High Add Valued Application of Turpentine in Crop Production through Structural Modification and QSAR Analysis

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Abstract: Turpentine is a volatile component of resin, which is an abundant forest resource in Southern China. As one of the most important components, the integrated application of β-pinene has been studied. The broad-spectrum evaluation of β-pinene and its analogues has, therefore, been necessary. In an attempt to expand the scope of agro-activity trials, the preparation and the evaluation of the herbicidal activity of a series of β-pinene analogues against three agricultural herbs were carried out. In accordance with the overall herbicidal activity, it is noteworthy that compounds 6k, 6l, and 6m demonstrated extreme activity with IC50 values of 0.065, 0.065, and 0.052 mol active ingredients/hectare against E. crus-galli. The preliminary structure–activity relationship (SAR) was analyzed and the compounds with the appropriate volatility and substituent type that had beneficial herbicidal activity were analyzed. Simultaneously, the quantitative structure–activity relationship (QSAR) model was built and the most important structural features were indicated, which was, to a certain extent, in line with the SAR study. The study aimed to study the application of the forest resource turpentine in agriculture as a potential and alternative approach for comprehensive utilization.

Keywords: turpentine; herbicidal activity; SAR; QSAR

1. Introduction

Weeds are considered worldwide as an infestation for crops. Some weed species interfere with the production of grain and vegetables and severely reduce the yield and quality [1]. Among them, Echinochloa crus-galli is one of the world’s worst weeds because it can seize the available soil nitrogen from the crop [2]. Effective and integrated control of these noxious weeds is essential for modern agriculture [3]. However, the wide use of single-type herbicides causes the serious occurrence of resistance weeds and a series of environmental issues [4]. Therefore, developing more effective and low toxic herbicides has become a priority for agriculture producers, researchers, and extension personnel [5–10]. As an important alternative to replacing traditional herbicides, forest resources have attracted increasing attention from researchers due to their unique qualities, such as being widely distributed, environmentally friendly, and diverse in chemical composition [11]. The acrylopimaric acid-based botanical herbicides have been synthesized from forest resource rosin [3]. The natural herbicide activity of Satureja hortensis L., an essential oil against two important weed species, has been
Mansonone E has potential as a new natural pesticide for agricultural plant pathogen management [13]. Therefore, taking advantage of forest resources for agriculture through chemical structural modification is a viable solution for the production of new herbicides.

Resin, an abundant forest resource, is obtained from pines, which use it to resist external insect bites and other damage. In the growth of plants, resin components (including pinene) play many special roles, such as plant defense, plant interspecific phytochemical roles, attracting insects for pollination, and so on. Just as plant secondary metabolites, resin is agriculturally active and the high value-added application of resin is meaningful. In the complex chemical components of resin, turpentine, a monoterpenic mixture of volatile oil, has excellent performance and potential for use in many fields. Turpentine has a high content of β-pinene and the cost to isolate β-pinene is low. However, studies on the biological activity of β-pinene derivatives are not rich. Therefore, further development of derivatives with bioactive properties using β-pinene as a starting material is gradually becoming a trend in turpentine processing and utilization. For instance, β-pinene and its analogues have agricultural activities, such as antifeedant, repellent, and antimicrobial properties [14–16]. The preparation of most compounds has been reported by us. Based on previous research, the reaction process has been improved. In order to further study the impact of heteroatoms on the activity, other novel heterocyclic compounds (6k–6n) have been synthesized [17,18]. The herbicidal activity of β-pinene and its analogues is worthy of study in order to develop some ecofriendly herbicides that can be applied in the control of noxious weeds.

In a program to design and synthesize herbicidal active substances via derivation of volatile β-pinene, there are many factors that need to be considered. First, the primary factor is the effective contact between the herbicide and the treated soil [19]. In order to improve the permeability (the penetration of the molecule through the plant epidermis, which is influenced by amphipathic characteristics, and better the biphasic solubility) and reduce volatilization, ester and amino groups were introduced into β-pinene. Second, new technology is needed to screen potential herbicides from mickle candidates for the weed control. QSAR, an efficient means to screen biological activity and mechanisms of action, was carried out through quantification software packages [20–22]. After the essential structural features for the activity have been defined, a study on the mechanisms of action should be targeted. The objective of this study was to continue expanding the application of volatile β-pinene in a high value-added field and to develop its broad-spectrum biological activity in agriculture.

