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ORIGINAL ARTICLE

Synthesis, antiviral activity, and 3D-QSAR study of novel chalcone derivatives containing malonate and pyridine moieties

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3D-QSAR

Abstract Several novel chalcone derivatives containing malonate and pyridine moieties were synthesized, and their structures were confirmed by ^{1}H nuclear magnetic resonance, ^{13}C nuclear magnetic resonance, ^{19}F nuclear magnetic resonance, infrared, and elemental analyses. Antiviral bioassays revealed that most of the compounds exhibited good antiviral activity against cucumber mosaic virus (CMV) at 500 μg/mL. In particular, compounds **5l** and **5n** showed significant curative activities against CMV *in vivo* with 50% effective concentration (EC_{50}) values of 186.2 and 211.5 μg/mL, respectively; these values are even better than that of ningnanmycin (330.5 μg/mL). A 3D quantitative structure–activity relationship study was carried out using the comparative molecular field analysis technique based on curative activities against CMV. Results revealed good predictive ability with cross-validated q^2 and non-cross-validated r^2 values of 0.517 and 0.990, respectively.

Abbreviations: TLC, thin layer chromatography; EC_{50}, 50% effective concentration; ^{1}H NMR, ^{1}H nuclear magnetic resonance; ^{13}C NMR, ^{13}C nuclear magnetic resonance; ^{19}F NMR, ^{19}F nuclear magnetic resonance; MS, mass spectroscopy; CMV, cucumber mosaic virus; 3D-SAR, three-dimensional quantitative structure–activity relationship; CoMFA, comparative molecular field analysis; *Nicotiana tabacum* L., *N. tabacum* L.; *Chenopodium amaranticolor*, *C. amaranticolor*; PLS, partial least-squares; ONC, optimal number of components; SEE, standard error of estimate.

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1. Introduction

Cucumber mosaic virus (CMV), a member of *Cucumovirus* that affects cucumber and other cucurbits, was first described in detail in 1916 and is known to affect 1200 species in over 100 plant families and cause significant economic losses in a great quantity of vegetable and horticultural crops (Palukaitis, 1992; Abad et al., 2000; Gallitelli, 2000; Stamova and Chetelat, 2000; Sulistyowati et al., 2004; Lin et al., 2010). Ningnanmycin, the most successful registered anti-plant viral agent, cannot achieve optimal cure rates for the virus (Chen et al., 2009; Wang et al., 2012; Luo et al., 2013; Ma et al., 2014). As such, the development of highly efficient, novel, and environmentally benign agents to control the refractory virus disease is a significant endeavor (Ritzenthaler, 2005; Song et al., 2009).

1,3-Diarylprop-2-en-1-ones, commonly known as chalcones, have received a great deal of interest in the pharmacological chemistry. Compounds with the chalcone backbone are reported to possess a wide range of biological activities, such as nematicide (González and Estévez-Braun, 1998), antifungal (Zhao et al., 2007), antiallergenic (Yoshimura et al., 2009), antimicrobial (Bandgar et al., 2010), anticancer (Vincenzo et al., 2000), antimalarial (Liu et al., 2001), and antifeedant (Thirunarayanan and Vanangamudi, 2014) properties.

Malonates are traditionally regarded as important materials for synthesizing the key intermediates of numerous active substances but rarely found as pharmacophores belonging to the target compounds (Wheeler, 1984; Woo et al., 1989; Ragoussis et al., 2004; Brandau et al., 2006). In our previous work, we designed and synthesized a series of β-amino acid ester derivatives containing the malonate moiety with improved antiviral activities against tobacco mosaic virus (TMV) (Xiao et al., 2014). Pyridine derivatives are an important class of bioactive compounds that have a wide spectrum of activities, including antimicrobial (Patel and Patel, 2012), herbicidal (Liu et al., 2008), antitumor (Ahmeda et al., 2009), and insecticidal (Kang et al., 2013) properties.

Using previous findings as a basis, we aimed to introduce malonate and pyridine fragments to the parent chalcone skeleton by Michael addition to build a novel family of bioactive molecules. Bioassay results of most of the resultant compounds revealed moderate to good anti-CMV activity. A 3D quantitative structure–activity relationship (3D-QSAR) study of 22 target compounds was carried out using the comparative molecular field analysis (CoMFA) technique based on their curative activities against CMV, and results revealed good predictive ability. To the best of our knowledge, the present work is the first to report chalcone derivatives containing malonate and pyridine moieties with potent effects against CMV determined through 3D-QSAR analysis.

