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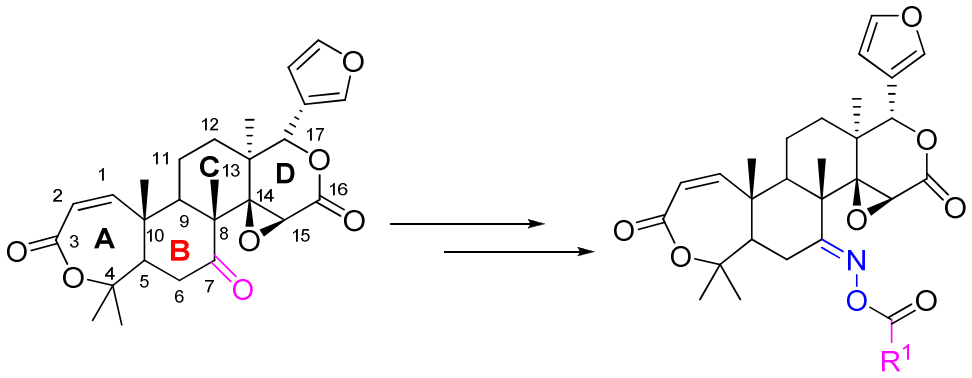

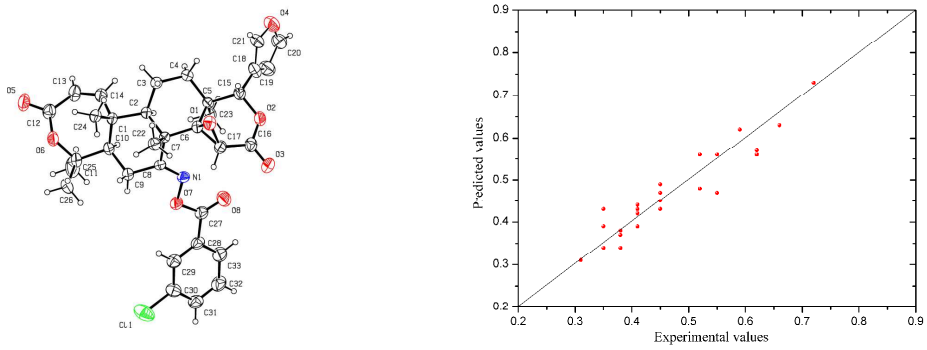

Synthesis and quantitative structure-activity relationship (QSAR) study of C7-oxime ester derivatives of obacunone as insecticidal agents†

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†Electronic supplementary information (ESI) available:

^{1}H-NMR, HRMS, optical rotation, melting point, and IR data for the target compounds.

Abstract

As part of our ongoing search for new insecticidal agents originating from natural products, in the present paper, we have prepared a series of C7-oxime ester derivatives of obacunone and evaluated their insecticidal activity at 1 mg/mL against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest. The structures of all target compounds were well characterized by ^{1}H NMR, HRMS, optical rotation, IR, and mp. More importantly, three key steric structures of **3m**, **3r**, and **3s** were unambiguously determined by single-crystal X-ray diffraction. Compounds **3e**, **3r**, **3s** and **3w** exhibited more promising insecticidal activity with the final mortality rates greater than 60%, when compared with their precursor obacunone and toosendanin (a positive control). To C7-oxime alkylester series, the proper length of their side chain at the C-7 position of C7-oximeobacunone to the insecticidal activity was very important; to C7-oxime arylester series, introduction of the chlorine atom on the phenyl ring at the C-7 position of C7-oximeobacunone could lead to the best potent compounds. According to the QSAR model, five descriptors such as RDF100v, RDF105u, Dm, Mor15m and R1u, were likely to affect the biological activity of these compounds. Among them, the most important one was RDF100v. The correlation coefficient (R^2), the cross-validation correlation coefficient (Q^2) and the standard deviation error in prediction (SDEP) are 0.891, 0.835 and 0.0358, respectively.

KEYWORDS: obacunone, insecticidal activity, natural-product-based insecticide, B-ring modification, chemical modification, QSAR, *Mythimna separata* Walker

Introduction

Lepidopteran insect pests could cause substantial reductions in crop yield and quality. In an effort to combat those pest infestations, various synthetic agrochemicals have been employed. However, repeat application of those synthetic agrochemicals has resulted in the emergence of insecticide-resistant lepidopteran pests and environmental problems.[1-3] Consequently, discovery and development of novel, selective and safe insecticidal agents for effective controlling lepidopteran insect pests is highly desirable. Recently, botanical insecticides have been attracting more and more attention,[4-8] for example, some botanical insecticides such as nicotine, pyrethrum and neem extracts have been made directly from plants as defenses against insects.[9]

Obacunone (**1**, Figure 1), a naturally occurring limonoid, has been isolated from many species of plants such as *Citrus* and its hybrids,[10] and *Dictamnus angustifolius*.[11] It was found that compound **1** exhibited some medicinal activities, including antiproliferative,[12] anticancer,[13-15] antimalarial,[16] and antioxidant activities.[17] Meanwhile, compound **1** also showed the interesting insecticidal activities, such as moult inhibiting activity against mosquito *Culex quinquefasciatus*,[18] and antifeedant activity against *Spodoptera frugiperda*.[19] Encouraged by the above results, and as part of our ongoing search for new potential natural-product-based insecticidal agents to control the lepidopteran pests, in the present paper, we designed and synthesized a series of C7-oxime ester derivatives of obacunone. Their insecticidal activity was evaluated against the oriental armyworm (*Mythimna separata* Walker), an important and typical lepidopteran pest.

Materials and methods

General. All chemical reagents were purchased and utilized without further purification.

Solvents were used directly or treated with standard methods before use. Melting points (mp) were determined on a XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were uncorrected. Infrared spectra (IR) were recorded on a Bruker TENSOR 27 spectrometer. Optical rotation was measured on a Rudolph Research Analytical Autopol III automatic polarimeter. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ on a Bruker Avance 400 or 500 MHz instrument, and tetramethylsilane (TMS) was used as the internal standard. High-resolution mass spectra (HR-MS) were obtained on an IonSpec 4.7 Tesla FTMS instrument.

