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ORIGINAL RESEARCH

# Mosquito larvicidal studies of some chalcone analogues and their derived products: structure–activity relationship analysis

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**Abstract** A series of chalcone analogues and some of their derivatives were synthesized and subjected to the mosquito larvicidal study. Chalcones having electron releasing group(s) on either ring A or ring B showed high toxicity. Electron withdrawing group(s), especially at ring B, reduced the activity of chalcones. The activity was abruptly decreased due to replacement of ring A by $CH_3$, extension of conjugation or blocking of $\alpha,\beta$-unsaturated ketone part of chalcones by derivatization. Quantitative structure–activity relationship (QSAR) studies of these compounds were performed using various spatial, electronic and physicochemical parameters. Genetic Function approximation with linear and spline options was used as the chemometric tool for developing the QSAR models.

**Keywords** Chalcones · *Culex quinquefasciatus* · Mosquito larvicidal activity · QSAR

## Introduction

Lymphatic filariasis, which may be caused by different species of filarial worm, e.g., *Wuchereria bancrofti*, has a scattered distribution in the tropics and subtropics. Nearly one billion people in the developing countries are at risk. Mosquito of *Culex* species is mainly the intermediate host and vector of Bancroftian filariasis (Kumar and Clark 2005). Diethylcarbamazine (DEC) kills both adult worms and microfilariae. However, serious allergic responses may occur as the parasites are killed and particular care is needed when one is using DEC in areas endemic for loiasis (lymphatic filariasis) (Kumar and Clark 2005). In Indian subcontinent, filariasis is quite common and *Culex quinquefasciatus* is the most prevalent species of mosquito in this region.

One of the primary ways to prevent this deadly disease is the control of mosquito larvae, for which several insect growth regulators, e.g., diflubenzuron and methoprene are used extensively along with other insecticides like organochlorinated compounds, organo-phosphates and carbamate type of compounds (Yang *et al.*, 2002; Chang *et al.*, 2003; Frear, 1955). But the constant and repeated applications of these controlling agents have some grave consequences toward the agriculture and public health programs including disruption the natural and biological control system, outbreak of other insect species, wide spread development of resistance, and undesirable effects toward the nontarget animals, e.g., acute poisoning and high mammalian toxicity (Yang *et al.*, 2002; Chang *et al.*, 2003; Frear, 1955). Thus, the unprecedented environmental persistence of these insecticides forced us to realize that they cannot be used indefinitely and in exponentially increasing quantities. Hence there is a tremendous need for new strategies for mosquito larval control which will be safe, cheaper, and more effective.

Chalcones are structurally simple compounds of the flavonoid family and are present in variety of plant species (Agarwala, 1989). Not only that, they can be readily synthesized in laboratory by Claisen-Schmidt reaction which is very easy and simple to conduct as well as inexpensive.

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Various substitution patterns can easily be incorporated on the two aromatic rings of chalcone to give large number of analogues which may have potential activity. Chemically chalcone is 1,3-diphenyl-2-propen-1-one. Depending on the substitution pattern on the two aromatic rings, a wide spectrum of pharmacological activities have been observed for chalcones (Liu *et al.*, 2001) encompassing anti-malarial (Liu *et al.*, 2001; Go *et al.*, 2004), anti-leshmanial, anti-fungal and immunosuppressive (Boeck *et al.*, 2006), anti-inflammatory (Herencia *et al.*, 1998) and anti-miotic (Ducki *et al.*, 1998) properties. Though chalcones have been widely studied, to our knowledge there is a lack of quantitative-structure–activity relationship study of the mosquito larvicidal activity of chalcones along with their derived products. This made us interested to study the larvicidal activities of some chalcone-type compounds with varying substitution pattern along with some of their derived products against the larvae of *Culex quinquefasciatus.*

In the present study, a series of chalcones (**1–28**) (Table 1) were synthesized by (i) varying the substitution pattern on ring A and ring B of chalcone (1,3-diphenyl-2-propen-1-one), (ii) varying the length of the conjugation in the $\alpha,\beta$-unsaturated ketone part, and (iii) by blocking the $\alpha,\beta$-unsaturated ketone part of chalcone by derivatization. Then, these products were evaluated for their mosquito larvicidal activity. Structure–activity relationship study of these synthesized compounds was also done based on the relevant physicochemical, spatial, and electronic parameters for these compounds. This would be our guiding tool for further exploration and lead optimization. Genetic function approximation (GFA) method with linear and spline options was applied as the chemometric tool to develop the QSAR models which would give us clear idea about the relationship between the larvicidal activity and the structural patterns of these compounds.

