides, the impact on human health and the environment was also taken into consideration with ADMETlab server.

2 Results and discussion

2.1 Chemistry

Urea derivatives were obtained in the first step as a result of the nucleophilic addition reaction of 4-aminoacetophenone and various isocyanate molecules. These compounds then formed chalcone derivatives according to the Claisen–Schmidt reaction mechanism [22] with various aldehyde derivatives (Scheme 1). IR spectroscopy, ^{1}H-NMR spectroscopy, and ^{13}C-NMR spectroscopy, as well as elemental analysis data, were used to confirm the chemical structures of the synthesized compounds.



Scheme 1: The synthetic pathway of target compounds. Reagents and conditions: (i) acetone, substituted isocyanate, 90 °C, 6 h; (ii) methanol, benzaldehyde derivative, 40 % NaOH, rt, 3 h.

In the IR spectrum of the synthesized target compounds, the N–H stretching bands of urea structures were detected at 3244–3336 cm^{-1}. The C=O stretching bands of urea and chalcone structures were recorded at 1600–1662 cm^{-1}. In the ^{1}H-NMR spectrum of the synthesized target compounds, the urea NH protons resonated in the range of 8.65–9.88 ppm as two separate singlet peaks. The –CH=CH– protons forming the chalcone structure were detected in the range of 6.78–8.30 ppm together with aromatic protons. In the ^{13}C-NMR spectrum of the synthesized target compounds, C=O peaks of chalcone and urea structures, which are important clues of the completion of the synthesis, were detected in the range of 186.24–187.81 ppm and 152.21–153.20 ppm, respectively. Elemental analysis data were found to be compatible with theoretical values.

Kurşun-Aktar et al. detected C=O stretching bands in the range of 1655–1693 cm^{-1} and N–H stretching bands in the range of 3062–3346 cm^{-1} in the IR spectrum of compounds bearing urea and chalcone structures. In the ^{1}H-NMR spectra of the same study, they observed chalcone protons in the range of 7.62–7.91 ppm and urea protons in the range of 6.40–10.54 ppm; in the ^{13}C-NMR spectra, they observed the C=O peak of the chalcone structure in the range of 187.04–189.00 ppm [23].

Sıcak et al. reported that they obtained C=O stretching bands in the range of 1627–1676 cm^{-1} in IR spectra of the chalcone structures they synthesized, chalcone protons in the range of 7.22–7.97 ppm in ^{1}H-NMR spectra, and C=O carbon in the range of 177.13–189.60 ppm in ^{13}C-NMR spectra [24].

In a previous study, in the IR spectra of a urea compound, N–H stretching bands were detected in the range of 3360 cm^{-1} and C=O stretching bands in the range of 1597 cm^{-1}. In the ^{1}H-NMR spectra, urea protons resonated in the range of 9.25–9.26 ppm [25]. All these literature data and the spectroscopic data obtained in this study are fully compatible with each other.

2.2 Prediction of toxicity studies

A drug candidate molecule to be developed as an insecticide or pesticide is expected to have minimal harmful effects on human and environmental health. The bioconcentration factor (BCF) is known as the ratio of chemical concentration in biota to that in steady-state water, as a result of absorption through the respiratory surface. It is used to assess the potential for secondary poisoning and to predict risks to human health through the food chain [26]. When the toxicity parameters were calculated through ADMETlab server, it was determined that the bioconcentration factor values of the compounds were in the range of 1.618–3.364. The IGC_{50} values of the compounds are close to each other and are in the range of 4.966–5.584. When the hepatotoxic potential of the compounds was evaluated, it was determined that the toxicity risk was low. When the corrosive or irritant properties of the compounds for the eye were evaluated, no significant risk was observed. Similarly, the toxic effect of the compounds on the respiratory system was estimated to be low, except for compound **12** (Table 1).

2.3 Toxicity of chalcone derivatives against female *A. suspensa*

The results of topical bioassays showed that all derivatives demonstrated significant toxicity against female adult *A. suspensa*, with varied mortality across all 14 derivatives (Figure 1) ($F = 9.83$; $df = 30, 44$; $P < 0.0001$). Derivative **2** showed the strongest toxicity with 73.0 % of mortality, followed by derivatives **1**, **3**, **8**, **12**, **14**, and **10**, which resulted in mortalities of 30.0, 27.0, 23.0, 23.0, 23.0, and 20.0 %, respectively, and all were significantly greater than that of control (DMSO) treatments, which resulted in 3.0 % mortality. Derivatives **4**, **11**, and **13** showed the lowest toxicity with 7.0 % mortality as same for all three, with no significant difference compared to control treatments. Derivatives **5**, **6**, **7**, and **9** showed mild toxicity against female *A. suspensa*, with 17.0, 17.0, 13.0, and 13.0 % mortality, although these values were not significantly different from control treatments.