Compound **1** was isolated from *Dictamnus dasycarpus* in our laboratory, and obtained in 0.163% yield and >99% purity measured by reverse phase high-performance liquid chromatography (RP-HPLC). CAS no. 751-03-1; white solid; R_f = 0.43 (petroleum ether/acetone = 2:1); mp 228-230 ^{o}C (lit.[14] 228-235 ^{o}C); $[\alpha]^{20}_D$ = -18 (*c* 3.7 mg/mL, acetone); IR cm^{-1} (KBr) 2990, 1736, 1707, 1464, 1394, 1371, 1282, 1121; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H, H-21), 7.40 (s, 1H, H-23), 6.53 (d, *J* = 11.6 Hz, 1H, H-1), 6.36 (s, 1H, H-22), 5.98 (d, *J* = 11.6 Hz, 1H, H-2), 5.46 (s, 1H, H-17), 3.65 (s, 1H, H-15), 3.02 (t, *J* = 14.0 Hz, 1H, H-6), 2.62 (dd, *J* = 14.0, 4.8 Hz, 1H, H-5), 2.31 (dd, *J* = 14.0, 4.8 Hz, 1H, H-6), 2.14 (m, 1H, H-9), 1.86-1.91 (m, 3H, H-11, 12), 1.50 (s, 6H, H-29, 30), 1.42-1.48 (m, 4H, H-12, 28), 1.24 (s, 3H, H-19), 1.12 (s, 3H, H-18); HRMS *m/z* calcd for $C_{26}H_{30}O_7Na$ ($[M+Na]^+$) 477.1884; found 477.1888.

*Synthesis of C7-Oximeobacunone (**2**).* A mixture of **1** (227.3 mg, 0.5 mmol) and hydroxylamine hydrochloride (347.5 mg, 5.0 mmol) in pyridine (0.5 mL) and absolute C_2H_5OH (10 mL) was stirred at 60 ^{o}C for 2 h. When the reaction was complete, checked by thin-layer chromatography (TLC) analysis, the solvent was removed under reduced pressure

and saturated aqueous $NaHCO_3$ (15 mL) was added to the residue, which was extracted with ethyl acetate (3×30 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (2:1, v/v) to afford **2** in 93% yield.

Data for ***2*****:** CAS no.123885-85-8; white solid, mp 268-270 °C (lit.[20] 268 °C); $[\alpha]^{20}_D$ = -12 (*c* 3.3 mg/mL, acetone); IR cm^{-1} (KBr): 3432, 2953, 1752, 1681, 1389, 1267, 1076; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.41 (s, 1H, H-21), 7.39 (s, 1H, H-23), 7.34 (s, 1H, -OH), 6.49 (d, *J* = 11.5 Hz, 1H, H-1), 6.36 (s, 1H, H-22), 5.90 (d, *J* = 11.5 Hz, 1H, H-2), 5.50 (s, 1H, H-17), 3.67 (s, 1H, H-15), 3.34 (dd, *J* = 14.5, 5.0 Hz, 1H, H-6), 2.30 (dd, *J* = 14.0, 5.0 Hz, 1H, H-5), 2.06-2.15 (m, 2H, H-6, 9), 1.81-1.94 (m, 3H, H-11, 12), 1.53 (s, 3H, H-29), 1.51 (s, 3H, H-28), 1.44-1.48 (m, 1H, H-11), 1.40 (s, 3H, H-19), 1.22 (s, 3H, H-18), 1.13 (s, 3H, H-30); HRMS *m/z* calcd for $C_{26}H_{32}NO_7$ ($[M+H]^+$) 470.2173, found 470.2176.

*General Procedure for the Synthesis of C7-Oxime Ester Derivatives of Obacunone (****3a-x****).* A mixture of **2** (0.15 mmol), carboxylic acids R^1CO_2H (0.21 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 0.18 mmol), and 4-dimethylaminopyridine (DMAP, 0.03 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature. When the reaction was complete according to TLC analysis, the mixture was diluted by CH_2Cl_2 (20 mL), and washed by water (15 mL), aqueous HCl (0.1 mol/L, 15 mL), 5% aqueous Na_2CO_3 (15 mL) and brine (15 mL). Finally, the organic phase was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by preparative thin-layer chromatography (PTLC) to give the pure target products **3a-x** in 64-97% yields. The example data of **3a-e** are shown as follows, whereas data of **3f-x** can be found in the Supporting Information.

Data for ***3a*****:** CAS no. 476331-10-9. Yield = 90%, white solid, mp 114-116 °C (lit.,[19] not

reported); $[\alpha]^{20}_D$ = -5 (*c* 3.1 mg/mL, acetone); IR cm^{-1} (KBr): 2953, 1744, 1698, 1632, 1374, 1282, 1075; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.43 (s, 1H, H-21), 7.40 (s, 1H, H-23), 6.49 (d, *J* = 11.5 Hz, 1H, H-1), 6.38 (s, 1H, H-22), 5.92 (d, *J* = 12.0 Hz, 1H, H-2), 5.51 (s, 1H, H-17), 3.72 (s, 1H, H-15), 3.20 (dd, *J* = 12.5, 3.0 Hz, 1H, H-6), 2.29-2.38 (m, 2H, H-5, 6), 2.24 (s, 3H, $-CH_3$), 2.11-2.26 (m, 1H, H-9), 1.86-1.96 (m, 3H, H-11, 12), 1.46-1.53 (m, 7H, H-11, 28, 29), 1.42 (s, 3H, H-19), 1.28 (s, 3H, H-18), 1.20 (s, 3H, H-30); HRMS *m/z* calcd for $C_{28}H_{33}NO_8Na$ ($[M+Na]^+$) 534.2098, found 534.2100.

Data for ***3b*****:** Yield = 98%, white solid, mp 191-192 °C; $[\alpha]^{20}_D$ = -3 (*c* 3.3 mg/mL, acetone); IR cm^{-1} (KBr): 2943, 1754, 1703, 1636, 1391, 1283, 1071; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.43 (s, 1H, H-21), 7.40 (t, *J* = 1.5 Hz, 1H, H-23), 6.49 (d, *J* = 12.0 Hz, 1H, H-1), 6.38 (d, *J* = 1.0 Hz, 1H, H-22), 5.92 (d, *J* = 11.5 Hz, 1H, H-2), 5.50 (s, 1H, H-17), 3.72 (s, 1H, H-15), 3.20 (dd, *J* = 13.0, 3.5 Hz, 1H, H-6), 2.46-2.57 (m, 2H, $-CH_2CH_3$), 2.28-2.37 (m, 2H, H-5, 6), 2.14-2.17 (m, 1H, H-9), 1.85-1.95 (m, 3H, H-11, 12), 1.52 (s, 3H, H-28), 1.48-1.50 (m, 4H, H-11, 29), 1.42 (s, 3H, H-19), 1.29 (s, 3H, H-18), 1.18-1.21 (s, 6H, H-30, $-CH_2CH_3$);. HRMS *m/z* calcd for $C_{29}H_{35}NO_8Na$ ($[M+Na]^+$) 548.2255, found 548.2255.