2. Experimental

2.1. Materials and methods

^{1}H nuclear magnetic resonance (^{1}H NMR), ^{19}F nuclear magnetic resonance (^{19}F NMR), and ^{13}C nuclear magnetic resonance (^{13}C NMR) (solvent $CDCl_3$) spectral analyses were performed on a JEOL-ECX500 NMR spectrometer operating at 500, 475 and 125 MHz at room temperature with tetramethylsilane as the internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Infrared (IR) spectra were recorded on a Bruker VECTOR 22 spectrometer by using KBr disks. Mass spectral studies were conducted on an Agilent 5973 organic mass spectrometer. The melting points of the products were determined by using an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and are reported uncorrected. Analytical thin-layer chromatography was performed on silica gel GF254 (400 mesh). Column chromatographic purification was carried out using silica gel. All reagents and reactants were purchased from commercial suppliers and were either of analytical reagent grade or of chemically pure. All solvents were dried, deoxygenated, and redistilled prior to use.

2.2. Chemistry

2.2.1. General procedure for synthesizing (E)-3-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-1-(aryl)prop-2-en-1-ones (4a–4k)

As shown in Scheme 1, intermediate **3** was synthesized following a previously reported method (Choudhary and Juyal, 2011). Substituted acetophenone **1** was reacted with *p*-hydroxy benzaldehyde **2** through Claisen–Schmidt aldol condensation at 20–25 °C to achieve intermediate **3** in good yield. Then, key intermediates **4a–4k** were synthesized as shown in Scheme 1 by using a previously described method (Zhao et al., 2007). Intermediate **3** (2.5 mmol), 2-chloro-5-(chloromethyl) pyridine (2.5 mmol), K_2CO_3 (7.5 mmol), and acetone (15 mL) were added to a 25 mL three-neck round-bottom flask fitted with a magnetic stirring bar. The resulting mixture was refluxed at 60 °C for 12 h, poured into ice water (20 mL), and then separated. The aqueous phase was acidified with 10% HCl to pH 6–7 and filtered. The residue was dried and recrystallized from ethanol to obtain the key intermediates **4a–4k**. The physical characteristics and IR, ^{1}H NMR, ^{13}C NMR, and elemental data of **4a–4k** are provided as supplementary data.

2.2.2. General procedure for synthesizing dimethyl (ethyl) 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(aryl)phlpropyl)malonates (5a–5v)

The target compounds **5a–5v** were synthesized as illustrated in Scheme 1. A 25 mL round-bottomed flask equipped with a magnetic stirrer was charged with intermediate **4** (0.3 mmol), dimethyl malonate (or diethyl malonate) (1.5 mmol), CH_3OH (or C_2H_5OH) (8 mL), and KOH (0.3 mmol). The resulting mixture was refluxed and stirred at 70–80 °C for 0.5 h. After pouring into ice water (10 mL), the solution was acidified to pH 6–7 with 10% HCl and then extracted with CH_2Cl_2 (5 mL × 3). The combined organic layer was concentrated under rotary evaporation, and the crude residue was separated through column chromatography on silica gel (ethyl acetate/petroleum ether = 1/5 [v/v]) to obtain the target compounds **5a–5v**. The physical characteristics and IR, ^{1}H NMR, ^{13}C NMR, ^{19}F nuclear magnetic resonance (^{19}F NMR), mass spectra (MS), and elemental data of target compounds **5a–5v** are provided below.

2.2.2.1. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-phenylpropyl)malonate (5a). Yield, 77%; white