**Table 1** Larvicidal activities of chalcones and some of the derived products[a]

| Compound | $LC_{50}$ (μmole $dm^{-3}$) |
|---|---|
| (2*E*)-1,3-diphenylprop-2-en-1-one (**1**) | 90.00 |
| (2*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (**2**) | 55.00 |
| (2*E*)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one (**3**) | 104.00 |
| (2*E*)-3-(4-hydroxyphenyl-3-methoxyphenyl)-1-phenylprop-2-en-1-one (**4**) | 1474.00 |
| (2*E*)-3-(4-hydroxyphenyl-3-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5**) | 684.90 |
| (2*E*)-3-(3-nitrophenyl)-1-phenylprop-2-en-1-one (**6**) | 994.40 |
| (2*E*)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**7**) | 526.60 |
| (2*E*)-3-(1,3-benzodioxol-5-yl)-1-phenylprop-2-en-1-one (**8**) | 5.00 |
| (2*E*)-3-(1,3-benzodioxol-5-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**9**) | 986.10 |
| (2*E*)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (**10**) | 238.00 |
| (2*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (**11**) | 5.00 |
| (2*E*)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (**12**) | 959.50 |
| (2*E*)-1-(4-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (**13**) | 91.00 |
| (2*E*)-1-(2-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one (**14**) | 881.60 |
| (2*E*)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**15**) | 81.00 |
| (2*E*)-1-(4-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (**16**) | 89.00 |
| (2*E*)-3-(furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**17**) | 19.00 |
| (3*E*)-4-phenylbut-3-en-2-one (**18**) | 479.00 |
| (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (**19**) | 2064.40 |
| (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one (**20**) | 437.00 |
| (2*E*,4*E*)-1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one (**21**) | 1121.10 |
| Phenylhydrazone of **1** (**22**) | 2134.50 |
| 2,4-Dinitrophenylhydrazone of **1** (**23**) | 2531.30 |
| Phenylhydrazone of **18** (**24**) | 465.00 |
| 2,4-Dinitrophenylhydrazone of **18** (**25**) | 2344.10 |
| Semicarbazone of **18** (**26**) | 2087.90 |
| Oxime of **18** (**27**) | 345.00 |
| 2,4-Dinitrophenylhydrazone of **19** (**28**) | 2638.20 |

[a] A and B indicate ring systems in the general structure of chalcones

## Results and discussion

### Chemical synthesis

Total 28 compounds (chalcones along with some of the derived products) were synthesized and tested for mosquito larvicidal activity against the third instar larvae of *Culex quinquefasciatus*. Table 1 displays structures as well as the $LC_{50}$ values (μmole $dm^{-3}$) of these compounds against the third instar larvae of *Culex quinquefasciatus*.

Compounds (**1–17** and **20–21**, except **4** and **5**) were prepared by Claisen-Schmidt reaction in the absence of solvent. Here the method of Toda *et al*. (1990) has been employed with some modifications. In this method, no organic solvent was used unless in the case of recrystallisation of the crude product. Moreover, it requires no preformed enolate. No heating, stirring, or cooling were required here.

### Mosquito larvicidal activity

On the basis of larvicidal assay, we have divided these compounds into three main categories:

(1) Compounds showing 100% mortality of mosquito larvae at 100 μg $cm^{-3}$ concentration at 30 ± 2°C after 24 h. There are five compounds under this category; which are (**1**), (**2**), (**3**), (**17**), and (**15**).
(2) Compounds, which showed no mortality of mosquito larvae at 100 μg $cm^{-3}$ concentration at 30 ± 2°C after 24 h. There are three compounds under this category; which are (**23**), (**25**), and (**28**).
(3) Compounds which showed variations of % mortality of mosquito larvae at 100 μg $cm^{-3}$ concentration at 30 ± 2°C after 24 h are included in this category. These are (**4**), (**5**), (**6**), (**7**), (**8**), (**9**), (**10**), (**11**), (**12**), (**13**), (**14**), (**16**), (**18**), (**19**), (**20**), (**21**), (**22**), (**24**), (**26**), and (**27**). Total 20 compounds are included in this category. These twenty compounds showed % mortality ranging from a very low value (∼10%) to a very high value (∼90%).

Present toxicity study showed that chalcones having electron releasing group on either ring A or on ring B showed high activities. Analysing the % mortality and $LC_{50}$ values for all the compounds, it is evident that the lead compounds were (2*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (**11**), i.e. 4-chlorochalcone, which has a chlorine substituent at 4 position of ring B and (2*E*)-3-(1,3-benzodioxol-5-yl)-1-phenylprop-2-en-1-one (**8**) with a methylenedioxy group at 3,4-position of ring B. Both of these two compounds showed potent larvicidal activity having $LC_{50}$ value of 5 μmole $dm^{-3}$. On the other hand, (2*E*)-3-(furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**17**), showed 100% mortality. Its $LC_{50}$ is also very low with a value of 19 μmole $dm^{-3}$. In this compound, ring B is replaced by a furan ring. Hydroxychalcones (**2**, **3**, **15**, and **16**) showed high activity.