Although no derivative, at the tested dosage, showed toxicity to cause 100 % mortality for female adult *A. suspensa*, derivative **2** had significantly greater toxicity than control and other derivatives. Current management of tephritid fruit flies relies heavily on the use of conventional insecticides, which has raised various concerns including environmental contamination by synthesized chemicals, negative impacts on non-target organisms, and resistance development of insect pests to conventional insecticides. The urgent need for safe and sound management of tephritid fruit flies is critical for subtropical and tropical fruit crop production, as well as for phytosanitary treatment of exported fruit produce from the USA. In our study, derivative **2** resulted in the highest mortality of *A. suspensa*, which was promising as it also demonstrated the lowest toxic risk with the lowest bioconcentration factor and respiratory toxicity (Table 1). Derivative **2** may have the potential to serve as a synergist along with other eco-friendly chemicals for pest management, by optimizing the composition with

Table 1: The toxicity risk profile of target compounds.

Compound	Environmental toxicity		H-HT	Eye corrosion	Eye irritation	Respiratory toxicity
	Bioconcentration factors	IGC_{50}				
1	2.116	5.051	0.109	0.003	0.41	0.177
2	1.618	5.027	0.035	0.003	0.676	0.079
3	2.287	5.000	0.235	0.003	0.280	0.229
4	2.523	5.308	0.059	0.003	0.247	0.066
5	2.514	5.268	0.116	0.003	0.174	0.147
6	2.505	5.313	0.065	0.003	0.210	0.130
7	3.364	5.584	0.050	0.003	0.238	0.158
8	2.812	5.442	0.044	0.003	0.418	0.067
9	2.434	4.966	0.532	0.003	0.130	0.239
10	2.271	5.027	0.324	0.003	0.157	0.266
11	2.709	5.307	0.223	0.003	0.138	0.221
12	2.182	4.993	0.281	0.003	0.401	0.912
13	2.043	5.091	0.590	0.003	0.129	0.548
14	2.315	5.058	0.561	0.003	0.121	0.262

H-HT: The human hepatotoxicity, Category 1: hepatotoxic chemicals; Category 0: non-hepatotoxic chemicals. Eye corrosion/Eye irritation: Category 1: corrosives/irritants chemicals; Category 0: non-corrosives/non-irritants chemicals. Respiratory toxicity: Category 1: respiratory toxicants; Category 0: non-respiratory toxicants.

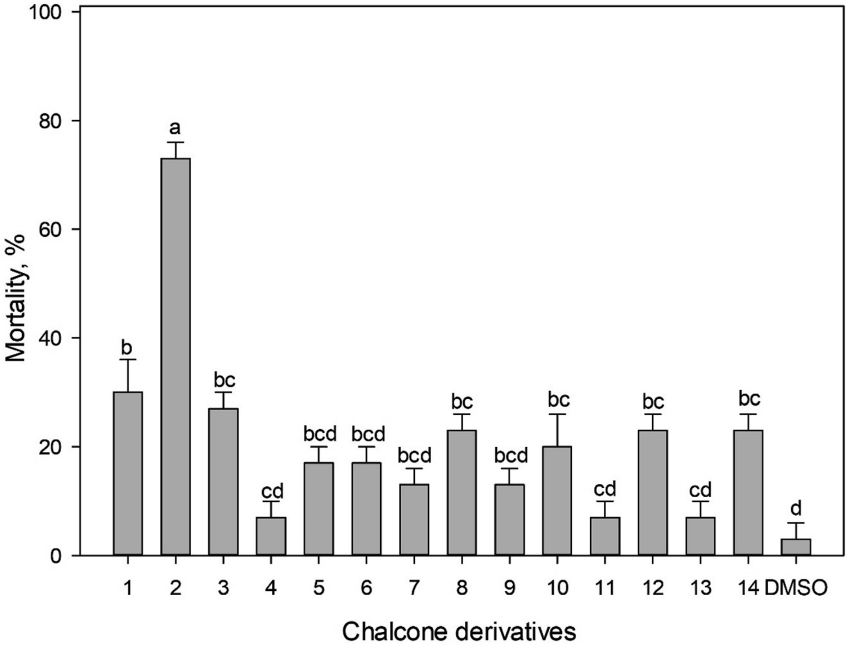


Figure 1: Mortality of female *Anastrepha suspensa* treated with 14 chalcone derivatives. Each of the 14 compounds and DMSO were applied at 1 μg/fly.