2. Results and Discussion

2.1. Synthesis of Derivatives of β-Pinene

The synthesis of two series of turpentine-based ester derivatives are shown in Schemes 1 and 2. The preparation of most of these compounds has been reported by us and, based on previous research, the reaction process has been improved. In order to further study the impact of heteroatoms on the activity, other novel heterocyclic compounds (6k–6n) were synthesized. The dehydrocumic acid was prepared from β-pinene through reactions of alkaline oxidation, dehydration, and isomerization, respectively. For the purpose of improving the reactivity of carboxylic acid, the dehydrocumic acid was activated by thionyl chloride into acid chloride. In the synthesis process of oxime esters, some of the intermediate oxime compounds have been prepared by us. In order to obtain a high yield, the synthesis process was optimized by regulating the reaction temperature and the reaction solvent and alkali. The optimal reaction conditions were ethanol as the reaction solvent, sodium carbonate as the base, and 25 °C as the reaction temperature. The structures of the title compounds have been well characterized by IR, 1H-NMR, MS, and elemental analysis.
2.2. Herbicidal Activity and Structure–Activity Relationships (SARs)

In Supplementary Materials (Table S1), the herbicidal activities of compounds 5a–5l and 6a–6n against E. crus-galli, Amaranthus retroflexus, and Brassica campestris L. var. amplexicaulis Makino were screened primarily at 200 g active ingredients/hectare. It is shown that all of the ester and oxime ester derivatives of β-pinene had good inhibition rates against E. crus-galli compared with another two herbs. From Table 1, the research on the herbicidal activity against E. crus-galli was done and the analogues displayed good herbicidal activity, which was more significant compared with compound 3. It is noteworthy that compounds 6k, 6l, and 6m demonstrated extreme activity with IC\text{50} values of 0.065, 0.065, and 0.052 mol active ingredients/hectare against E. crus-galli, which were similar to that for sulfentrazone, a commercialized agriculture herbicide. For different types of β-pinene analogues, the level of herbicidal activity was oxime esters (6a–6n) > monoesters (5a–5l). Nevertheless, the environmental toxicology research on the newly synthesized compounds was very necessary. On the one hand, in order to avoid pesticide residues of herbicides in the soil, water, and air,
the chemical stability and products of metabolism of the candidates must be experimentally tested under the influence of prolonged exposure to ambient temperature, sunlight, microflora or selected microorganisms, and plants. On the other hand, the impact of herbicides on non-target organisms such as bird, fish, and bees should also be assessed. Research in this area will be carried out in the follow-up work.

Table 1. Herbicidal activity of compounds against *E. crus-galli* in a seed germination method.

| Compd. | Herbicidal Activity at a Concentration of (g Active Ingredients/Hectare) | IC50 | y = a + bx | R² | log IC50 |
|--------|-------------------------------------------------------------|------|--------|---|--------|
|        | 200 100 50 25 12.5 g Active Ingredients/ Hectare |  | | | |
| 3      | 80 68 55 45 32 49.880 0.300 y = -0.643 + 0.011x 0.962 -0.523 |
| 5a     | 100 91 72 57 42 19.886 0.110 y = -0.617 + 0.031x 0.991 -0.959 |
| 5b     | 100 90 71 56 41 21.204 0.109 y = -0.643 + 0.030x 0.989 -0.963 |
| 5c     | 100 90 70 55 41 22.024 0.106 y = -0.667 + 0.030x 0.993 -0.975 |
| 5d     | 100 90 70 54 40 23.099 0.111 y = -0.712 + 0.031x 0.993 -0.955 |
| 5e     | 96 80 68 50 39 25.505 0.115 y = -0.498 + 0.020x 0.983 -0.939 |
| 5f     | 90 78 64 50 39 25.679 0.116 y = -0.373 + 0.015x 0.949 -0.936 |
| 5g     | 100 90 69 53 40 23.908 0.108 y = -0.736 + 0.031x 0.996 -0.967 |
| 5h     | 91 78 65 49 40 23.335 0.114 y = -0.383 + 0.015x 0.959 -0.943 |
| 5i     | 92 81 64 50 38 26.605 0.113 y = -0.442 + 0.017x 0.951 -0.947 |
| 5j     | 88 76 60 50 40 27.573 0.103 y = -0.360 + 0.013x 0.959 -0.987 |
| 5k     | 75 60 49 42 35 64.910 0.309 y = -0.570 + 0.009x 0.972 -0.510 |
| 5l     | 80 60 48 41 35 63.125 0.249 y = -0.656 + 0.010x 0.994 -0.604 |
| 6a     | 100 96 77 61 40 18.905 0.069 y = -0.768 + 0.041x 0.990 -1.161 |
| 6b     | 100 96 76 61 43 17.510 0.061 y = -0.674 + 0.038x 0.994 -1.215 |
| 6c     | 95 84 65 58 37 21.708 0.068 y = -0.416 + 0.019x 0.954 -1.167 |
| 6d     | 93 81 64 55 40 21.115 0.071 y = -0.345 + 0.016x 0.965 -1.149 |
| 6e     | 85 74 59 50 41 26.975 0.076 y = -0.305 + 0.011x 0.950 -1.139 |
| 6f     | 100 95 74 60 40 19.723 0.073 y = -0.738 + 0.037x 0.989 -1.137 |
| 6g     | 90 76 61 54 40 23.711 0.107 y = -0.323 + 0.014x 0.967 -0.971 |
| 6h     | 88 73 60 54 41 23.411 0.100 y = -0.282 + 0.012x 0.969 -1.000 |
| 6i     | 98 85 69 57 40 20.229 0.075 y = -0.460 + 0.023x 0.990 -1.125 |
| 6j     | 93 79 63 55 42 20.196 0.071 y = -0.314 + 0.016x 0.982 -1.149 |
| 6k     | 100 98 78 60 45 17.182 0.065 y = -0.741 + 0.043x 0.993 -1.187 |
| 6l     | 100 97 79 63 44 16.004 0.065 y = -0.665 + 0.042x 0.995 -1.187 |
| 6m     | 100 95 77 60 46 15.714 0.052 y = -0.564 + 0.036x 0.998 -1.284 |
| 6n     | 100 95 75 61 40 19.077 0.070 y = -0.719 + 0.038x 0.987 -1.155 |
| Sulflentrazone | 100 80 69 55 39 15.743 0.044 y = -0.820 + 0.052x 0.999 -1.387 |