Data for ***3c*****:** Yield = 97%, white solid, mp 116-118 °C; $[\alpha]^{20}_D$ = -5 (*c* 3.3 mg/mL, acetone); IR cm^{-1} (KBr): 2965, 1750, 1703, 1635, 1391, 1282, 1072; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.42 (s, 1H, H-21), 7.39 (t, *J* = 1.5 Hz, 1H, H-23), 6.49 (d, *J* = 11.5 Hz, 1H, H-1), 6.38 (d, *J* = 1.0 Hz, 1H, H-22), 5.92 (d, *J* = 11.5 Hz, 1H, H-2), 5.50 (s, 1H, H-17), 3.72 (s, 1H, H-15), 3.15 (dd, *J* = 12.5, 3.0 Hz, 1H, H-6), 2.41-2.48 (m, 2H, $-CH_2CH_2CH_3$), 2.28-2.37 (m, 2H, H-5, 6), 2.14-2.17 (m, 1H, H-9), 1.85-1.92 (m, 3H, H-11, 12), 1.67-1.72 (m, 2H, $-CH_2CH_2CH_3$), 1.52 (s, 3H, H-28), 1.47-1.51 (m, 4H, H-11, 29), 1.42 (s, 3H, H-19), 1.29 (s, 3H, H-18), 1.20 (s, 3H, H-30), 0.98 (t, *J* = 7.5 Hz, 3H, $-CH_2CH_2CH_3$); HRMS *m/z* calcd for $C_{30}H_{37}NO_8Na$ ($[M+Na]^+$)

562.2411, found 562.2403.

Data for ***3d*****:** Yield = 79%, white solid, mp 146-148 °C; $[\alpha]^{20}_{D}$ = -3 (*c* 3.3 mg/mL, acetone); IR cm^{-1} (KBr): 2958, 1750, 1703, 1636, 1392, 1282, 1073; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42 (s, 1H, H-21), 7.39 (t, *J* = 1.5 Hz, 1H, H-23), 6.49 (d, *J* = 12.0 Hz, 1H, H-1), 6.38 (d, *J* = 1.0 Hz, 1H, H-22), 5.92 (d, *J* = 11.5 Hz, 1H, H-2), 5.50 (s, 1H, H-17), 3.72 (s, 1H, H-15), 3.15 (dd, *J* = 12.5, 3.0 Hz, 1H, H-6), 2.46-2.50 (m, 2H, -C*H*$_2$CH$_2$CH$_2$CH$_3$), 2.30-2.37 (m, 2H, H-5, 6), 2.14-2.17 (m, 1H, H-9), 1.85-1.92 (m, 3H, H-11, 12), 1.62-1.67 (m, 2H, -CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.52 (s, 3H, H-28), 1.47-1.50 (m, 4H, H-11, 29), 1.38-1.42 (m, 5H, H-19, -CH$_2$CH$_2$C*H*$_2$CH$_3$), 1.29 (s, 3H, H-18), 1.20 (s, 3H, H-30), 0.89 (t, *J* = 7.5 Hz, 3H, -CH$_2$CH$_2$CH$_2$C*H*$_3$); HRMS *m/z* calcd for $C_{31}H_{39}NO_8Na$ ($[M+Na]^+$) 576.2568, found 576.2557.

Data for ***3e*****:** Yield = 83%, white solid, mp 100-102 °C; $[\alpha]^{20}_{D}$ = 0.48 (*c* 3.8 mg/mL, acetone); IR cm^{-1} (KBr): 2954, 1751, 1702, 1636, 1391, 1282, 1073; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.42 (s, 1H, H-21), 7.39 (t, *J* = 1.5 Hz, 1H, H-23), 6.49 (d, *J* = 12.0 Hz, 1H, H-1), 6.38 (d, *J* = 1.0 Hz, 1H, H-22), 5.92 (d, *J* = 12.0 Hz, 1H, H-2), 5.50 (s, 1H, H-17), 3.72 (s, 1H, H-15), 3.15 (dd, *J* = 12.5, 3.0 Hz, 1H, H-6), 2.45-2.49 (m, 2H, -C*H*$_2$CH$_2$(CH$_2$)$_2$CH$_3$), 2.30-2.34 (m, 2H, H-5, 6), 2.14-2.17 (m, 1H, H-9), 1.85-1.88 (m, 3H, H-11, 12), 1.65-1.68 (m, 2H, -CH$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), 1.52 (s, 3H, H-28), 1.47-1.51 (m, 4H, H-11, 29), 1.42 (s, 3H, H-19), 1.33-1.36 (m, 4H, -CH$_2$CH$_2$(C*H*$_2$)$_2$CH$_3$), 1.29 (s, 3H, H-18), 1.20 (s, 3H, H-30), 0.89 (t, *J* = 7.0 Hz, 3H, -CH$_2$CH$_2$(CH$_2$)$_2$C*H*$_3$); HRMS *m/z* calcd for $C_{32}H_{41}NO_8Na$ ($[M+Na]^+$) 590.2724, found 590.2724.

Biological assay. The insecticidal activity of **1**, **2** and **3a-x** was evaluated as the mortality rate values, as per leaf-dipping method described previously,[21,22] against the pre-third-instar larvae

of *Mythimna separata*. For each compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions of **1**, **2**, **3a-x** and toosendanin (a positive control) were made up at 1 mg/mL. Fresh wheat leaf discs (1×1 cm) were dipped into the corresponding solution for 3 s, then taken out and dried. Leaf discs treated with acetone alone were used as a blank control group. Several pieces of treated leaf discs were kept in each dish (10 larvae were raised in each dish), which was then placed in a conditioned room (25 ± 2 ^{o}C, 65-80% relative humidity (RH), 12 h/12 h (light/dark) photoperiod). If the treated leaf discs were consumed, additional treated ones were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until adult emergence. The corrected mortality rate values were obtained by the formula

$$\text{corrected mortality rate } (\%) = (T - C) \times 100/(100\% - C)$$

Where T is the mortality rate in the group treated with the tested compounds, and C is the mortality rate in the blank control group (T and C were all expressed as the percentage).