Hydroxylated chalcones showed potent activity especially when there is –OH group at 2′-position on ring A as in the cases of compounds (**2**), (**15**), and (**17**). In general, it was noticed that the larvicidal activity of chalcones was reduced when there was electron withdrawing group on ring B, e.g., (**6**), (**7**), (**12**), and (**14**). Compounds (**13**) and (**16**) showed high activity though there is $–NO_2$ group at 4 and 3 positions, respectively, on ring B. But in addition to $–NO_2$ group, there is also one –OH group at the 4′-position on ring A; which may be the cause of their enhanced activity. But when there is a $–NO_2$ group on ring B and at the same time there is a –OH group at 2′-position on ring A, low activity was observed as in the case of (**7**) and (**14**). It was observed that when there is a chlorine substituent at the 4-position of ring B, activity is enhanced, e.g., (**11**). Another trend was observed: when there was electron releasing group on both rings A and B, the activity of chalcones was abruptly decreased as in the case of (2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one (**4**) and (2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**5**). Activity was also reduced when ring A is replaced by a methyl group or $C_6H_5$–CH=CH– group, e.g., 4-phenyl-but-3-en-2-one (**18**) and 1,5-diphenyl-penta-1,4-dien-3-one (**19**). Larvicidal activity was also reduced when ring B was replaced by $C_6H_5$–CH=CH– group, e.g., compounds (**20**) and (**21**). But it was observed that when ring B is replaced by a furan ring, the activity was highly enhanced as in the case of compound (**17**). However, compound (**18**) showed higher activity than (**19**), (**20**), and (**21**). Actually in (**19**), (**20**), and (**21**), there was an extension of conjugation of the α,β-unsaturated ketone part of chalcone and this might be the cause of their lower larvicidal activity. When the α,β-unsaturated ketone part was blocked by derivatization, activity was drastically reduced as in the case of compounds (**22**), (**23**) (**25**), and (**28**), though some toxicity was noticed in case of (**27**). This result was in agreement with a previous report (Das *et al*., 2005).

### Structure–activity relationship study

Biological activity of a compound depends on the types and magnitude of interactions between the target site and the molecule. Various structural attributes of the drug molecule like electronic distribution, steric feature, etc., are the determining factors regulating the interactions. Quantitative structure activity relationship (QSAR) studies (van de Waterbeemd, 1995a, b) are based on the notion that biological activity is function of structure and/or property.

The goals of QSAR studies include better understanding of the modes of actions, prediction of new analogues with better activity, and optimization of the lead compound to reduce toxicity and increase selectivity. In order to have a clear understanding about the relationship between the larvicidal activity and structures of the synthesized chalcones, we have performed a preliminary QSAR study using structural (numbers of rotatable bonds, hydrogen bond donors, and acceptors and chiral centers), physicochemical [lipophilicity (AlogP98) and molar refractivity (MolRef)], spatial (radius of gyration, Jurs descriptors, shadow indices, area, density, and molar volume) and electronic (principal moment of inertia, dipole moment, energies of highest occupied and lowest unoccupied molecular orbitals, and superdelocalizability) parameters. For the QSAR work, the larvicidal concentration data (μg $cm^{-3}$) were first converted into the molar scale and then the negative logarithm of the inverse of concentration [$pLC_{50}$(M)] was used as the response variable.

Genetic function approximation with linear and spline options was used as the chemometric tool for developing the QSAR models. GFA (Rogers and Hopfinger, 1994) involves a combination of multivariate adaptive regression splines (MARS) algorithm with genetic algorithm to evolve a population of equations. In the GFA technique, an initial population of equations is first generated by random selection of descriptors followed by random crossover between pairs of equations chosen from the population whereby new progeny equations are formed. The fitness of the equations formed is measured by the "lack of fit" (LOF) value. The equations with lower LOF value show higher significance. The LOF is given by:

$$\mathrm{LOF} = \frac{\mathrm{LSE}}{\left(1 - \frac{c + d*p}{M}\right)^2},$$

where LSE is the least square error, $c$ is the number of basis functions, $d$ is the smoothing parameter which was set at the default value of 1, $p$ is the number of descriptors, and $m$ is the number of observations in the training set. GFA builds models not only with linear functions, but also use higher order polynomials, splines, etc. The spline option enables nonlinear modeling of activity as the function of the different variables. For example, $<f(x) - a>$ is equal to zero if the value of $<f(x) - a>$ is negative, else it is equal to $f(x) - a$. The constant '$a$' is called the knot of the spline.

Descriptor calculation and model development was done using Cerius2 software (http://www.accelrys.com/) running on a Silicon Graphics O2 workstation. The values of important descriptors are shown in Table 2.