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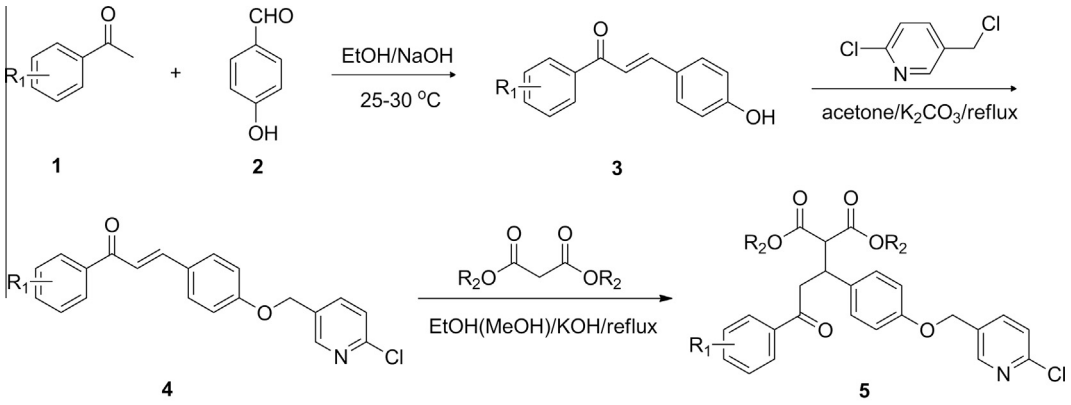


5a: R_1=H, R_2=CH_3; **5b**: R_1=2-CH_3, R_2=CH_3; **5c**: R_1=3-CH_3, R_2=CH_3; **5d**: R_1=4-CH_3, R_2=CH_3;
5e: R_1=2-Cl, R_2=CH_3; **5f**: R_1=3-Cl, R_2=CH_3; **5g**: R_1=4-Cl, R_2=CH_3; **5h**: R_1=2,4-di-Cl, R_2=CH_3;
5i: R_1=4-OC_2H_5, R_2=CH_3; **5j**: R_1=4-F, R_2=CH_3; **5k**: R_1=4-*i*-Pr, R_2=CH_3; **5l**: R_1=H, R_2=C_2H_5;
5m: R_1=2-CH_3, R_2=C_2H_5; **5n** :R_1=3-CH_3, R_2=C_2H_5; **5o**: R_1=4-CH_3, R_2=C_2H_5; **5p**: R_1=2-Cl, R_2=C_2H_5;
5q: R_1=3-Cl, R_2=C_2H_5; **5r**: R_1=4-Cl, R_2=C_2H_5; **5s**: R_1=2,4-di-Cl, R_2=C_2H_5; **5t**: R_1=4-OC_2H_5, R_2=C_2H_5;
5u: R_1=4-F, R_2=C_2H_5; **5v**: R_1=4-*i*-Pr, R_2=C_2H_5.

Scheme 1 Synthesis of the target compounds **5a**–**5v**.

solid; m.p. 89–91 °C; IR (KBr, cm^{-1}): *v* 2841–2996 (C—H), 1734 (—O—C═O), 1674 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1298 (C—N), 1234 (Ar—C—O), 1157 (CH_3—O—C═O), 818 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.25 Hz, Py-2-H), 7.90 (d, 2H, J = 7.10 Hz, Ar′-2,6-H), 7.71 (dd, 1H, J_1 = 2.35 Hz, J_2 = 2.40 Hz, Py-4-H), 7.54 (t, 1H, J_1 = 7.35 Hz, J_2 = 7.40 Hz, Ar′-4-H), 7.43 (t, 2H, J_1 = 7.85 Hz, J_2 = 7.60 Hz, Ar′-3,5-H), 7.34 (d, 1H, J = 8.25 Hz, Py-5-H), 7.20 (d, 2H, J = 8.75 Hz, Ar-2,6-H), 6.84 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.17–4.13 (m, 1H, Ar—CH—), 3.82 (d, 1H, J = 9.35 Hz, —CO_2—CH—), 3.74 (s, 3H, —CO_2—CH_3), 3.54–3.50 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.44 (dd, 1H, J_1 = J_2 = 9.20 Hz, —CO—CH—); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.6, 168.7, 168.2, 157.1, 151.1, 148.7, 138.1, 136.7, 133.3, 133.2, 131.5, 129.3, 128.6, 128.1, 124.3, 114.7, 66.6, 57.4, 52.7, 52.5, 42.4, 40.1; MS (ESI) *m/z*: 504.3 ($[M + Na]^+$). Anal. Calcd for $C_{26}H_{24}ClNO_6$: C, 64.80; H, 5.02; N, 2.91. Found: C, 64.86; H, 5.19; N, 2.98.