QSAR model development.

Data set. The experimental data used in this study contained 26 compounds (**1**, **2** and **3a-x**). The biological activity of 26 compounds, expressed by the final mortality rate, was used as the dependent variable in the following QSAR study.

Descriptor generation. The structures of 26 compounds were sketched in HyperChem program (Version 7.0 Hypercube, Inc.)[23] and pre-optimized using the MM+ molecular mechanics force field. Then, the semi-empirical AM1 method was applied to carry out more precise optimization.[24] Then, 1664 descriptors were calculated by the DRAGON software (Version 5.4), including 0 dimension (0D), 1 dimension (1D), 2 dimension (2D) and 3 dimension (3D) molecular descriptors.[23] The abbreviations and meanings of these molecular

descriptors could be seen in the *Handbook of Molecular Descriptors*.[25] In the preliminary screening step, constants, near-constant values, and descriptors with a correlation coefficient greater than 0.99 were excluded.[24] 615 molecular descriptors were retained for the subsequent variable selection.

Feature selection and model construction by GA-MLR. Genetica algorithm (GA)[26] combined with multiple linear regression (MLR) was carried out in the MobyDigs software (version 1.2 for Windows)[27] in the QSAR model development.[25] In GA calculation, stepwise addition and regression of the descriptors were performed to find the best multivariable regression model with the best value of statistical criteria. Multiple linear regression (MLR) was used to build QSAR models based on the descriptors selected by the GA. The parameters of GA process were selected as follows: a population size of 100, a maximum of 5 allowed descriptors in the final model and a reproduction/mutation trade-off radio of 0.5. The rest parameters were set as the defaults.

Validation and applicability domain of the QSAR model. The squared correlation coefficient (R^2) and the leave-one-out cross-validation coefficient (Q^2_{LOO}) were used to evaluate the fitting ability and the predictive ability of the proposed model respectively.[26] The applicability domain (AD) of the generated model was assessed by the leverage (h) value.[27] The Williams plot was used to verify the presence of response outliers (i.e., compounds with cross validate standardized residuals greater than three times of standard deviation units, $>3\sigma$) and X outliers (i.e., compounds with leverage values greater than warning leverage h^*). The warning leverage was defined as $h^*=3(k+1)/n$, where k was the number of model variables and n was the number of the compounds used to develop the model.

Results and discussion

Synthesis. As shown in Scheme 1, first, obacunone (**1**) reacting with hydroxylamine hydrochloride gave C7-oximeobacunone (**2**). Second, compound **2** reacted with different carboxylic acids to afford the target compounds, C7-oxime ester derivatives of obacunone (**3a-x**). The structures of all target compounds were well characterized by ^{1}H NMR, HRMS, optical rotation, IR, and mp. The steric configuration of **3m**, **3r**, and **3s** was further confirmed by X-ray crystallography (Figures 2-4). It obviously showed that, due to the steric hindrance of D-ring, the substituents on the C=N double bond of **3m**, **3r** and **3s** all adopted *E* configuration. Crystallographic data (excluding structure factors) for the structures of **3m**, **3r**, and **3s** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 998485, 998483, and 998484, respectively. These data can be obtained free of charge on application to CCDC [fax +44 (0)1223 336033 or e-mail deposit@ccdc.cam.ac.uk].

Insecticidal activity. As shown in Table 1, the insecticidal activity of **3a-x** against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker) in vivo was evaluated as the mortality rates at 1 mg/mL. Toosendanin was used as the positive control at 1 mg/mL, and leaves treated with acetone alone were used as a blank control group. It was found that the obacunone derivatives exhibited the delayed insecticidal activity against *M. separata*. For example, the corrected mortality rates of **3r** against *M. separata* after 10 and 20 days were 20% and 43.3%, respectively; whereas after 35 days it was sharply increased to 72.4%, which was more than 3-fold of that after 10 days. In addition, the symptoms of *M. separata* treated by the obacunone derivatives were observed in the same way as our previous studies.[21,22] For instance, in the treated groups, many larvae generally died with the slim and wrinkled bodies during the stage of larva (Figure 5); a majority of larvae molted to malformed

pupae or died during the pupation period (Figure 6); some malformed moths were with imperfect wings during the stage of adult emergence (Figure 7). Among all the derivatives, compounds **3b**, **3e**, **3j**, **3o**, **3p**, **3r**, **3s**, **3w**, and **3x** showed the more potent insecticidal activity than their precursor **1** and toosendanin (a positive control). Especially compounds **3e**, **3r**, **3s** and **3w** exhibited the best promising insecticidal activity with the final mortality rates greater than 60%. For example, the final mortality rates of **3e**, **3r**, **3s** and **3w** were 62.1%, 72.4%, 65.5% and 62.1%, respectively. In addition, the relationship between the structures of the obacunone derivatives (**3a-x**) and their insecticidal activity was also investigated. (1) To C7-oxime alkylester series (**3a-k**), the proper length of their side chain at the C-7 position of **2** to the insecticidal activity was very important. For example, the final mortality rates of **3a** (R^1 = CH_3), **3c** (R^1 = $(CH_2)_2CH_3$), **3d** (R^1 = $(CH_2)_3CH_3$), **3f** (R^1 = $(CH_2)_5CH_3$), **3g** (R^1 = $(CH_2)_6CH_3$), **3h** (R^1 = $(CH_2)_8CH_3$), **3i** (R^1 = $(CH_2)_9CH_3$), and **3k** (R^1 = $(CH_2)_{16}CH_3$) were 38%, 34.5%, 41.4%, 44.8%, 38%, 31.1%, 41.4%, and 34.5%, respectively; whereas the final mortality rates of **3b** (R^1 = CH_2CH_3), **3e** (R^1 = $(CH_2)_4CH_3$), and **3j** (R^1 = $(CH_2)_{14}CH_3$) were 55.2%, 62.1%, and 55.2%, respectively. (2) To C7-oxime arylester series (**3l-x**), introduction of the chlorine atom on the phenyl ring of **3l** could lead to the best potent compounds **3r** and **3s**, whose final mortality rates were 72.4% and 65.5%, respectively. On the contrary, introduction of the fluorine or bromine atom on the phenyl ring of **3l** resulted in the less promising compounds **3q** and **3t**, and the final mortality rates of **3q** and **3t** were only 44.8% and 34.5%, respectively. (3) In our previous report, it demonstrated that introduction of the (1-naphthylacetyl)oxy group at the C-4 position of the corresponding podophyllotoxin derivatives could give the more potential compounds.[22] However, herein introduction of a (1-naphthylacetyl)oxy group at the C-7 position of **2** did not afford the potent compound (e.g.,