Genetic function approximation with 5,000 iterations and linear terms generated the following best equation:

$$\begin{aligned} pLC_{50} &= 19.913(\pm 4.722) - 0.025(\pm 0.005)\mathrm{Jurs\_PPSA\_1} \\ &\quad - 6.784(\pm 2.125)\mathrm{Jurs\_RPCG} + 0.692(\pm 0.312)\mathrm{HOMO} \\ n &= 28, R^2 = 0.590, R_a^2 = 0.538, F = 11.5(df\,3, 24), \\ s &= 0.529, Q^2 = 0.455, \mathrm{PRESS} = 8.9 \end{aligned} \quad (1)$$

The standard errors of the regression coefficients are shown within parentheses. All regression coefficients are significant at 95% level. Equation 1 could explain 53.8% of the variance of the larvicidal activity while the predicted variance was found to be 45.5%.

Equation 1 contains two Jurs descriptors and one electronic parameter, which is energy of the highest occupied molecular orbital (HOMO). Jurs descriptors combine shape and electronic information to characterize the molecules. The descriptors are calculated by mapping atomic partial charges on solvent-accessible surface areas of individual atoms. Both Jurs descriptors present in Eq. 1 show negative coefficients while the HOMO energy shows positive contribution.

Jurs_PPSA_1 is partial positive surface area, which is the sum of the solvent-accessible surface areas of all positively charges atoms. Jurs_RPCG is the partial charge of the most positive atom divided by the total positive charge. The negative coefficients of these terms indicate that compounds with higher positively charged surface areas (because of presence of electron withdrawing substituents) will have less larvicidal activity.

HOMO is the energy of highest occupied molecular orbital of a molecule. It is crucially important in governing molecular reactivity and properties. Molecules with high HOMO energies are more able to donate their electrons and are hence relatively reactive compared to molecules with low-lying HOMOs; thus descriptor HOMO should measure the nucleophilicity of a molecule. The positive coefficient of HOMO in Eq. 1 indicates that electron withdrawing substituents are not favorable for the activity.

GFA with spline option gave the following best equation:

$$\begin{aligned} pLC_{50} &= 4.068(\pm 0.326) - 24.477(\pm 4.145) \\ &\quad \langle 1.120 - \mathrm{Density} \rangle + 7.687(\pm 1.667) \\ &\quad \langle \mathrm{Jurs\_FNSA\_2} + 0.697 \rangle - 0.001(\pm 0.0.001)\mathrm{Jurs\_PPSA\_2} \\ n &= 28, R^2 = 0.709, R_a^2 = 0.672, F = 19.5(df\,3, 24), \\ s &= 0.446, Q^2 = 0.633, \mathrm{PRESS} = 6.0 \end{aligned} \quad (2)$$

Note that on using spline option, there has been considerable increase in statistical quality in comparison to Eq. 1. The predicted variance of Eq. 2 is 63.3% compared to 45.5% in case of Eq. 1.

Density is a 3D spatial descriptor, which is defined as the ratio of molecular weight to molecular volume. The density reflects the types of atoms and how tightly they are