other insecticidal compounds, demonstrating potential for developing novel compounds for tephritid fruit fly management. Alternative environmentally friendly chemicals to conventional insecticides for pest management are not only conducive to plant safety but also lower the selection pressure for resistance development of insect pests by continued utilization of conventional pesticides. Future studies are required to evaluate higher dosages of derivatives with alternative solvents and their lethal toxicity against different life stages of *A. suspensa*, as well as other insect pests.

3 Conclusions

Fourteen chalcone derivatives were synthesized, and their characteristics were studied using spectroscopic methods. All derivatives did not exhibit significant toxic effects on human health and the environment, except for derivative **12**. Furthermore, this study is the first report on the insecticidal activity of 14 chalcone derivatives against female *A. suspensa*, while only derivative **2** resulted in 73 % mortality. The other derivatives demonstrated mild insecticidal toxicity that is possibly due to their lower ability to penetrate the insect cuticle. Although the majority of these novel chalcone derivatives were less potent as insecticides against this pest, this study revealed that derivative **2** could serve as the basis to further design synthesize novel derivatives with a broad spectrum of activity for controlling CFF and other insect pests. Our results suggested that this compound (**2**) could be potential source for developing insecticides for further studies.

4 Experimental section

All chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA). The melting points of the compounds were determined by Schmelzpunktbestimmer SMP II (Gottfried-Keller-Weg, Überlingen, Germany). Infrared spectra were obtained with an FTIR-8400S Shimadzu spectrometer (Tokyo, Japan). ^{1}H-NMR and ^{13}C-NMR spectra were recorded against an internal reference standard of TMS in deuterium DMSO using a Bruker Avance III HD 600 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, respectively. Elemental analyses were performed using Leco CHNS-932.

4.1 General synthesis of urea compounds

4-Aminoacetophenone (10 mmol) was dissolved in 15 mL of acetone, and substituted isocyanate derivative (10 mmol) was added dropwise and heated in a water bath for 6–8 h. Excess solvent was evaporated under vacuum. The solid formed at the end of the reaction was filtered, dried, and crystallized from acetone [27].

4.2 General synthesis of chalcone compounds

1-(4-Acetylphenyl)-3-substituted urea (1 mmol) was dissolved in 10 mL of methanol. Benzaldehyde derivative (1 mmol) was added to the mixture taken on a magnetic stirrer. NaOH (40 %, 1 mL) solution was added to the mixture as a catalyst. Colored substances that precipitate over time are collected by washing with water and crystallized from ethanol [28].

Synthesized compounds **1**, **2**, **4**, and **6** are registered in the literature and their melting points are given below [29, 30]. All other compounds were synthesized for the first time in this study.

4.2.1 1-(4-(3-(4-Fluorophenyl)acryloyl)phenyl)-3-(p-tolyl)urea (1): Yield: 75 %, white solid, m.p. = 223 °C [29].

4.2.2 1-(4-Cinnamoylphenyl)-3-(p-tolyl)urea (2): Yield: 70 %, yellow solid, m.p. = 207 °C [29].

4.2.3 1-(4-Fluorophenyl)-3-(4-(3-(4-fluorophenyl)acryloyl)phenyl)urea (3): Yield: 75 %, gray solid, m.p. = 246 °C. FTIR (ATR, cm^{-1}): 3288 (N–H), 3078 (=C–H), 1656 (C=O urea), 1643 (C=O), 1587, 1506 (C=C), 1340 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.16–8.16 (m, 14H, ArH and chalcone CH=CH), 8.89 (s, 1H, NH), 9.18 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 113.17, 115.62, 115.84, 116.16, 116.26, 116.38, 117.79, 120.43, 120.51, 120.83, 122.79, 125.74, 130.60, 131.18, 131.27, 131.62, 136.49, 140.63, 153.20, 154.39, 156.63, 158.99, 186.24. Anal. calcd for $C_{22}H_{16}F_2N_2O_2$: C 69.84, H 4.26, N 7.40. Found: C 69.42, H 4.20, N 7.52 %.