Values in columns followed by similar letters were not significantly different according to Fisher’s protected; LSD test (P = 0.05).

In the process of herbicides playing a role, there are many factors that restrict the interaction with the herbs. For instance, the suitable volatility and electrostatic interaction may be mainly affected [1,23]. In order to improve the volatility, the structure of the carboxylic acid (compound 3) was modified with a hydroxy group to obtain ester analogues. From the activity data shown in Table 1, the shorter branched chained compounds were intrinsically more toxic, for the monoesters compounds (5a–5l), and the activity sequence was 5a > 5b > 5c > 5d > 5g > 5e ≈ 5f > 5h > 5i > 5j > 5k > 5l. In light of the point of view of Li et al. and Fan et al. aliphatic compounds are more volatile than aromatic compounds, and the volatility decreases with an increase in the molecular weight of the compounds [8,24]. Therefore, with regard to the same series of derivatives, along with the branched chain length increasing, the herbicidal activity showed a downward trend, and a similar trend was observed with oxime esters compounds (6a–6n). The electrostatic interaction of compounds is another important factor for herbicidal activity, and the type and position of the substituent has a great impact on the electrostatic effect of the compound. From investigations of the relationship of charge density and drug activity, the heteroatoms N, O, and S can play electron-withdrawing or electron-donating actions. Therefore, heterocyclic substituents such as thiophene, furan, and pyrrole were introduced to the derivatives. Based on the activity results, the rough order of herbicidal activity was aromatic substituted compounds was higher...
than aliphatic substituted compounds, and specifically heterocyclic substituted compounds (6a, 6b, 6f, 6k, 6l, 6m and 6n) was higher than benzene substituted compounds (6c, 6d, 6i and 6j). As a preliminary conclusion, during the molecules playing an effect on E. crus-galli, the ring of thiophene and furan accepts the electron, which forms an electron transfer process with a protein or enzyme to achieve the herbicidal effect. To sum up, the volatility and substituent type are two important compound properties influencing the herbicidal activity.

2.3. QSAR Study on Herbicidal Activity Against E. crus-galli

There were six groups of descriptors that reflected all of the features of the compounds, such as molecular composition, connectivity, 3D-coordinates, charge distribution, and quantum chemical data. Through calculation and selection, several significant molecular descriptors were determined during the model development process. The most important descriptors in this work were the quantum-chemical descriptors. In an attempt to establish a relationship between activity and molecular descriptors through several regression approaches, the heuristic regression was chosen to obtain a QSAR model with satisfactory values of $R^2$, $F$, and $S^2$. Through the “breaking point” rule and the number of molecular descriptors being no more than one-third of the sample number plus one, the descriptor number was determined. The “breaking point” rule results are shown in Figure 1. Finally, the four-descriptor QSAR model was determined as the best model and is shown in Table 2. The four significant descriptors and their values are listed in the Supplementary Materials (Table S2).

![Figure 1](image-url)  
Figure 1. The “breaking point” rule results. The black code indicates a statistically notable improvement in the correlation coefficient, and the red code indicates it was not improving obviously.

| Descriptor No. | X       | ±ΔX          | t-Test     | Descriptor          |
|---------------|---------|--------------|------------|---------------------|
| 0             | −4.5445 | 7.3386 $\times 10^{-1}$ | −6.1926  | Intercept           |
| 1             | −2.5592 $\times 10^{-3}$ | 2.3118 $\times 10^{-4}$ | −11.0701 | $\Delta H_f$ a     |
| 2             | 2.8334  | 3.8752 $\times 10^{-1}$ | 7.3163   | $P_{\mu\mu}$ b     |
| 3             | 7.7801 $\times 10^{-2}$ | 1.0981 $\times 10^{-2}$ | 7.0850  | $\mu$ c            |
| 4             | −3.4698 $\times 10^{-1}$ | 1.5347 $\times 10^{-1}$ | −2.2609 | $q_{max}$ d        |

a Final heat of formation of atoms. b Max atomic orbital electronic population. c Tot dipole of the molecule. d Max net atomic charge for a O atom.