*2.2.2.2. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(o-tolyl)propyl)malonate (**5b**)*. Yield, 68%; white solid; m.p. 79–81 °C; IR (KBr, cm^{-1}): *v* 2855–2955 (C—H), 1748 (—O—C═O), 1682 (Ar′—C═O), 1456–1609 (C═C and benzene and Py-ring), 1327 (C—N), 1248 (Ar—C—O), 1157 (CH_3—O—C═O), 829 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.40 (d, 1H, J = 2.20 Hz, Py-2-H), 7.69 (dd, 1H, J_1 = J_2 = 2.40 Hz, Py-4-H), 7.55 (d, 1H, J = 7.70 Hz, Ar′-6-H), 7.31–7.29 (m, 2H, Ar′-4-H, Py-5-H), 7.21 (t, 1H, J_1 = 7.55 Hz, J_2 = 7.50 Hz, Ar′-5-H), 7.14–7.12 (m, 3H, Ar′-3-H, Ar-2,6-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.07–4.02 (m, 1H, Ar—CH—), 3.78 (d, 1H, J = 9.65 Hz, —CO_2—CH—), 3.73 (s, 3H, —CO_2—CH_3), 3.50–3.45 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.29 (dd, 1H, J_1 = J_2 = 9.90 Hz, —CO—CH—), 2.16 (s, 3H, Ar′—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 202.0, 168.7, 168.1, 157.2, 151.0, 148.8, 138.2, 138.0, 137.9, 133.1, 131.8, 131.7, 131.2, 129.5, 128.3, 125.7, 124.3, 114.8, 66.7, 57.5, 52.7, 52.4, 45.5, 40.5, 20.7; MS (ESI) *m/z*: 518.3 ($[M + Na]^+$). Anal. Calcd. for $C_{27}H_{26}ClNO_6$: C, 65.39; H, 5.28; N, 2.82. Found: C, 65.36; H, 5.40; N, 2.89.

*2.2.2.3. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(m-tolyl)propyl)malonate (**5c**)*. Yield, 44%; white solid; m.p. 75–77 °C; IR (KBr, cm^{-1}): *v* 2849–2955 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–1616 (C═C and benzene and Py-ring), 1289 (C—N), 1242 (Ar—C—O), 1152 (CH_3—O—C═O), 827.5 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.41 (d, 1H, J = 1.60 Hz, Py-2-H), 7.71–7.70 (m, 3H, Py-4-H, Ar′-2,6-H), 7.35–7.28 (m, 3H, Ar′-4,5-H, Py-5-H), 7.21 (d, 2H, J = 8.15 Hz, Ar-2,6-H), 6.84 (d, 2H, J = 8.20 Hz, Ar-3,5-H), 4.97 (s, 2H, Py—CH_2—), 4.18–4.14 (m, 1H, Ar—CH—), 3.84 (d, 1H, J = 8.50 Hz, —CO_2—CH—), 3.73 (s, 3H, —CO_2—CH_3), 3.55–3.42 (m, 5H, —CO_2—CH_3, —CO—CH—), 2.37 (s, 3H, Ar′—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.8, 168.8, 168.3, 157.2, 151.1, 148.8, 138.4, 138.3, 136.8, 134.0, 133.5, 131.7, 129.4, 128.7, 128.6, 125.4, 124.3, 114.7, 66.7, 57.5, 52.8, 52.6, 42.6, 40.1, 21.4; MS (ESI) *m/z*: 518.3 ($[M + Na]^+$). Anal. Calcd for $C_{27}H_{26}ClNO_6$: C, 65.39; H, 5.28; N, 2.82. Found: C, 65.28; H, 5.52; N, 2.96.

*2.2.2.4. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(p-tolyl)propyl)malonate (**5d**)*. Yield, 44%; white solid; m.p. 87–89 °C; IR (KBr, cm^{-1}): *v* 2886–3032 (C—H), 1749 (—O—C═O), 1670 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1271 (C—N), 1232 (Ar—C—O), 1159 (CH_3—O—C═O), 822 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.25 Hz, Py-2-H), 7.80 (d, 2H, J = 8.15 Hz, Ar′-2,6-H), 7.72 (dd, 1H, J_1 = J_2 = 2.40 Hz, J = 2.35 Hz, Py-4-H), 7.35 (d, 1H, J = 8.15 Hz, Py-5-H), 7.23–7.19 (m, 4H, Ar-2,6-H, Ar′-3,5-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.16–4.12 (m, 1H, Ar—CH—), 3.82 (d, 1H,