the final mortality rate of corresponding compound **3v** was only 41.4%). Therefore, by chemical modifications to obtain the more promising derivatives, the introduced functional groups and the parent compounds should be considered together. (4) Introduction of the heterocyclic rings at the C-7 position of **2** led to the potent compounds **3w** and **3x** (e.g., the final mortality rates: 62.1% for **3w**; 58.6% for **3x**).

QSAR model. By using GA-MLR analysis, a multivariable linear model with the highest cross-validation correlation coefficient (Q^2) was built as follows:

$$Y = 0.062 * RDF100v + 1.379 * Dm - 0.297 * Mor15m - 2.287 * R1u - 0.006 * RDF105u + 4.386$$

$$N_{dateset} = 26, R^2 = 0.891, Q^2 = 0.835, SDEP = 0.0358 \quad (1)$$

where Y represents the final mortality rate, $N_{dateset}$ is the number of selected compounds, R^2 represents the correlation coefficient, Q^2 is the cross-validation correlation coefficient, and SDEP represents the standard deviation error in prediction.

All the five selected descriptors were 3D descriptors and were belonged to four categories: the RDF descriptor (RDF100v and RDF105u), the 3D-MoRSE descriptor (Mor15m), the WHIM descriptor (Dm) and the GETAWAY descriptor (R1u). The statistical parameters and meanings of the descriptors were shown in Table 2. Based on the above model, the predicted biological activity values (final mortality rates) of 26 compounds by this model were listed in Table 3. Figure 8 was the plot of the experimental versus predicted values of activity for 26 compounds by the GA-MLR model. It can be seen that the experimental values has a good correlation with the predicted ones. The applicability domain of the derived model evaluated by Williams plot (the cross-validated standardized residual versus hat values) was shown in Figure 9. Only one compound (compound **3t**) had a hat value higher than the

warning h^* value of 0.692. Therefore, it can be seen as a structural outlier of this model. So this model could be taken as a useful tool to predict the activity of compounds with structural modifications at C-7 position of C7-oximeobacunone. The meaning and statistical parameter of each descriptor was shown in Table 2. Among these descriptors, RDF100v and Dm positively correlated with the activity, while Mor15m, GETAWAY and RDF105u negatively correlated with the activity. In order to find the most relevant variable, the standardized coefficient of each descriptor was calculated. Descriptors with a higher absolute value of standardized coefficient tended to have a greater effect on the response value. As shown in Table 2, the most relevant descriptor was RDF100v, belonging to the radial distribution function descriptors. In the above discussion, compounds **3b**, **3e**, **3j**, **3o**, **3p**, **3r**, **3s**, **3w**, and **3x** exhibited more potent insecticidal activity than their precursor **1** and toosendanin. Comparing the experiment activity with the value of RDF100v from Table 3, we found that all these compounds with higher insecticidal activity had the relatively larger RDF100v values.

Conclusions

In summary, a series of C7-oxime ester derivatives of obacunone were prepared and evaluated their insecticidal activity at 1 mg/mL against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker). Three key steric structures of **3m**, **3r**, and **3s** were determined by single-crystal X-ray diffraction. Especially compounds **3e**, **3r**, **3s** and **3w** exhibited the more promising insecticidal activity with the final mortality rates greater than 60%. To C7-oxime alkylester series, the proper length of their side chain at the C-7 position of C7-oximeobacunone to the insecticidal activity was very important; to C7-oxime arylester series, introduction of the chlorine atom on the phenyl ring at the C-7 position of

C7-oximeobacunone could lead to the best potent compounds; introduction of the heterocyclic rings at the C-7 position of C7-oximeobacunone led to the potent compounds**.** QSAR model suggested that five descriptors such as RDF100v, RDF105u, Dm, Mor15m and R1u, were likely to affect the biological activity of these compounds. Among them, the most important descriptor was RDF100v. It will pave the way for further design and chemical modifications of obacunone as botanical insecticidal agents.

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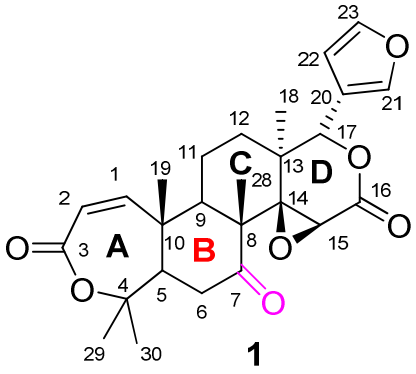


Fig. 1 The chemical structure of obacunone (**1**).

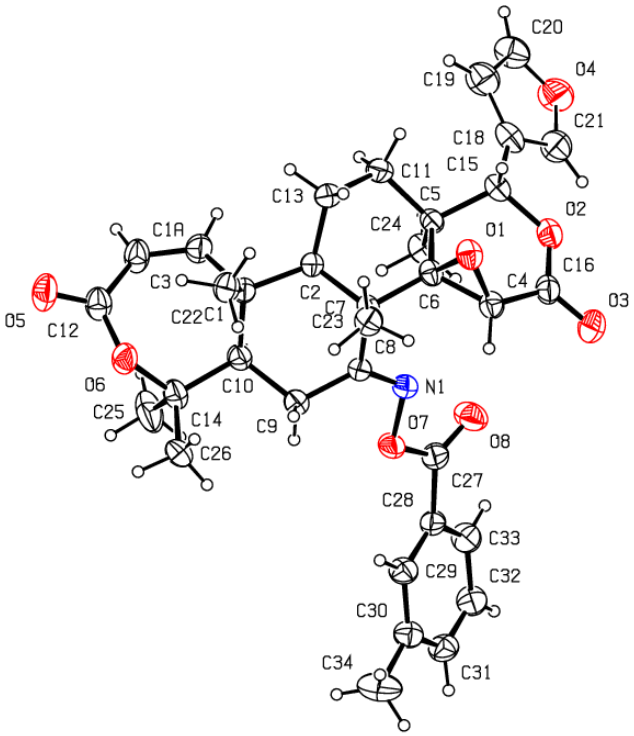


Fig. 2 X-ray crystal structure of **3m**.