The final four-descriptor QSAR model contained the sample number $N = 26$, and had the following statistical characteristics: $R^2 = 0.9428$, $F = 86.49$, $S^2 = 0.0014$. The experimental and predicted log IC$_{50}$ values are shown in Table 3, which were also compared in Figure 2. The following equation

$$
\text{IC}_{50} = a \cdot X_1 + b \cdot X_2 + c \cdot X_3 + d \cdot X_4 + e
$$
(Equation (1)) describes the four-descriptor QSAR model. In the model, a positive sign (+) indicates that the descriptor value had a positive correlation with log IC₅₀, and that the log IC₅₀ value was higher as the descriptor value was larger. In contrast, a negative sign (−) indicates a negative correlation.

\[
\log \text{IC₅₀} = -4.5445 - 0.0026 \times \Delta H_f + 2.8334 \times P_{\mu\mu} + 0.0778 \times \mu - 0.3470 \times q_{\text{max}}^\circ
\]  (1)

\[N = 26, R^2 = 0.9428, F = 86.49, S^2 = 0.0014\]

Figure 2. The comparison between experimental log IC₅₀ and predicted log IC₅₀.

Table 3. The difference between the experimental log IC₅₀ and predicted log IC₅₀.

| No. | Compd. | Calc. Log IC₅₀ | Exp. Log IC₅₀ | Difference |
|-----|--------|----------------|---------------|------------|
| 1   | 5a     | −0.884         | −0.959        | 0.075      |
| 2   | 5b     | −0.907         | −0.963        | 0.056      |
| 3   | 5c     | −0.926         | −0.975        | 0.049      |
| 4   | 5d     | −0.964         | −0.955        | −0.009     |
| 5   | 5e     | −0.942         | −0.939        | −0.003     |
| 6   | 5f     | −0.952         | −0.936        | −0.016     |
| 7   | 5g     | −0.975         | −0.967        | −0.008     |
| 8   | 5h     | −0.979         | −0.943        | −0.036     |
| 9   | 5i     | −0.949         | −0.947        | −0.002     |
| 10  | 5j     | −0.990         | −0.987        | 0.003      |
| 11  | 5k     | −0.534         | −0.510        | −0.024     |
| 12  | 5l     | −0.628         | −0.604        | −0.024     |
| 13  | 6a     | −1.163         | −1.161        | −0.002     |
| 14  | 6b     | −1.220         | −1.215        | −0.005     |
| 15  | 6c     | −1.023         | −1.167        | 0.044      |
| 16  | 6d     | −1.079         | −1.149        | 0.070      |
| 17  | 6e     | −1.102         | −1.119        | 0.017      |
| 18  | 6f     | −1.067         | −1.137        | 0.070      |
| 19  | 6g     | −1.011         | −0.971        | −0.040     |
| 20  | 6h     | −1.027         | −1.000        | −0.027     |
| 21  | 6i     | −1.183         | −1.125        | −0.058     |
| 22  | 6j     | −1.225         | −1.149        | −0.076     |
| 23  | 6k     | −1.152         | −1.187        | 0.035      |
| 24  | 6l     | −1.109         | −1.187        | 0.078      |
| 25  | 6m     | −1.338         | −1.284        | −0.054     |
| 26  | 6n     | −1.162         | −1.155        | −0.007     |
The validation of the established QSAR model was carried out by two validation methods. The internal validation method was described as the compounds 1, 4, 7, etc. were assigned to group A; compounds 2, 5, 8, etc. were assigned to group B. The other compounds belonged to subset C. The training set was two of these three groups, and the test set was the other group. The values of the corresponding test sets were predicted by the obtained correlation equation from the training set using the identical descriptors. The results of the internal validation are listed in Supplementary Materials (Table S3). The difference between $R_{\text{Training}}^2$ and $R_{\text{Test}}^2$ for the three sets was tiny enough to be ignored, and the average values of $R_{\text{Training}}^2$ and $R_{\text{Test}}^2$ were essentially identical to the overall $R^2$ value, which meant the approving predictive power of the obtained model. In the “leave-one-out” method, the compounds 1, 5, 9, etc. were the external test set and the others were the training set. The $R^2$ value of the training set and test set were close and the QSAR developed in this study had good predictability.

With regard to the descriptors related to the QSAR model, the first important descriptor was the heat of formation of the molecule ($\Delta H_f$), which gives the energy of the molecule in the thermodynamic standard scale (elements in ideal gas state at 298.15 K and 101.325 Pa). It measures the change of enthalpy during the formation of 1 mol of the compound from its constituent elements with all substances in their standard states. This quantum-chemical descriptor indicates the level of reactivity of the molecule and can find the standard enthalpy change of any reaction [21]. The value of $\Delta H_f$ signifies the difficulty and heat change of the chemical reaction. A large absolute value $\Delta H_f$ implies a prone reaction and a negative value $\Delta H_f$ means an exothermic reaction. The converse is also true. From Equation (1), the $\Delta H_f$ value had a negative effect on log IC$_{50}$ values.