J = 9.40 Hz, —CO_2—CH—), 3.73 (s, 3H, —CO_2—CH_3), 3.52–3.46 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.40 (dd, 1H, J_1 = 9.20 Hz, J_2 = 9.15 Hz, —CO—CH—), 2.39 (s, 3H, Ar′—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.8, 168.8, 168.3, 157.2, 151.1, 148.8, 138.4, 138.3, 136.8, 134.0, 133.5, 131.7, 129.4, 128.7, 128.6, 125.4, 124.3, 114.7, 66.7, 57.5, 52.8, 52.6, 42.6, 40.1, 21.4; MS (ESI) m/z: 518.3 ($[M + Na]^+$). Anal. Calcd for $C_{27}H_{26}ClNO_6$: C, 65.39; H, 5.28; N, 2.82. Found: C, 65.17; H, 5.57; N, 2.90.

*2.2.2.5. Dimethyl 2-(3-(2-chlorophenyl)-1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxopropyl)malonate (**5e**)*. Yield, 70%; white solid; m.p. 97–99 °C; IR (KBr, cm^{-1}): ν 2849–3065 (C—H), 1721 (—O—C═O), 1701 (Ar′—C═O), 1458–1611 (C═C and benzene and Py-ring), 1319 (C—N), 1258 (Ar—C—O), 1161 (CH_3—O—C═O), 820 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.43 (d, 1H, J = 2.15 Hz, Py-2-H), 7.73 (dd, 1H, J_1 = 2.35 Hz, J_2 = 2.30 Hz, Py-4-H), 7.36–7.32 (m, 3H, Ar′-4,6-H, Py-5-H), 7.26–7.21 (m, 1H, Ar′-5-H), 7.19 (d, 1H, J = 7.05 Hz, Ar′-3-H), 7.16 (d, 2H, J = 8.65 Hz, Ar-2,6-H), 6.84 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 5.01 (s, 2H, Py—CH_2—), 4.07–4.02 (m, 1H, Ar—CH—), 3.78–3.75 (m, 4H, —CO_2—CH—, —CO_2—CH_3), 3.53–3.48 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.42 (dd, 1H, J_1 = 9.55 Hz, J_2 = 9.65 Hz, —CO—CH—); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 200.7, 168.5, 168.0, 157.2, 151.1, 148.7, 139.0, 138.1, 132.8, 131.7, 131.5, 130.7, 130.4, 129.5, 129.0, 126.8, 124.3, 114.7, 66.6, 57.3, 52.8, 52.5, 46.5, 40.1; MS (ESI) m/z: 538.3 ($[M + Na]^+$). Anal. Calcd for $C_{26}H_{23}Cl_2NO_6$: C, 60.48; H, 4.49; N, 2.71. Found: C, 60.55; H, 4.64; N, 2.83.

*2.2.2.6. Dimethyl 2-(3-(3-chlorophenyl)-1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxopropyl)malonae (**5f**)*. Yield, 50%; white solid; m.p. 77–79 °C; IR (KBr, cm^{-1}): ν 2849–3065 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1311 (C—N), 1244 (Ar—C—O), 1155 (CH_3—O—C═O), 831 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.41 (d, 1H, J = 2.15 Hz, Py-2-H), 7.84 (s, 1H, Ar′-2-H), 7.79 (d, 1H, J = 7.90 Hz, Ar′-6-H), 7.72 (dd, 1H, J_1 = 2.30 Hz, J_2 = 2.40 Hz, Py-4-H), 7.51–7.49 (m, 1H, Ar′-4-H), 7.39–7.33 (m, 2H, Ar′-5-H, Py-5-H), 7.20 (d, 2H, J = 8.60 Hz, Ar-2,6-H), 6.85 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.99 (s, 2H, Py—CH_2—), 4.15–4.10 (m, 1H, Ar—CH—), 3.82 (d, 1H, J = 9.45 Hz, —CO_2—CH—), 3.74 (s, 3H, —CO_2—CH_3), 3.53–3.48 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.41 (dd, 1H, J_1 = 9.25 Hz, J_2 = 9.20 Hz, —CO—CH—); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.5, 168.8, 168.2, 157.3, 151.0, 148.7, 138.4, 138.2, 134.9, 133.2, 133.1, 131.7, 130.7, 130.1, 129.4, 128.2, 126.3, 124.4, 114.8, 66.6, 57.3, 52.9, 52.6, 42.7, 40.1; MS (ESI) m/z: 538.3 ($[M + Na]^+$). Anal. Calcd for $C_{26}H_{23}Cl_2NO_6$: C, 60.48; H, 4.49; N, 2.71. Found: C, 60.58; H, 4.72; N, 2.75.