4.2.4 1-(2,4-Dichlorophenyl)-3-(4-(3-(p-tolyl)acryloyl)phenyl)urea (4): Yield: 70 %, yellow solid, m.p. = 206–207 °C [30].

4.2.5 1-(2,4-Dichlorophenyl)-3-(4-(3-(4-fluorophenyl)acryloyl)phenyl)urea (5): Yield: 72 %, white solid, m.p. = 220 °C. FTIR (ATR, cm^{-1}): 3308 (N–H), 3070 (=C–H), 1639 (C=O urea), 1600 (C=O), 1579, 1506 (C=C), 1300 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.02–8.24 (m, 13H, ArH and chalcone CH=CH), 9.16 (bs, 1H, NH), 9.88 (bs, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 114.86, 116.27, 116.49, 117.87, 122.33, 122.92, 123.54, 127.01, 128.15, 129.10, 130.50, 130.66, 131.13, 131.64, 131.79, 132.00, 135.41, 142.41, 144.53, 152.28, 162.55, 165.02, 187.70. Anal. calcd for $C_{22}H_{15}Cl_2FN_2O_2$: C 61.56, H 3.52, N 6.53. Found: C 60.04, H 3.53, N 6.13 %.

4.2.6 1-(2,4-Dichlorophenyl)-3-(4-(3-(4-methoxyphenyl)acryloyl)phenyl) urea (6): Yield: 68 %, white solid, m.p. = 212 °C [30].

4.2.7 1-(2,4-Dichlorophenyl)-3-(4-(3-(3,4-dichlorophenyl)acryloyl)phenyl) urea (7): Yield: 60 %, yellow solid, m.p. = 208 °C. FTIR (ATR, cm^{-1}): 3336, 3302 (N–H), 3076 (=C–H), 1647 (C=O urea), 1593, 1519 (C=C), 1301 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.54–8.26 (m, 12H, ArH and chalcone CH=CH), 9.13–9.36 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 117.72, 117.85, 123.01, 123.64, 124.52, 126.98, 128.04, 128.13, 129.09, 129.58, 130.20, 130.53, 130.82, 131.18, 131.45, 132.85, 135.49, 136.23, 140.82, 144.44, 152.34, 187.81. Anal. calcd for $C_{22}H_{14}Cl_4N_2O_2$: C 55.03, H 2.94, N 5.83. Found: C 55.02, H 2.94, N 5.70 %.

4.2.8 1-(4-(3-(4-Bromophenyl)acryloyl)phenyl)-3-(2,4-dichlorophenyl) urea (8): Yield: 58 %, white powder, m.p. = 290 °C. FTIR (ATR, cm^{-1}): 3275 (N–H), 3086 (=C–H), 1643 (C=O urea), 1600 (C=O), 1585, 1543 (C=C), 1300 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.36–8.24 (m, 13H, ArH and chalcone CH=CH), 9.20–9.47 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 117.88, 122.90, 123.23, 123.52, 124.26, 126.19, 127.08, 128.18, 129.12, 130.73, 131.19, 131.73, 132.33, 134.60, 135.30, 142.28, 144.58, 152.21, 187.68. Anal. calcd for $C_{22}H_{15}BrCl_2N_2O_2$: C 53.91, H 3.08, N 5.72. Found: C 54.72, H 3.15, N 5.53 %.

4.2.9 1-(2,4-Difluorophenyl)-3-(4-(3-(4-fluorophenyl)acryloyl)phenyl) urea (9): Yield: 65 %, white solid, m.p. = 209 °C. FTIR (ATR, cm^{-1}): 3309 (N–H), 3078 (=C–H), 1645 (C=O), 1597, 1552 (C=C), 1307 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.06–8.17 (m, 13H, ArH and chalcone CH=CH), 8.67 (s, 1H, NH), 9.49 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.09, 104.35, 104.59, 111.48, 111.70, 114.85, 116.27, 116.48, 117.74, 122.35, 122.86, 124.19, 130.65, 131.12, 131.54, 131.65, 131.99, 142.38, 144.64, 152.48, 162.54, 165.01, 187.69. Anal. calcd for $C_{22}H_{15}F_3N_2O_2$: C 66.67, H 3.81, N 7.07. Found: C 65.61, H 3.73, N 7.23 %.