The second important descriptor is the max atomic orbital electronic population ($\mu_{\text{electronic}}$), and it is a simplified index to describe the nucleophilicity of a molecule. According to the molecular orbital theory and frontier orbital theory, the highest occupied molecular orbital (HOMO) and the lowest unoccupied orbital (LUMO) are influential on the activity of the molecule [21]. When a molecule reacts with a target molecule, it occurs near the molecular front-orbital; therefore, the frontier orbital energy can greatly affect the herbicidal activity of a molecule. The suitable values of HOMO and LUMO are beneficial for the pesticide activity. From Figure 3, the frontier molecular orbitals (FMOs) of compounds 6k, 6l, and 6m are shown, which indicate the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The HOMO and LUMO of the molecule mainly distribute in heterocyclic and benzene rings, which means that these active groups accept or provide electrons during the interaction with the receptor.

![Figure 3. The HOMO and LUMO energy maps for compounds 6k, 6l and 6m.](image-url)
The third most important descriptor in the model is the tot dipole of the molecule, which measures the separation of positive and negative electrical charges within a molecule, that is, a measure of the overall polarity of the molecule [23,25,26]. The electric field strength of the dipole is proportional to the magnitude of the dipole moment. In Figure 4, the molecular electrostatic potential and contour maps of compounds 6k, 6l and 6m are illustrated. The electron withdrawing substitute of O and S atoms, demonstrated by the green parts, represents the positive molecular orbitals. The electron donating substitute of N atoms shown in red, represents the negative molecular orbitals. The electron donating substitute of N atoms shown in red, represents the negative molecular orbitals. From Equation (1), the $\mu$ value has a positive influence on the herbicidal activity.

![Image](image_url)

**Figure 4.** The molecular electrostatic potential and contour maps of compounds 6k, 6l and 6m.

The last important descriptor in the model is the max net atomic charge for an O atom. This electrostatic descriptor reflects the characteristics of the charge distribution of the molecule and is related to the strength of the intermolecular bonding interactions and characterizes the stability of the molecules, their conformational flexibility, and the other valency-related properties [24,25]. Figure 5 shows the optimized geometries and charge distribution of compounds 6k, 6l and 6m.

![Image](image_url)

**Figure 5.** The optimized geometries and charge distribution of compounds 6k, 6l and 6m.
3. Materials and Methods

3.1. Synthesis and Characterizations

β-pinene (1) was obtained from Jiangxi Jishui Hongda Natural Spices Ltd. (Jishui, China) of a commercial grade. All the other chemicals used in the synthesis were of reagent grade and the purity was chemically pure (≥ 99.5%). A Nicolet IS 10 spectrophotometer (Thermo Nicolet Co., Ltd., Madison, WI, USA) was used for the Fourier transform infrared (FT-IR) spectra. A Bruker AV-300 nuclear magnetic resonance spectrometer (Bruker Co., Ltd., Karlsruhe, Germany) was used to measure the $^1$H-NMR spectra. A Bruker AV-300 nuclear magnetic resonance spectrometer (Bruker Co., Ltd., Karlsruhe, Germany) was used to measure the $^1$H-NMR spectra. An Agilent-5973 spectrophotometer was (Agilent Technologies Inc., Santa Clara, CA, USA) utilized for the MS spectra. A Bruker Q-TOF mass spectrometer (Bruker Bruker Co., Ltd., Karlsruhe, Germany) equipped with an electro spray ionization source was applied for ESI mass spectral data. Thin-layer chromatography (TLC) was employed to monitor the progress of reactions and determine the reaction’s end, which was carried out on Merck silica gel 60 GF254 plates (Qingdao Bangkai Hi-Tech Materials Co., Ltd., Qingdao, China) with eluent of petroleum ether/ethylacetate (v/v = 8:1). The preparation and the structures of title compounds are shown in Schemes 1 and 2.

3.1.1. Synthesis of 4-Isopropylcyclohexa-1,3-dienecarboxylic acid (Dehydrocumic acid, 3)

β-pinene was used as the raw material. Through alkaline oxidation, dehydration, and isomerism, the nopinic acid (2) and dehydrocumic acid (3) were prepared according previously [24]. During this process, the colorless crystal of 2 was obtained by recrystallization with organic solvent (such as ethanol or toluene) [25]. After dehydration and isomerization with concentrated sulfuric acid, the hydroxyl of the carboxylic acid was eliminated and the crude product 3 was obtained, which was used in the subsequent reaction after purification by recrystallization. Crystal Data for C$_{10}$H$_{16}$O$_3$ (compound 2, M = 184.23 g/mol): monoclinic, $a = 26.796$ (5) Å, $b = 6.6560$ (13) Å, $c = 12.250$ (3) Å, $\beta = 112.23$ (3)°, $V = 2022.5$ (9) Å$^3$, $Z = 8$, $\mu = 0.009$ mm$^{-1}$, $T = 293$ K, $R [F^2 > 2\sigma (F^2)] = 0.056$, $wR(F^2) = 0.153$, $S = 1.00$, $\lambda = 0.71073$ Å, $F(000) = 800$.

3.1.2. Synthesis of 4-Isopropylcyclohexa-1,3-dienecarboxylates (Compounds 5a–5l)

From the lead compound 3, the 12 resulting compounds 5a–5l were obtained by an esterification reaction between dehydrocumic acid chloride and the corresponding alcohols [26].