*2.2.2.7. Dimethyl 2-(3-(4-chlorophenyl)-1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxopropyl)malonate (**5g**)*. Yield, 57%; white solid; m.p. 103–105 °C; IR (KBr, cm^{-1}): ν 2887–3088 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–1616 (C═C and benzene and Py-ring), 1308 (C—N), 1248 (Ar—C—O), 1159 (CH_3—O—C═O), 833 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.35 Hz, Py-2-H), 7.84 (d, 2H, J = 8.55 Hz, Ar′-2,6-H), 7.71 (dd, 1H, J_1 = 2.55 Hz, J_2 = 2.40 Hz, Py-4-H), 7.41 (d, 2H, J = 8.60 Hz, Ar′-3,5-H), 7.35 (d, 1H, J = 8.15 Hz, Py-5-H), 7.18 (d, 2H, J = 8.65 Hz, Ar-2,6-H), 6.84 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.13–4.09 (m, 1H, Ar—CH—), 3.80 (d, 1H, J = 9.35 Hz, —CO_2—CH—), 3.74 (s, 3H, —CO_2—CH_3), 3.52–3.48 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.38 (dd, 1H, J_1 = 9.30 Hz, J_2 = 9.35 Hz, —CO—CH—); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.4, 168.7, 168.1, 157.2, 151.2, 148.7, 139.6, 138.1, 135.0, 133.0, 131.5, 129.5, 129.3, 128.9, 124.3, 114.7, 66.6, 57.3, 52.7, 52.5, 42.4, 40.1; MS (ESI) m/z: 538.3 ($[M + Na]^+$). Anal. Calcd for $C_{26}H_{23}Cl_2NO_6$: C, 60.48; H, 4.49; N, 2.71. Found: C, 60.53; H, 4.69; N, 2.86.

*2.2.2.8. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(2,4-dichlorophenyl)-3-oxopropyl)malonate (**5h**)*. Yield, 51%; white solid; m.p. 109–111 °C; IR (KBr, cm^{-1}): ν 2864–3069 (C—H), 1741 (—O—C═O), 1699 (Ar′—C═O), 1456–1609 (C═C and benzene and Py-ring), 1280 (C—N), 1252 (Ar—C—O), 1159 (CH_3—O—C═O), 831 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.43 (d, 1H, J = 2.30 Hz, Py-2-H), 7.73 (dd, 1H, J_1 = J_2 = 2.40 Hz, Py-4-H), 7.38 (d, 1H, J = 1.85 Hz, Ar′-6-H), 7.36 (d, 1H, J = 8.15 Hz, Py-5-H), 7.23 (dd, 1H, J_1 = 1.80 Hz, J_2 = 1.95 Hz, Ar′-3-H), 7.17 (d, 1H, J = 8.25 Hz, Ar′-5-H), 7.14 (d, 2H, J = 8.70 Hz, Ar-2,6-H), 6.84 (d, 2H, J = 8.70 Hz, Ar-3,5-H), 5.00 (s, 2H, Py—CH_2—), 4.04–4.00 (m, 1H, Ar—CH—), 3.76–3.74 (m, 4H, —CO_2—CH—, —CO_2—CH_3), 3.52–3.47 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.39 (dd, 1H, J_1 = 9.70 Hz, J_2 = 9.60 Hz, —CO—CH—); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 199.5, 168.5, 168.0, 148.7, 138.1, 132.7, 130.3, 129.5, 127.2, 124.3, 114.8, 66.7, 57.2, 52.8, 52.5, 46.5, 40.2; MS (ESI) m/z: 572.2 ($[M + Na]^+$). Anal. Calcd for $C_{26}H_{22}Cl_3NO_6$: C, 56.69; H, 4.03; N, 2.54. Found: C, 56.55; H, 4.11; N, 2.82.