**Table 2** Values of important descriptors and observed and calculated larvicidal activity of synthesized chalcones

| Sl. No. | Descriptors | | | | | | Larvicidal activity [$pLC_{50}$] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Jurs-PPSA-1 | Jurs-PPSA-2 | Jurs-RPCG | Density | HOMO | Jurs-FNSA-2 | Obs. | Calc.[a] | Calc.[b] |
| 1 | 262.683753 | 252.79364 | 0.192591 | 1.02629 | −11.996 | −0.39215 | 4.046 | 3.669 | 3.755 |
| 2 | 269.847858 | 333.418953 | 0.176666 | 1.064626 | −11.9302 | −0.50744 | 4.26 | 3.642 | 3.686 |
| 3 | 278.61443 | 335.396975 | 0.181327 | 1.062644 | −11.7095 | −0.47318 | 3.983 | 3.542 | 3.898 |
| 4 | 315.655653 | 464.497803 | 0.148406 | 1.075214 | −11.2893 | −0.5438 | 2.831 | 3.12 | 3.47 |
| 5 | 312.68571 | 545.563021 | 0.125165 | 1.100116 | −11.4136 | −0.68522 | 3.164 | 3.267 | 2.871 |
| 6 | 230.122828 | 380.533502 | 0.345244 | 1.119427 | −12.4616 | −0.88399 | 3.002 | 3.134 | 3.497 |
| 7 | 228.571569 | 440.421277 | 0.296288 | 1.14441 | −12.4428 | −1.05295 | 3.25 | 3.518 | 3.41 |
| 8 | 284.61765 | 397.922957 | 0.132566 | 1.114274 | −11.2293 | −0.57585 | 5.301 | 4.053 | 4.279 |
| 9 | 289.581378 | 483.985482 | 0.130606 | 1.142259 | −11.1764 | −0.69747 | 3.006 | 3.978 | 3.345 |
| 10 | 259.968908 | 310.793236 | 0.182561 | 1.06047 | −11.9079 | −0.52502 | 3.623 | 3.867 | 3.483 |
| 11 | 233.168295 | 220.090305 | 0.196353 | 1.120704 | −11.9415 | −0.47314 | 5.301 | 4.427 | 5.464 |
| 12 | 230.417008 | 380.923501 | 0.345318 | 1.119554 | −12.5628 | −0.87586 | 3.018 | 3.056 | 3.499 |
| 13 | 229.016357 | 432.003539 | 0.302637 | 1.146881 | −12.3171 | −1.02922 | 4.041 | 3.551 | 3.423 |
| 14 | 237.997626 | 458.484168 | 0.296341 | 1.147448 | −12.435 | −1.01143 | 3.055 | 3.285 | 3.383 |
| 15 | 240.260772 | 292.431791 | 0.179342 | 1.149717 | −11.8843 | −0.60603 | 4.092 | 4.403 | 4.334 |
| 16 | 224.849464 | 424.237472 | 0.302582 | 1.145965 | −12.2394 | −1.04386 | 4.051 | 3.71 | 3.434 |
| 17 | 258.536841 | 356.834536 | 0.158154 | 1.1135 | −10.5769 | −0.54441 | 4.721 | 4.99 | 4.563 |
| 18 | 261.199661 | 251.365425 | 0.192591 | 1.027586 | −11.996 | −0.40197 | 3.32 | 3.707 | 3.713 |
| 19 | 287.961821 | 305.957081 | 0.167687 | 1.016621 | −11.7946 | −0.4523 | 2.685 | 3.339 | 2.976 |
| 20 | 294.884399 | 320.504267 | 0.170505 | 1.015957 | −11.3031 | −0.44996 | 3.359 | 3.485 | 2.956 |
| 21 | 298.560574 | 406.075985 | 0.16049 | 1.049525 | −11.2413 | −0.56303 | 2.95 | 3.503 | 2.781 |
| 22 | 384.279004 | 541.029735 | 0.135005 | 1.026731 | −10.0302 | −0.50007 | 2.67 | 2.349 | 2.505 |
| 23 | 331.850475 | 964.70279 | 0.197215 | 1.147253 | −10.9925 | −1.47362 | 2.597 | 2.585 | 2.626 |
| 24 | 352.729549 | 405.185612 | 0.165455 | 1.003391 | −10.1393 | −0.37092 | 3.333 | 2.864 | 3.13 |
| 25 | 305.38771 | 815.398821 | 0.21472 | 1.152992 | −11.0301 | −1.23996 | 2.63 | 3.109 | 2.85 |
| 26 | 301.907006 | 440.25256 | 0.227714 | 1.045263 | −10.8069 | −0.46336 | 2.68 | 3.263 | 3.392 |
| 27 | 292.272598 | 321.939157 | 0.225437 | 1.040324 | −11.0415 | −0.41403 | 3.462 | 3.36 | 3.827 |
| 28 | 355.694528 | 1076.11087 | 0.189501 | 1.132231 | −10.7025 | −1.50889 | 2.579 | 2.236 | 2.46 |

[a] From Eq. 1

[b] From Eq. 2

packed in a molecule. The negative coefficient of the term <1.120-Density> indicates that the larvicidal activity increases when the value of Density is more than 1.120.

The positive coefficient of <Jurs_FNSA_2 + 0.697> indicates that the value of Jurs_FNSA_2 should be less negative than −0.697. Jurs_FNSA_2 is fractional charged partial negative surface area which is obtained by dividing total charge weighted negative surface area (partial negative solvent-accessible surface area multiplied by total negative charge) by the total solvent-accessible surface area.

Jurs_PPSA_2 is total charge weighted positive surface area which is obtained by multiplying partial positive solvent-accessible surface area with total positive charge. The negative coefficient of Jurs_PPSA_2 indicates that it has negative contribution on the larvicidal activity. The positive and negative coefficients of the terms <Jurs_FNSA_2 + 0.697> and Jurs_PPSA_2 indicate that there should be optimum charge distribution over the molecular surface for the optimum activity. The calculated activity values obtained from Eqs. 1 and 2 are shown in Table 2.