3.1.3. Synthesis of Oximyl 4-Isopropylcyclohexa-1,3-dienecarboxylates (Compounds 6a–6n)

From the lead compound 3, the 14 resulting compounds 6a–6n were obtained by an esterification reaction between dehydrocumic acid chloride and the corresponding oximes [8].

3.2. Herbicidal Activity Assay of β-Pinene Analogues

β-pinene analogues were evaluated for herbicidal activity of the representative monocotyledonous weed E. crus-galli and the dicotyledonous weeds A. retroflexus and B. campestris L. The herbs were grown in a greenhouse and the test method was the conventional plate method (in vitro) [25–27]. In the evaluation, all of the test compounds were dissolved in mixed solution of DMF and distilled water (v/v = 8:1). The inhibition percentage of seed germination was the mean value obtained through three independent experiments and the standard deviation (SD) values were limited within ±5%. On the basis of the series inhibition percentage, the IC$_{50}$ results were calculated by the SPSS statistics program version 17.0 (SPSS Inc., Chicago, IL, USA). For stringency, the commercial herbicide sulfentrazone (Shanghai Aladdin Bio-Chem Technology Co., Ltd., Shanghai, China) was chosen as the positive control, and a pure solution without test compound was tested as the negative control.

The initial concentration was set at 200 g active ingredients/hectare and the lower required concentrations were diluted with water (100, 50, 25 and 12.5 g active ingredients/hectare). For better dispersion all the solutions were prepared with Tween (final concentration 0.1%) as surfactant. In petri
dishes of 9 cm diameter, 2 pieces of filter paper were placed and 2 mL test compound solution with certain concentrations was sprayed evenly. For the accuracy of the results, the negative control was the same amount of DMF mixed with Tween-80 and water. Ten just-sprouting seeds of each weed species including *E. crus-galli*, *A. retroflexus* and *B. campestris* L. were grown in the test plate and allowed to germinate for 72 h at 28 ± 1 °C. Finally, the average values of the height of the ground portion of the above mentioned seedlings growing in each plate were calculated. For reproducibility and credibility, all of the tests at each concentration were repeated three times. The inhibition percentage (IP) was used to describe the control efficiency of the compounds and was calculated according to Equation (2):

\[
IP(\%) = \left( \frac{H_0 - H_1}{H_0} \right) \times 100
\]

where \(H_0\) is the height of control test and \(H_1\) is the height of treated test. Through regression analysis using SPSS Statistics 17.0, the IC\(_{50}\) values were calculated by the inhibition percentage of *E. crus-galli* at five concentrations for each test compound [28].

### 3.3. Building and Verification of the Quantitative Structure–Activity Relationship (QSAR) Model

The QSAR model was built and validated in accordance with the common procedure. Briefly, optimization of the most stable configuration was obtained with a Gaussian 03W package of programs (Gaussian Inc., Wallingford, CT, USA.), and all the molecular descriptors were calculated by CODESSA 2.7.15 (The copyright of this version is held by the Center of Heterocyclic Chemistry, University of Florida.) [29]. The “breaking point” rule for determining the number of the descriptors is described in Figure 1. The best multilinear regression showed a significant increase in \(R^2\) when the number of the descriptors was less than or equal to 4. However, there was negligible change in \(R^2\) when the number of descriptors increased from 4 to 5. Descriptors with high t values were accepted and those with low t values were rejected. A “breaking point” indicates that the improvement of the regression model has become non-significant (\(R^2 < 0.02-0.04\)). For the determination of the most significant structural features of herbicidal activity against *E. crus-galli*, the heuristic analysis was to build the QSAR model. During the model development process, the related statistical parameters (\(R^2\), \(S^2\) and \(F\)) were determined to assess the predictability of a given model. In this study, a model displaying good predictability between the compounds’ structures and the log IC\(_{50}\) values was developed. To ensure high quality of the final QSAR results, two verification methods, internal validation, and the “leave-one-out” cross-validation were used for determination [30].

### 4. Conclusions

In order to expand the efficient application of the forest resource \(\beta\)-pinene to agriculture, two series of \(\beta\)-pinene analogues—ester and oxime esters—were prepared with suitable volatility and a substituent type through structural modification. The herbicidal activity against *E. crus-galli*, *A. retroflexus* and *B. campestris* L. was evaluated. Among them, the activity against *E. crus-galli* was more ideal; therefore, a study on the structure–activity relationship (SAR) against *E. crus-galli* was focused on. The preliminary conclusion is that compounds with esterification modification and a short chain length are beneficial for herbicidal activity. In addition, the heteroatoms N, O and S can play an active role. Simultaneously, the quantitative structure–activity relationship (QSAR) model (\(R^2 = 0.9428, F = 86.49, S^2 = 0.0014\)) was built and indicates that the most important structural feature is the heat of formation of the molecule (\(\Delta H_f\)). In view of these results, for the further exploitation of ecofriendly herbicides and the better application of volatile \(\beta\)-pinene, short chain esters, and heterocyclic-substituted derivatives could be the direction of the future development of new structures. It is noteworthy that environmental toxicology should be focused on in future work.