4.2.10 1-(2,4-Difluorophenyl)-3-(4-(3-(4-methoxyphenyl)acryloyl) phenyl)urea (10): Yield: 70 %, white solid, m.p. = 212 °C. FTIR (ATR, cm^{-1}): 3292 (N–H), 3049 (=C–H), 2978 (C–H), 1647 (C=O), 1595, 1566 (C=C), 1292 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 7.02–8.15 (m, 13H, ArH and chalcone CH=CH), 8.87–9.35 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 55.84, 104.35, 104.61, 111.49, 111.71, 114.86, 117.73, 119.92, 122.81, 122.91, 124.20, 127.93, 130.48, 131.13, 131.94, 143.60, 144.45, 147.34, 152.52, 161.69, 187.67. Anal. calcd for $C_{23}H_{18}F_2N_2O_3$: C 67.64, H 4.44, N 6.86. Found: C 68.12, H 4.42, N 6.44 %.

4.2.11 1-(4-(3-(3,4-Dichlorophenyl)acryloyl)phenyl)-3-(2,4-difluorophenyl) urea (11): Yield: 77 %, white solid, m.p. = 220 °C. FTIR (ATR, cm^{-1}): 3244 (N–H), 3066 (=C–H), 1645 (C=O), 1599, 1552 (C=C), 1303 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.06–8.30 (m, 12H, ArH and chalcone CH=CH), 8.69 (s, 1H, NH), 9.51 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.10, 104.37, 104.60, 111.50, 111.67, 117.72, 122.78, 124.02, 124.06, 124.50, 129.55, 130.52, 130.81, 131.43, 132.27, 132.98, 136.22, 140.80, 144.84, 151.76, 152.47, 154.20, 156.47, 158.87, 187.50. Anal. calcd for $C_{22}H_{14}Cl_2F_2N_2O_2$: C 59.08, H 3.16, N 6.26. Found: C 58.52, H 3.12, N 6.41 %.

4.2.12 1-(2,4-Difluorophenyl)-3-(4-(3-(4-(dimethylamino)phenyl) acryloyl)phenyl)urea (12): Yield: 70 %, orange solid, m.p. = 175 °C. FTIR (ATR, cm^{-1}): 3323 (N–H), 3080 (=C–H), 2910 (C–H), 1662 (C=O), 1593, 1535 (C=C), 1311 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.95–3.22 (m, 6H, $N(CH_3)_2$), 6.78–8.13 (m, 13H, ArH and chalcone CH=CH), 8.90–9.13 (bs, 1H, NH), 9.67 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 19.03, 56.50, 104.09, 104.36, 104.60, 111.50, 111.71, 112.22, 116.47, 117.70, 122.62, 122.72, 124.15, 124.25, 130.22, 131.08, 132.44, 144.04, 144.77, 151.70, 152.33, 152.51, 154.14, 156.40, 158.80, 187.40. Anal. calcd for $C_{24}H_{21}F_2N_3O_2$: C 68.40, H 5.02, N 9.97. Found: C 66.97, H 5.09, N 9.68 %.

4.2.13 1-(2,4-Difluorophenyl)-3-(4-(3-(4-(trifluoromethyl)phenyl) acryloyl)phenyl)urea (13): Yield: 72 %, light yellow solid, m.p. = 210 ° C. FTIR (ATR, cm^{-1}): 3304 (N–H), 3099 (=C–H), 1658 (C=O), 1597, 1546 (C=C), 1323 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.00–8.29 (m, 13H, ArH and chalcone CH=CH), 8.89–9.30 (bs, 2H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 104.09, 104.36, 104.60, 111.49, 111.71, 117.76, 122.86, 123.20, 124.16, 125.13, 125.90, 126.16, 129.80, 130.19, 130.50, 130.80, 131.39, 139.34, 141.59, 144.86, 151.74, 152.46, 154.18, 156.46, 158.86, 187.63. Anal. calcd for $C_{23}H_{15}F_5N_2O_2$: C 61.89, H 3.39, N 6.28. Found: C 60.59, H 3.31, N 6.14 %.