## Conclusion

Present investigation has clearly shown that certain chalcone analogues had potent mosquito larvicidal activity. Most of the hydroxyl chalcones showed toxicity against the third instar larvae of *Culex quinquefasciatus*. The favorable chemical structures were found to be a hydroxyl substituent in ring A at 2′-position which may be hydrogen bonded with the electron pair on α,β-unsaturated ketone moiety,

thereby decreasing the electrophilicity of this part. Presence of hydroxyl group at 2′-position of ring A and replacement of ring B (phenyl) by furan ring also increased the larvicidal activity. Besides that 3-chlorine substitution in ring B was also another feature of favorable activity. Presence of methylenedioxy group at 3,4 positions of ring B also enhanced the larvicidal activity of chalcone-type compound. However, extension of conjugation and blocking of α,β-unsaturated ketone part of chalcones had bad effects toward the activity of these compounds. In conclusion, QSAR analysis suggests that charge distribution on molecular surface and surface area are important determinants of the larvicidal activity. The derived models suggest that for the good larvicidal activity positively charged surface areas of the compounds should be limited. Moreover, there should be a balanced distribution of positive and negative charges on the molecular surfaces of the compounds.

## Experimental

### General experimental procedure

Melting points were determined using an electrothermal apparatus and were uncorrected. All reagents used were of A.R. Grade and supplied by E. Merck. All the synthesized products were purified by recrystallization from ethanol and their purity was routinely checked by thin layer chromatography. Thin layer chromatography of the compounds was carried out in silica gel GF 254 precoated plates using $CHCl_3$ or $CHCl_3$ and petroleum ether (60–80°C) mixture (5:2 or 4:1) as eluting solvents. The structures of the compounds were confirmed on the basis of melting point and $^1H$ NMR data. $^1H$ NMR spectra were recorded at 400 MHz in a Varian-400 spectrometer in $CDCl_3$ or MeOH-$d_4$ solutions using tetramethylsilane as an internal standard. $^1H$ NMR spectra revealed that all the chalcones were geometrically pure and configured E ($J_{H\alpha\text{-}H\beta}$ = 15–16 Hz).

### Chemical synthesis

For compound (**1**), a slurry mixture of benzaldehyde (10 mmol), acetophenone (10 mmol), and solid NaOH (10 mmol) were ground together by pestle in a mortar at r.t. for 2–3 min whereby the reaction mixture turned into pale yellow solid. The solid was washed with aq. acid solution and recrystallised from ethanol. The yield was 91.2%. M.p. 58°C [literature m.p. 58°C (Pollock and Stevens, 1965)].

The same procedure was also adopted for the synthesis of other substituted chalcones (**2–3, 6–17**, and **20–21**) using appropriately substituted acetophenone and benzaldehydes as the starting materials; except in the case of compounds (**17**), (**20**) and (**21**). For compound (**17**), the starting materials were *o*-hydroxyactetophenone and furfuraldehyde. Compound (**20**) was prepared by this solid phase aldol condensation reaction between acetophenone and cinnamaldehyde. For compound (**21**), *o*-hydroxyactetophenone and cinnamaldehyde were the starting materials.

Compounds (**4**) and (**5**) were synthesized by the method of Liu *et al.* (2001) with slight modification. Vanillin (10 mmol) was dissolved in 10 $cm^3$ methanol. A methanolic solution of NaOH (1% w/v, 10 $cm^3$) was added to it and the reaction mixture was stirred at 0–5°C for 30 min. A methanolic solution of acetophenone [in case of (**5**), *o*-hydroxyactetophenone] (10 mmol, 10 $cm^3$) was added drop wisely and the reaction mixture was stirred at ice cold condition for 3 h. Orange red colored solution was obtained; which was diluted with water, neutralized with HCl and then extracted with ether. The organic layer was dried with anhydrous $Na_2SO_4$ and removed by evaporation under reduced pressure to yield bright yellow precipitate of (**4**) and (**5**). In both case, the crude product was recrystallized from ethanol. Yields were in the range of 61–62%.

In compounds (**18**) and (**19**), ring A of chalcone has been replaced by $–CH_3$ group and $C_6H_5–CH=CH–$ group, respectively. These two compounds were prepared by Claisen–Schmidt reaction between acetone and benzaldehyde in different proportion in dilute alkali medium followed by recrystalization in ethanol medium (Furniss *et al*., 1984), yield: 77.0%. M.p. of (**18**) was found to be 42°C [literature m.p. 42°C (Furniss *et al.*, 1984)]. In case of (**19**), the yield was 92.19% and m.p. of (**19**) was found to be 112°C [literature m.p. 112°C (Furniss *et al*., 1984)]. For the preparations of compounds (**22–28**), standard methods with slight modifications were followed (Furniss *et al*., 1984). The yield was in the range of 70.00–80.00%; the structures of these compounds were confirmed by comparing their m.p. data with literature data (Pollock and Stevens, 1965; Furniss *et al*., 1984).