*2.2.2.9. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(4-ethoxyphenyl)-3-oxopropyl)malonate (**5i**)*. Yield, 46%; white solid; m.p. 93–95 °C; IR (KBr, cm^{-1}): ν 2891–2990 (C—H), 1732 (—O—C═O), 1667 (Ar′—C═O), 1458–1603 (C═C and benzene and Py-ring), 1308 (C—N), 1234 (Ar—C—O), 1179 (—CH_2—O—Ar′), 1157 (CH_3—O—C═O), 831 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.25 Hz, Py-2-H), 7.88 (d, 2H, J = 8.95 Hz, Ar′-2,6-H), 7.71 (dd, 1H, J_1 = 2.50 Hz, J_2 = 2.40 Hz, Py-4-H), 7.34 (d, 1H, J = 8.25 Hz, Py-5-H), 7.19 (d, 2H, J = 8.70 Hz, Ar-2,6-H), 6.88 (d, 2H, J = 8.90 Hz, Ar′-3,5-H), 6.83 (d, 2H, J = 8.70 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.16–4.05 (m, 3H, Ar—CH—, Ar′—OCH_2—), 3.81 (d, 1H, J = 9.40 Hz, —CO_2—CH—), 3.73 (s, 3H, —CO_2—CH_3), 3.52 (s, 3H, —CO_2—CH_3), 3.45 (dd, 1H, J_1 = 4.70 Hz, J_2 = 4.65 Hz, —CO—CH—), 3.36 (dd, 1H, J_1 = J_2 = 9.25 Hz, —CO—CH—), 1.43 (t, 3H, J_1 = J_2 = 7.05 Hz, Ar′—OCH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.0, 168.8, 168.2, 162.9, 157.1, 153.7, 148.7, 138.1, 133.4, 131.5, 130.4, 129.6, 129.3, 124.3, 114.6, 114.1, 66.6, 63.7, 57.4, 52.7, 52.5, 42.1, 40.3, 14.7; MS (ESI) m/z: 548.3 ($[M + Na]^+$). Anal. Calcd for $C_{28}H_{28}ClNO_7$: C, 63.94; H, 5.37; N, 2.66. Found: C, 63.92; H, 5.46; N, 2.78.

*2.2.2.10. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(4-fluorophenyl)-3-oxopropyl)malonate (**5j**)*. Yield, 65%; white solid; m.p. 74–76 °C; IR (KBr, cm^{-1}): ν 2849–3067 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–

1616 (C═C and benzene and Py-ring), 1319 (C—N), 1260 (C—F), 1227 (Ar—C—O), 1155 (CH_3—O—C═O), 831 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.20 Hz, Py-2-H), 7.95–7.92 (m, 2H, Ar′-2,6-H), 7.71 (dd, 1H, J_1 = 2.40 Hz, J_2 = 2.45 Hz, Py-4-H), 7.34 (d, 1H, J = 8.15 Hz, Py-5-H), 7.18 (d, 2H, J = 8.70 Hz, Ar-2,6-H), 7.10 (t, 2H, J_1 = 8.55 Hz, J_2 = 8.60 Hz, Ar′-3,5-H), 6.84 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.14–4.10 (m, 1H, Ar—CH—), 3.81 (d, 1H, J = 9.35 Hz, —CO_2—CH—), 3.74 (s, 3H, —CO_2—CH_3), 3.53–3.49 (m, 4H, —CO_2—CH_3, —CO—CH—), 3.39 (dd, 1H, $J_1 = J_2$ = 9.40 Hz, —CO—CH—); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.1, 168.8, 168.2, 166.8, 164.8, 157.3, 151.2, 148.8, 138.2, 133.2, 131.6, 130.9, 130.8, 129.4, 124.4, 115.9, 115.7, 114.8, 66.7, 57.4, 52.8, 52.6, 42.5, 40.3; ^{19}F NMR (470 MHz, $CDCl_3$, ppm): δ −104.91; MS (ESI) m/z: 522.3 ($[M + Na]^+$). Anal. Calcd for $C_{26}H_{23}ClFNO_6$: C, 62.47; H, 4.64; N, 2.80. Found: C, 62.09; H, 4.83; N, 2.91.