4.2.14 1-(2,4-Difluorophenyl)-3-(4-(3-(4-(methylthio)phenyl)acryloyl) phenyl)urea (14): Yield: 66 %, creamy powder, m.p. = 222 °C. FTIR (ATR, cm^{-1}): 3292 (N–H), 3078 (=C–H), 2924 (C–H), 1645 (C=O), 1587, 1562 (C=C), 1303 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 2.59 (s, 3H, CH_3S), 7.11–8.17 (m, 13H, ArH and chalcone CH=CH), 8.65 (s, 1H, NH), 9.47 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.63, 104.10, 104.37, 104.61, 111.50, 111.71, 117.74, 121.26, 122.81, 124.10, 126.00, 129.75, 130.57, 131.67, 131.79, 142.21, 143.23, 144.56, 151.77, 152.50, 154.20, 156.45, 158.86, 187.66. Anal. calcd for $C_{23}H_{18}F_2N_2O_2S$: C 65.08, H 4.27, N 6.60. Found: C 65.89, H 4.22, N 6.84 %.

4.3 Prediction of toxicity studies

ADMET lab 2.0 online program (https://admetmesh.scbdd.com/) was used to determine the toxicity profiles of the synthesized compounds. An estimation of the toxic effects of the compounds on the environment and evaluation of a negative effect on the liver, eye, and respiratory system were calculated using ADMET lab 2.0 program.

4.4 Toxicity of chalcone derivatives 1–14 against female *A. suspensa*

To determine the toxicities of newly derivatized chalcones **1**–**14**, topical application bioassays to adult female *A. suspensa* were conducted under the laboratory conditions as described previously [19]. For each derivative, a stock solution was first prepared by dissolving 10 mg of each compound (**1**–**14**) in 1 mL of dimethyl sulfoxide (DMSO) to establish a 10 mg/ml solution. Before a serial dilution with lower concentrations was made, 1 µL of the stock solution, containing 1 µL of compound, was first applied via topical bioassay on female adult *A. suspensa* to evaluate its toxicity. Serial dilutions based on the stock solution will be prepared once 100 % mortality has been observed. However, based on our results, no derivative demonstrated toxicity causing 100 % mortality of *A. suspensa*. Therefore, the lethal dosage of each derivative was not determined in the current study.

To prepare the female adult *A. suspensa* for the topical bioassay, pupae of *A. suspensa* were collected from the colony and placed in a tray inside a screen cage (30 × 30 × 30 cm) under laboratory conditions (28 ± 2 °C, 75 ± 5 R H and 14:10 light:dark photoperiod) to collect newly emerged female adults. After adult emergence, female adults were provided with food (sugar and yeast hydrolysate mixture at 4:1 per weight, 1 cm^3) and water agar. At 2 weeks old, female adults were collected into a plastic vial (3 cm in diameter × 8 cm in height) using an aspirator. Female adults in the vial were first cooled at −10 °C in a refrigerator for 5 min to anesthetize the flies, which were then removed from the refrigerator to a Petri dish for topical application. On each fly, a repeating dispenser equipped with gastight and microliter syringe (50 µL) (PB600, Hamilton Company, Reno, NV, USA) was used to apply 1 µL of solution of each derivative (**1**–**14**) on the dorsal thorax of the anaesthetized adult flies. After topical application, the adult flies were immediately transferred into a plastic cup (6 cm in diameter × 7.4 cm in height) and covered with a mesh screen for post-treatment observation. A block of sugar and yeast hydrolysate mixture and a block of water agar (1 cm^3) were placed on top of the mesh screen to supply the food and water for the tested flies. To remove the cooling effect from the treatment, only adult flies recovered from cooling were used for the experiment after topical bioassay. After 24 h, the number of live and dead flies was counted, and mortality of *A. suspensa* in each treatment was calculated. Untreated female adults and those treated with DMSO alone were used as controls. Ten female flies were tested per derivative, and the experiment was replicated three times.

4.5 Statistical analysis

Mortality data of female *A. suspensa* were calculated based on the number of live and dead flies. Mortality data for each treatment were first arcsin $\sqrt{x}$ transformed and subjected to a normality test using the Shapiro–Wilk test. The corrected mortality data were then analyzed using the general linear model (PROC GLM, SAS Institute), and separated means were analyzed by using Tukey's test. The statistical analysis was performed using SAS version 9.4 [31].

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