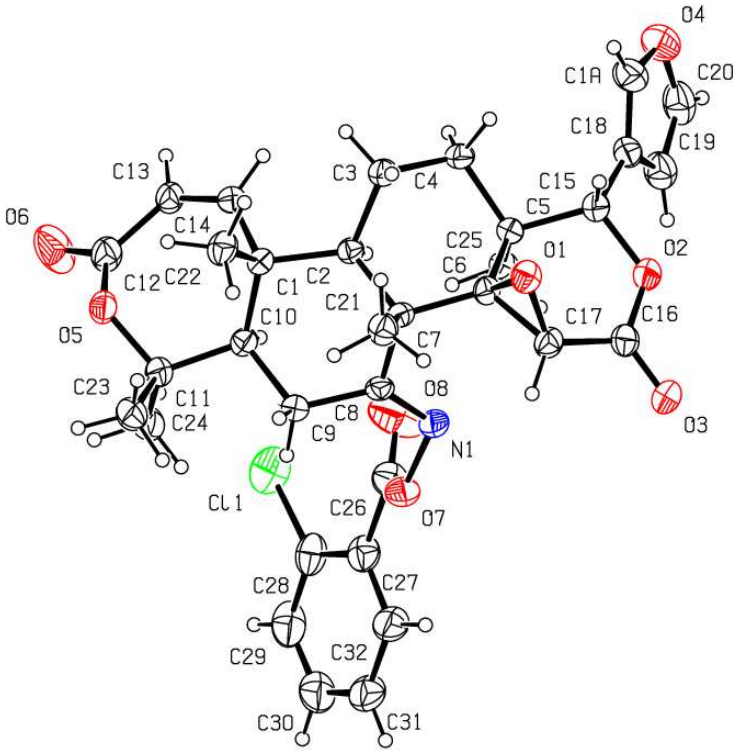


Fig. 3 X-ray crystal structure of **3r**.

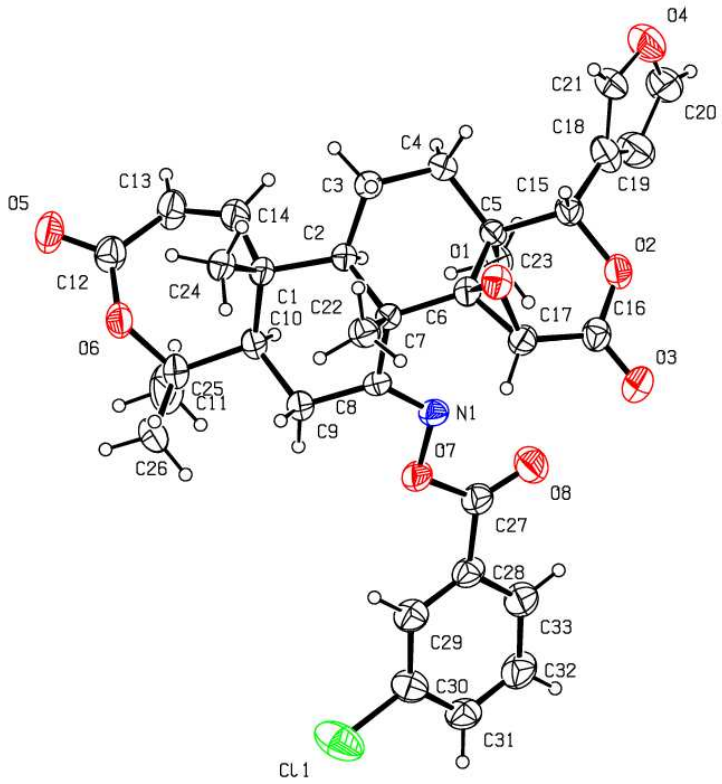


Fig. 4 X-ray crystal structure of **3s**.