**Supplementary Materials:** The following are available online. IR, \(^1\)H-NMR, MS and elemental analysis data for the target compounds. The herbicidal activity and structure descriptors of the title compounds are also available as supplementary information.
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References
1. Bangarwa, S.K.; Norsworthy, J.K. Herbicidal activity of three isothiocyanates against yellow nutsedge and their dissipation under two plastic mulches. Crop Prot. 2015, 74, 145–149. [CrossRef]
2. Ji, Z.Q.; Zhou, F.X.; Wei, S.P. Synthesis and herbicidal activities of benzothiazole N,O-acetals. Bioorganic Med. Chem. Lett. 2015, 25, 4065–4068. [CrossRef] [PubMed]
3. Gao, Y.Q.; Li, L.L.; Li, J.; Chen, H.; Song, Z.Q.; Song, J.; Shang, S.B.; Xiao, G.M.; Wang, Z.D.; Rao, X.P. High value-added application of rosin as a potential renewable source for the synthesis of the acropirimic acid-based botanical herbicides. Ind. Crops Prod. 2015, 78, 131–140. [CrossRef]
4. Epp, J.B.; Alexander, A.L.; Balko, T.W.; Buysee, A.M.; Brewster, W.K.; Bryan, K.; Daesumble, J.E.; Fields, S.C.; Gast, R.E.; Green, R.A.; et al. The discovery of Arylex TM active and Rinskor TM active: Two novel auxin herbicides. Bioorg. Med. Chem. 2016, 24, 362–371. [CrossRef] [PubMed]
5. Jin, Y.; Watkins, D.; Degtyareva, N.N.; Green, K.D.; Spano, M.N.; Garneau-Tsodikova, S.; Arya, D.P. Arginine-linked neomycin B dimers: Synthesis, rRNA binding, and resistance enzyme activity. MedChemComm 2015, 7, 164–169. [CrossRef] [PubMed]
6. Li, Z.S.; Wang, W.M.; Lu, W.; Niu, C.W.; Li, Y.H.; Li, Z.M.; Wang, J.G. Synthesis and biological evaluation of nonsymmetrical aromatic disulfides as novel inhibitors of acetohydroxyacid synthase. Bioorg. Med. Chem. Lett. 2013, 23, 3723–3727. [CrossRef] [PubMed]
7. Mara, C.; Dempsey, E.; Bell, A.; Barlow, J.W. Synthesis and evaluation of phosphor amidate and phosphor othioamidate analogues of amiprophos methyl as potential antimalarial agents. Bioorg. Med. Chem. Lett. 2011, 21, 6180–6183. [CrossRef] [PubMed]
8. Meng, X.Q.; Zhang, J.J.; Liang, X.M.; Zhu, W.J.; Dong, Y.H.; Wu, X.M.; Huang, J.X.; Rui, C.H.; Fan, X.L.; Chen, H.F.; et al. Synthesis and herbicidal activity of 12-(Arloxyacyloxyimino)-1,15-pentadecanlactone derivatives. J. Agric. Food Chem. 2009, 57, 610–617. [CrossRef] [PubMed]
9. Wang, Q.M.; Sun, H.K.; Cao, H.Y.; Cheng, M.R.; Huang, R.Q. Synthesis and herbicidal activity of 2-cyano-3-substituted-pyridinemethylaminoacrylates. J. Agric. Food Chem. 2003, 51, 5030–5035. [CrossRef] [PubMed]
10. Wang, Q.M.; Li, H.; Li, Y.H.; Huang, R.Q. Synthesis and herbicidal activity of 2-cyano-3-(2-chlorothiazol-5-yl) methylaminoacrylates. J. Agric. Food Chem. 2004, 52, 1918–1922. [CrossRef] [PubMed]
11. Jiang, L.; Watkins, D.; Jin, Y.; Gong, C.; King, A.; Washington, A.Z.; Green, K.D.; Garneau-Tsodikova, S.; Adegbuyegba, K.O.; Arya, D.P. Rapid synthesis, RNA binding, and antibacterial screening of a peptidic-aminosugar (PA) library. ACS Chem. Biol. 2015, 10, 1278–1289. [CrossRef] [PubMed]
12. Hossein, H.; Mohammad, J.S.; Mehrdad, N.; Mahmoodreza, M. Natural herbicide activity of Satureja hortensis L. essential oil nanoemulsion on the seed germination and morphophysiological features of two important weed species. Ecotoxicol. Environ. Saf. 2017, 142, 423–430.
13. Rachsawan, M.; Warinthorn, C. Antimicrobial, herbicidal and antifeedant activities of mansalone E from the heartwoods of Mansonia gagei Drumm. J. Integr. Agric. 2016, 15, 2795–2802.
14. Juárez, Z.N.; Hernández, L.R.; Bach, H.; Sánchez-Arreola, E. Antifungal activity of essential oils extracted from Agastache Mexicana ssp. xolocotziana and Porophyllumlinaria against post-harvest pathogens. Ind. Crops Prod. 2015, 74, 178–182. [CrossRef]
15. Kasuga, N.C.; Sekino, K.; Ishikawa, M.; Honda, A.; Yokoyama, M.; Nakano, S.; Shimada, N.; Koumo, C.; Nomiya, K. Synthesis, structural characterization and antimicrobial activities of 12 zinc(II) complexes with four thiosemicarbazone and two semicarbazone ligands. J. Inorg. Biochem. 2003, 96, 298–310. [CrossRef] [PubMed]
16. Wang, Z.D.; Song, J.; Chen, J.Z.; Song, Z.Q.; Shang, S.B.; Jiang, Z.K.; Han, Z.J. QSAR study of mosquito repellents from terpenoid with a six-member-ring. Bioorg. Med. Chem. Lett. 2008, 18, 2854–2859. [CrossRef] [PubMed]
17. Li, J.; Gao, Y.Q.; Shang, S.B.; Feng, J.T.; Zhang, X. A value-added use of volatile turpentine: Antifungal activity and QSAR study of b-pinene derivatives against three agricultural fungi. RSC Adv. 2015, 5, 66947–66955. [CrossRef]