### Spectroscopic data for the synthesized compounds

**(2*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one (2)**: $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 12.79 (bs, 1H, OH), 7.89 (d, 1H, $J$ = 16.10 Hz, $H_\beta$), 7.63 (d, 1H, $J$ = 16.10 Hz, $H_\alpha$), 7.03 (dd, 1H, $J$ = 1.20 Hz, 8.4 Hz, H-3′), 7.93 (dd, 1H, $J$ = 1.20 Hz, 8.40 Hz, H-6′), 7.49–7.51 (m, 1H, H-4′), 6.91–6.95 (m, 1H, H-5′), 7.41–7.49 (m, 3H, H-3, H-4, H-5), 7.64–7.67 (m, 2H, H-2, H-6); yellow crystals; Yield 88.0%; m.p. 88–89°C.

**(2*E*)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one (3)**: $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 6.58 (bs, 1H, OH), 8.17 (d, 1H, $J$ = 16.40 Hz, $H_\beta$), 7.71 (d, 1H, $J$ = 16.40 Hz, $H_\alpha$), 6.90 (d, 1H, $J$ = 8.00 Hz, H-6′),

7.55–7.59 (m, 2H, H-4, H-5), 6.94 (t, 1H, $J = 8.00$ Hz, H-4′), 7.28 (d, 1H, $J = 8.00$ Hz, H-2′),7.47–7.51 (m, 2H, H-3′, H-5′), 8.01–8.03 (m, 2H, H-2, H-4, H-5), greenish yellow crystals; yield 80.0%; m.p. 150°C.

**(2*E*)-3-(3-nitrophenyl)-1-phenylprop-2-en-1-one (6):** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.84 (d, 1H, $J = 16.00$ Hz, $H_\beta$), 7.67 (d, 1H, $J = 16.00$ Hz, $H_\alpha$), 7.92 (d, 1H, $J = 7.60$ Hz, H-6), 8.23–8.51 (m, 2H, H-2, H-4), 7.51–7.60 (m, 4H, H-5, H-3′, H-4′, H-5′), 8.02–8.05 (m, 2H, H-2′, H-6′), white crystals; yield 88.0%; m.p. 162–163°C.

**(2*E*)-1-(2-hydroxyphenyl)-3-(3-nitrophenyl)prop-2-en-1-one (7)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 12.60 (bs, 1H, OH), 7.94 (d, 1H, $J = 15.60$ Hz, $H_\beta$), 7.78 (d, 1H, $J = 15.60$ Hz, $H_\alpha$), 8.28–8.53 (m, 2H, H-2, H-4), 7.05 (dd, 1H, $J = 0.80$ Hz, 8.40 Hz, H-3′), 7.65 (dd, 1H, $J = 0.80$ Hz, 8.40 Hz, H-6′), 6.95–6.99 (m, 1H, H-5′), 7.50–7.52 (m, 1H, H-4′), 7.53–7.55 (m, 2H, H-5, H-6); yellow crystals; yield 52.0%; m.p. 164°C.

**(2*E*)-3-(1,3-benzodioxol-5-yl)-1-phenylprop-2-en-1-one (8)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.74 (d, 1H, $J = 15.60$ Hz, $H_\beta$), 7.37 (d, 1H, $J = 15.60$ Hz, $H_\alpha$), 6.01 (s, 2H, –O–C<u>H</u>$_2$–O–), 6.84 (d, 1H, $J = 8.00$ Hz, H-5), 7.09–7.15 (m, 2H, H-2, H-6), 7.46–7.57 (m, 3H, H-3′, H-4′, H-5′); pale yellow crystals; yield 92.0%; m.p. 121–122°C.

**(2*E*)-3-(1,3-benzodioxol-5-yl)-1-(2-hydroxyphenyl) prop-2-en-1-one (9)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 12.88 (bs, 1H, OH), 7.85 (d, 1H, $J = 15.60$ Hz, $H_\beta$), 7.497 (d, 1H, $J = 15.60$ Hz, $H_\alpha$), 6.03 (s, 2H, –O–C<u>H</u>$_2$–O–), 7.90 (dd, 1H, $J = 1.60$ Hz, 8.20 Hz, H-6′), 7.15 (dd, 1H, $J = 1.60$ Hz, 8.20 Hz, H-3′), 6.90–6.94 (m, 3H, H-2,H-5, H-6), 7.15–7.17 (m, 1H, H-5′), 7.45–7.47 (m, 1H, H-4′); yellow crystals; yield 50.0%; m.p. 133–134°C.

**(2*E*)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (10)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.90 (bs, 1H, OH), 7.81 (d, 1H, $J = 15.60$ Hz, $H_\beta$), 7.54 (d, 1H, $J = 15.60$ Hz, $H_\alpha$), 7.98–8.00 (m, 2H, H-2′, H-6′), 7.61–7.63 (m, 3H, H-3, H-4, H-5), 7.39–7.40 (m, 2H, H-2, H-6), 6.91–6.93 (m, 2H, H-3′, H-5′),; pale yellow crystals; yield 88.0%; m.p. 172–173°C.