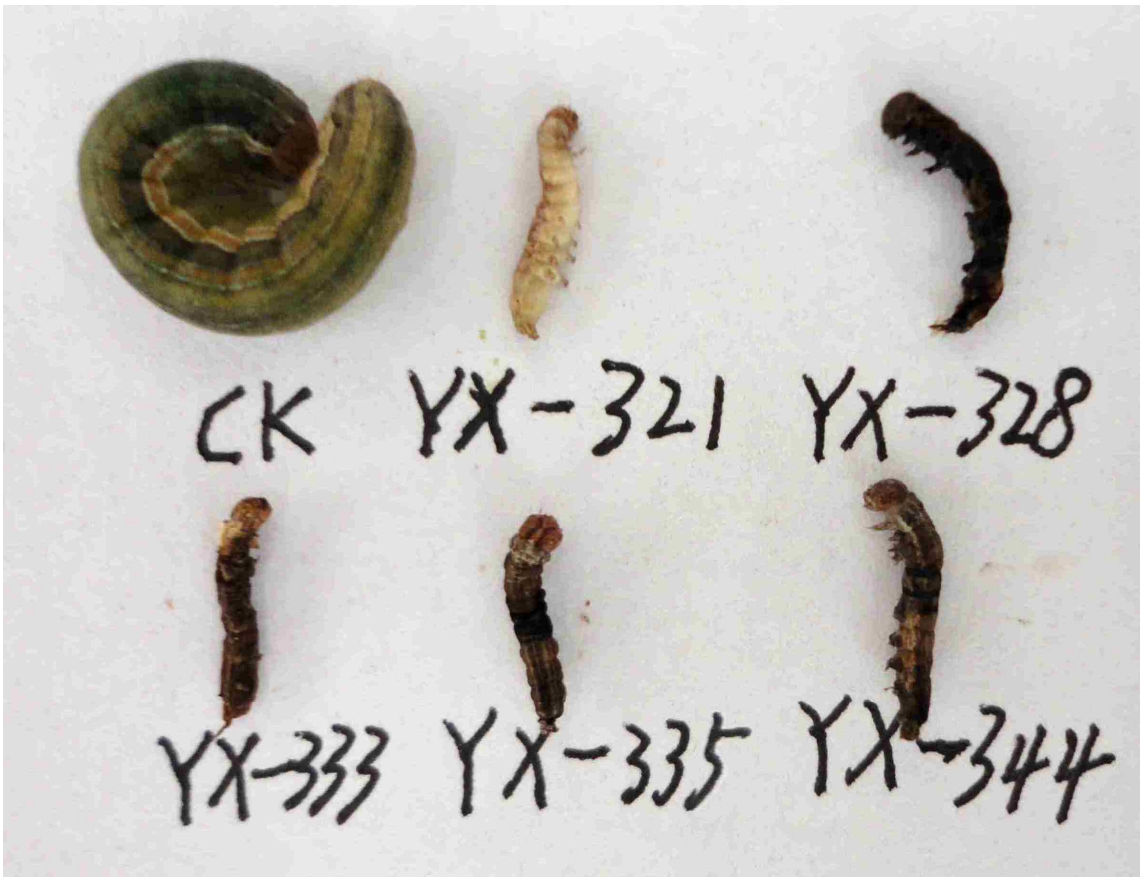


Fig. 5 The representative abnormal larvae pictures of **3r (YX-321)**, **3t (YX-328)**, **3p (YX-333)**, **3w (YX-335),** and **3b (YX-344)** during the larval period (**CK**: blank control group).

Fig. 6 The representative malformed pupae pictures of **3s (YX-322)**, **3o (YX-327)**, **3w (YX-335)**, **3b (YX-344)**, **3e (YX-346)**, **3j (YX-352)**, and **3k (YX-353)** during the pupation period (**CK**: blank control group).

Fig. 7 The representative malformed moth pictures of **3v (YX-320)**, **3r (YX-321)**, **3o (YX-327)**, **3w (YX-335)**, **3b (YX-344)**, **3e (YX-346)**, and **3g (YX-350)** during the stage of adult emergence (**CK**: blank control group).

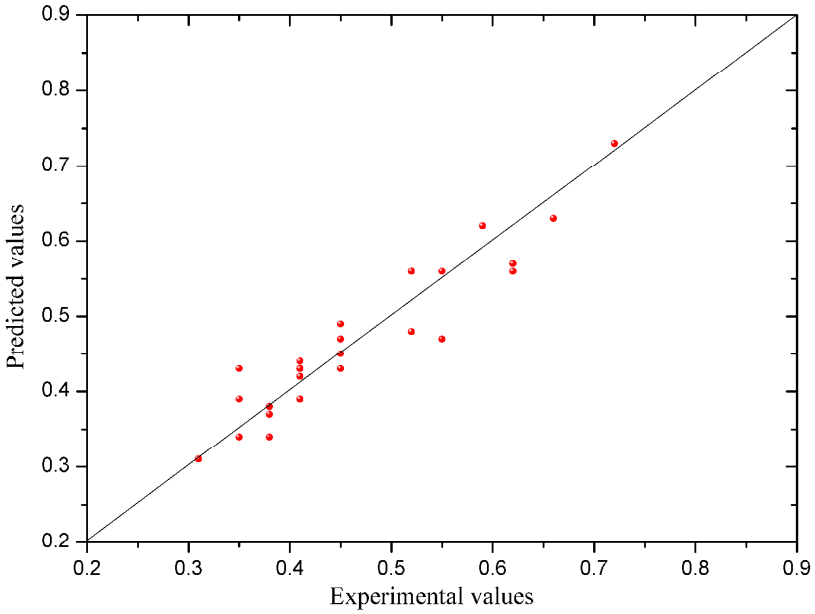


Fig. 8 The experimental values versus the predicted values by GA-MLR model for dataset.

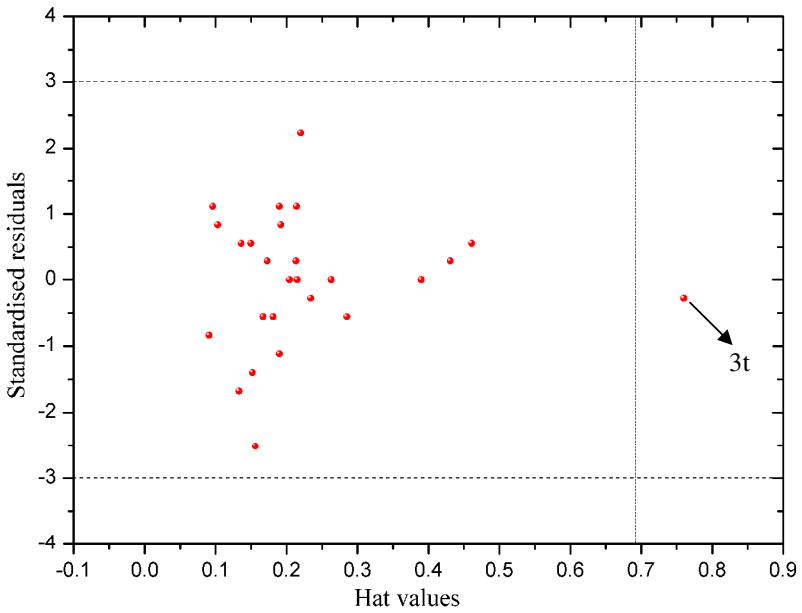
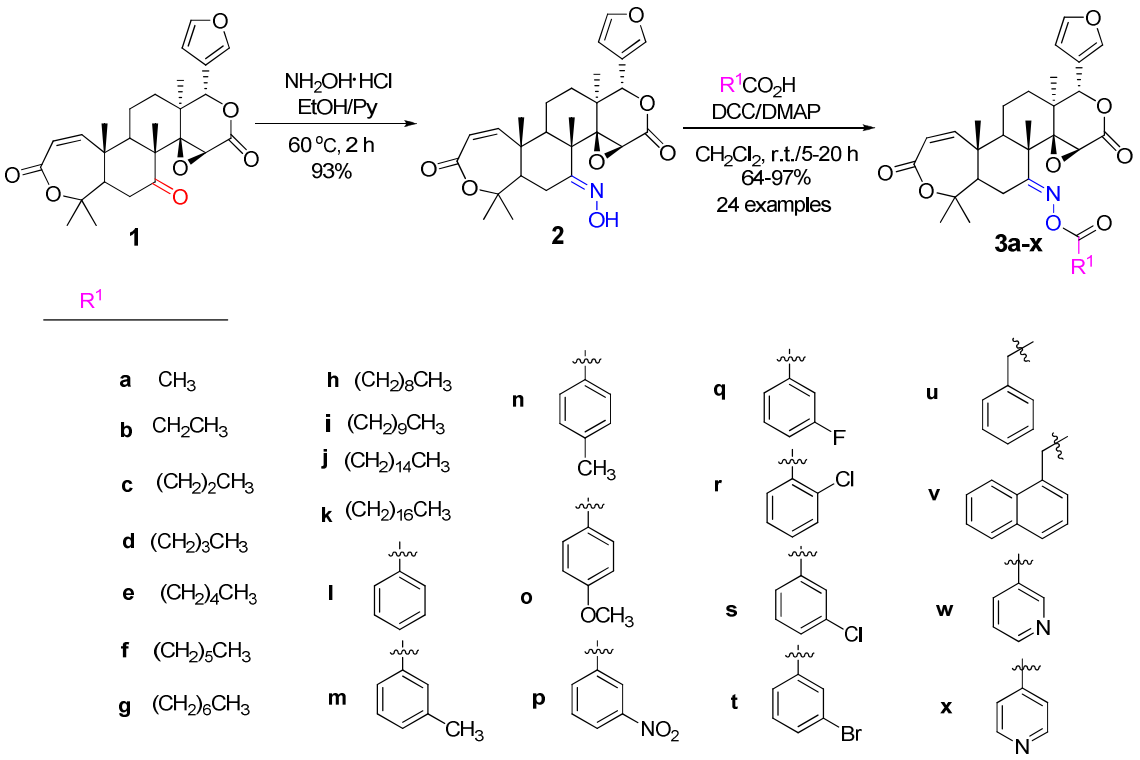