18. Gao, Y.Q.; Tian, X.R.; Li, J.; Shang, S.B.; Song, Z.Q.; Shen, M.G. Study on amphipathic modification and QSAR of volatile turpentine analogues as value-added botanical fungicides against crop-threatening pathogenic fungi. ACS Sustain. Chem. Eng. 2016, 4, 2741–2747. [CrossRef]

19. Che, J.Y.; Xu, X.Y.; Tang, Z.L.; Gu, Y.C.; Shi, D.Q. Synthesis and herbicidal activity evaluation of novel α-amino phosphonate derivatives containing a uracil moiety. Bioorg. Med. Chem. Lett. 2016, 26, 1310–1313. [CrossRef] [PubMed]

20. Robert, D.C. A perspective on the role of quantitative structure-activity and structure-property relationships in herbicide discovery. Pest Manag. Sci. 2012, 68, 513–518.

21. Prasad, R.K.; Sharma, R. 2D QSAR Analysis of pyrazinecarbox amides derivatives as an herbicidal agent. J. Comput. Methods Mol. Des. 2011, 1, 7–13.

22. Soung, M.G.; Park, K.Y.; Sung, N.D. 3D-QSAR Study on the influence of alryl amino (R) substituents on herbicidal activity of thiourea analogues. Bull. Korean Chem. Soc. 2010, 31, 1469–1473. [CrossRef]

23. Zhu, H.L.; Shi, J.; Huang, Z.Q.; Lv, L.J.; Duan, J.W. Structures, spectecroscopic analysis, herbicidal activities and enamine-aminone tautomerism of new β-diketone derivatives modified with glycerylglycine methyl ester. J. Mol. Struct. 2015, 1089, 170–177. [CrossRef]

24. Gao, Y.Q.; Li, J.; Shang, S.B.; Wang, D. Synthesis and insecticidal activity of acylthiourea derivatives from β-pinene. Lett. Drug Des. Discov. 2015, 11, 59–66.

25. Chen, X.B.; Shi, D.Q.; Zhu, X.F. Synthesis, crystal structure and biological activities of O, O-dialkylα-[1-(2-chlorothiazol-5-ylmethyl)-5-methyl-1H-1,2,3-triazol-4-ylcarbonyloxy]alkylphosphonates. Chin. J. Chem. 2007, 25, 1854–1858. [CrossRef]

26. Liu, Y.X.; Wei, D.G.; Zhu, Y.R.; Liu, S.H.; Zhang, Y.L.; Zhao, Q.Q.; Cai, B.L.; Li, Y.H.; Song, H.B.; Liu, Y.; et al. Synthesis, herbicidal activities, and 3D-QSAR of 2-cyanoacrylates containing aromatic methylamine moieties. J. Agric. Food Chem. 2008, 56, 204–212. [CrossRef] [PubMed]

27. Ugi, I.; Meyr, R.; Lipinski, M.; Bodesheim, F.; Rosendahl, F. Cyclobexyl isocyanide. Org. Synth. 1961, 41, 13–15.

28. SPSS Inc. SPSS Base Version 17.0 for User’s Guide; SPSS Inc. Press: Chicago, IL, USA, 2008.

29. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Montgomery, J.A.; Vreven, T.; Kudin, K.N.; Burant, J.C.; et al. Gaussian 03, Revision C.02; Gaussian, Inc. Press: Wallingford, CT, USA, 2004.

30. Shang, J.; Wang, W.M.; Li, Y.H.; Song, H.B.; Li, Z.M.; Wang, J.G. Synthesis, crystal structure, in vitro acetohydroxy acid synthase inhibition, in vivo herbicidal activity, and 3D-QSAR of new asymmetric aryl disulfides. J. Agric. Food Chem. 2012, 60, 8286–8293. [CrossRef] [PubMed]

Sample Availability: Samples of the compounds 3, 5a–5l and 6a–6n are available from the authors.

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