**(2*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one(11)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.76 (d, 1H, $J = 16.00$ Hz, $H_\beta$), 7.55–7.58 (m, 3H, H-2′, H-6′ $H_\alpha$), 7.47–7.51 (m, 3H, H-3′, H-4′, H-5′), 7.98–8.01 (m, 2H, H-3, H-5), 7.37–7.39 (m, 2H, H-2, H-6); pale yellow crystals; yield 88.3%; m.p. 113°C.

**(2*E*)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one (12)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.82 (d, 1H, $J = 16.40$ Hz, $H_\beta$), 7.65 (d, 1H, $J = 16.40$ Hz, $H_\alpha$), 7.67–7.79 (m, 3H, H-2′, H-6′), 7.52–7.54 (m, 2H, H-3′, H-5′), 7.59–7.61 (m, 1H, H-4′), 8.26–8.28 (m, 2H, H-3, H-5), 8.01–8.03 (m, 2H, H-2, H-6); white crystals; yield 88.0%; m.p. 164°C.

**(2*E*)-3-(furan-2-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (17)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 12.88 (bs, 1H, OH), 7.69 (d, 1H, $J = 15.20$ Hz, $H_\beta$), 7.56 (d, 1H, $J = 15.20$ Hz, $H_\alpha$), 7.92 (dd, 1H, $J = 1.20$ Hz, 8.20 Hz, H-6′), 6.94 (dd, 1H, $J = 1.20$ Hz, 8.20 Hz, H-3′), 6.99–7.01 (m, 2H, H-3, H-5), 6.53 (dd, 1H, $J = 1.80$ Hz, 3.60 Hz, H-4), 7.45–7.49 (m, 2H, H-4′, H-5′); deep yellow crystals; yield 68.0%; m.p. 102–103°C.

**(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one (20)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.64 (d, 1H, $J = 15.8$ Hz, $H_\alpha$), 7.97–7.98 (m, 3H, H-2′, H-6′, $H_\beta$), 7.49–7.64 (m, 7H, H-2, H-3, H-5, H-6, H-3′, H-4′, H-5′), 7.33–7.40 (m, 3H, H-$\gamma$, H-$\delta$, H-4); greenish yellow crystals; yield 89.0%; m.p. 99–100°C.

**(2*E*,4*E*)-1-(2-hydroxyphenyl)-5-phenylpenta-2,4-dien-1-one (21)**: $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 12.88 (bs, 1H, OH), 7.84 (d, 1H, $J = 15.60$ Hz, $H_\beta$), 7.69 (d, 1H, $J = 15.60$ Hz, $H_\alpha$), 7.74 (dd, 1H, $J = 1.60$ Hz, 8.00 Hz, H-6′), 7.01 (dd, 1H, $J = 1.60$ Hz, 8.00 Hz, H-3′), 7.47–7.51 (m, 3H, H-4′, H-2, H-6),7.33–7.36 (m, 5H, H-3, H-4, H-5, H-5′), 6.93 (t, 1H, $J = 12.10$ Hz, 10.10 Hz, H-$\gamma$), 6.98 (d, 1H, $J = 12.10$ Hz, H-$\delta$); deep yellow crystals; yield 88.3%; m.p. 156–157°C.

## Method of mosquito larvicidal assay

Larvae of *Culex quinquefasciatus* were reared at 30 ± 2°C with a photo period of 12 h light and 12 h dark and at 70 ± 10% relative humidity. Fifteen percentage yeast suspension was used as the growth medium. Larvicidal tests of the pure compounds (**1**–**28**) were performed on the third instar larvae of *Culex quinquefasciatus* in water medium according to the procedure of WHO (Rahuman *et al*., 2008) with some modification and as per the method of Cheng *et al.* (2004). Then, 0.5% alcoholic solution of each compound was added in thin stream with gentle stirring into the beaker containing 25 third instar larvae in the degassed distilled water, so that the final concentration became 100 μg cm$^{-3}$ at 30 ± 2°C. In the similar manner, same amount of alcohol was added to the control. Five replications were performed for each set. Percent (%) mortality was calculated after 24 h during which no foods was given to the larvae. The % mortality was corrected for control mortality applying Abbott's formula (Yang *et al.*, 2002; Abbott, 1925). The % mortality of the larvae was also measured at different concentrations at 30 ± 2°C and probit analysis (Finney, 1971) for these compounds were done accordingly to determine their $LC_{50}$ (μg cm$^{-3}$) values. Analysis of variance (ANOVA) calculations were done for those compounds which showed variations of % mortality at 100 μg cm$^{-3}$ concentration and were apparently found not to be equivalent. From ANOVA calculations, the critical difference (CD) values were determined. The order of toxicity was determined on the basis of CD and $LC_{50}$ values.

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