Fig. 9 Williams plot for the GA-MLR model with five descriptors.



Scheme 1 Synthesis of C7-oxime ester derivatives of obacunone (**3a-x**).

Table 1 Insecticidal activity of oxime ester derivatives of obacunone (**3a-x**) against *M. separata* on leaves treated with a concentration of 1 mg/mL.

Compound	Corrected mortality rate (%)		
	10 days	20 days	35 days
1	10.0 ±0	23.3 ±3.3	41.4 ±3.3
2	16.7 ±3.3	30.0 ±0	44.8 ±3.3
3a	13.3 ±3.3	30.0 ±0	38.0 ±5.8
3b	13.3 ±3.3	33.3 ±3.3	55.2 ±3.3
3c	6.7 ±3.3	26.7 ±6.7	34.5 ±3.3
3d	13.3 ±3.3	30.0 ±5.8	41.4 ±3.3
3e	20.0 ±0	46.7 ±3.3	62.1 ±3.3
3f	13.3 ±3.3	30.0 ±5.8	44.8 ±3.3
3g	3.3 ±3.3	16.7 ±3.3	38.0 ±5.8
3h	10.0 ±0	26.7 ±6.7	31.1 ±6.7
3i	10.0 ±5.8	26.7 ±3.3	41.4 ±3.3
3j	10.0 ±0	36.7 ±3.3	55.2 ±3.3
3k	10.0 ±0	23.3 ±3.3	34.5 ±3.3
3l	10.0 ±0	33.3 ±3.3	44.8 ±3.3
3m	13.3 ±3.3	26.7 ±3.3	38.0 ±5.8
3n	16.7 ±3.3	23.3 ±3.3	41.4 ±3.3
3o	13.3 ±3.3	36.7 ±3.3	51.7 ±3.3
3p	13.3 ±3.3	30.0 ±0	51.7 ±3.3
3q	6.7 ±3.3	33.3 ±3.3	44.8 ±3.3
3r	20.0 ±0	43.3 ±6.7	72.4 ±3.3
3s	23.3 ±3.3	40.0 ±5.8	65.5 ±3.3
3t	16.7 ±3.3	23.3 ±3.3	34.5 ±3.3
3u	3.3 ±3.3	20.0 ±0	34.5 ±6.7
3v	20.0 ±5.8	30.0 ±0	41.4 ±3.3
3w	20.0 ±5.8	36.7 ±3.3	62.1 ±3.3
3x	10.0 ±0	36.7 ±6.7	58.6 ±0
toosendanin	23.3 ± 3.3	26.7 ± 3.3	48.3 ± 5.8
blank control	0 ± 0	0 ± 0	3.3 ± 3.3

Table 2 Parameters of the GA-MLR model.

Variables	Meaning of variables	Regression coefficient	Error coefficient	Standardized coefficient
Intercept	constant	4.386	0.596	-
RDF100v	Radial distribution function-10.0/weighted by atomic van der waals volumes	0.062	0.005	1.566
Dm	D total accessibility index/weighted by atomic masses	1.379	0.292	0.459
Mor15m	3D-MoRSE-signal 15/weighted by atomic masses	-0.297	0.031	-0.894
R1u	R autocorrelation of lag1/unweighted	-2.287	0.313	-0.889
RDF105u	Radial distribution function-10.5/unweighted	-0.006	0.001	-0.672

Table 3 Experimental, predicted activity and RDF100v value of each compound.

Number	Compound	Experimental activity	Predicted activity	RDF100v value
1	**1**	0.414	0.433	3.050
2	**2**	0.448	0.448	2.885
3	**3a**	0.38	0.363	6.050
4	**3b**	0.552	0.559	9.820
5	**3c**	0.345	0.382	9.340
6	**3d**	0.414	0.415	10.848
7	**3e**	0.621	0.551	12.149
8	**3f**	0.448	0.464	11.916
9	**3g**	0.38	0.331	10.146
10	**3h**	0.311	0.308	10.337
11	**3i**	0.414	0.423	12.037
12	**3j**	0.552	0.46	12.312
13	**3k**	0.345	0.419	12.190
14	**3l**	0.448	0.423	9.195
15	**3m**	0.38	0.38	8.537
16	**3n**	0.414	0.435	11.715
17	**3o**	0.517	0.555	14.505
18	**3p**	0.517	0.479	11.564
19	**3q**	0.448	0.486	11.026
20	**3r**	0.724	0.728	11.693
21	**3s**	0.655	0.628	11.821
22	**3t**	0.345	0.334	11.970
23	**3u**	0.345	0.336	8.096
24	**3v**	0.414	0.388	9.508
25	**3w**	0.621	0.568	12.020
26	**3x**	0.586	0.611	12.859