*2.2.2.11. Dimethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)-phenyl)-3-(4-isopropylphenyl)-3-oxopropyl)malonate (**5k**).* Yield, 52%; white solid; m.p. 61–63 °C; IR (KBr, cm^{-1}): ν 2874–3046 (C—H), 1748 (—O—C═O), 1674 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1314 (C—N), 1262 (Ar—C—O), 1150 (CH_3—O—C═O), 830 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.25 Hz, Py-2-H), 7.84 (d, 2H, J = 8.35 Hz, Ar′-2,6-H), 7.72 (dd, 1H, J_1 = 2.55 Hz, J_2 = 2.40 Hz, Py-4-H), 7.34 (d, 1H, J = 8.25 Hz, Py-5-H), 7.28 (d, 2H, J = 8.25 Hz, Ar-2,6-H), 7.20 (d, 2H, J = 8.70 Hz, Ar′-3,5-H), 6.83 (d, 2H, J = 8.75 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.17–4.12 (m, 1H, Ar—CH—), 3.82 (d, 1H, J = 9.55 Hz, —CO_2—CH—), 3.73 (s, 3H, —CO_2—CH_3), 3.52 (s, 3H, —CO_2—CH_3), 3.48 (dd, 1H, J_1 = 4.85 Hz, J_2 = 4.80 Hz, —CO—CH—), 3.41 (dd, 1H, $J_1 = J_2$ = 9.15 Hz, —CO—CH—), 2.99–2.90 (m, 1H, Ar′—CH—), 1.25 (d, 6H, J = 6.90 Hz, Ar′—CH—$(CH_3)_2$); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.1, 168.7, 168.2, 157.1, 154.7, 151.1, 148.7, 138.2, 134.6, 133.4, 131.5, 129.3, 128.3, 126.7, 124.3, 114.6, 66.6, 57.4, 52.7, 52.5, 42.3, 40.1, 34.2, 23.7; MS (ESI) m/z: 546.3 ($[M + Na]^+$). Anal. Calcd for $C_{29}H_{30}ClNO_6$: C, 66.47; H, 5.77; N, 2.67. Found: C, 66.56; H, 5.77; N, 2.78.

*2.2.2.12. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-phenylpropyl)malonate (**5l**).* Yield, 71%; white solid; m.p. 82–84 °C; IR (KBr, cm^{-1}): ν 2874–3065 (C—H), 1740 (—O—C═O), 1676 (Ar′—C═O), 1458–1616 (C═C and benzene and Py-ring), 1292 (C—N), 1262 (Ar—C—O), 1150 (—CH_2—O—C═O), 824 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.41 (d, 1H, J = 2.15 Hz, Py-2-H), 7.90 (d, 2H, J = 7.75 Hz, Ar′-2,6-H), 7.72 (dd, 1H, J_1 = 2.40 Hz, J_2 = 2.30 Hz, Py-4-H), 7.54 (t, 1H, J_1 = 7.35 Hz, J_2 = 7.45 Hz, Ar′-4-H), 7.43 (t, 2H, J_1 = 7.65 Hz, J_2 = 7.70 Hz, Ar′-3,5-H), 7.34 (d, 1H, J = 8.20 Hz, Py-5-H), 7.20 (d, 2H, J = 8.60 Hz, Ar-2,6-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.26–4.17 (m, 2H, —CO_2—CH_2—), 4.16–4.11 (m, 1H, Ar—CH—), 3.97 (q, 2H, —CO_2—CH_2—), 3.78 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.52 (dd, 1H, J_1 = 4.30 Hz, J_2 = 4.20 Hz, —CO—CH—), 3.41 (dd, 1H, J_1 = 9.60 Hz, J_2 = 9.55 Hz, —CO—CH—), 1.25 (t, 3H, J_1 = 7.25 Hz, J_2 = 7.00 Hz, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.25 Hz, J_2 = 7.00 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.6, 168.3, 167.7, 157.1, 151.1, 148.7, 138.1, 136.7, 133.3, 133.1, 131.5, 129.5, 128.6, 128.1, 124.2, 114.6 66.6, 61.7, 61.4, 57.7, 42.8, 40.1, 14.1, 13.8; MS (ESI) m/z: 532.4 ($[M + Na]^+$). Anal. Calcd for $C_{28}H_{28}ClNO_6$: C, 65.94; H, 5.53; N, 2.75. Found: C, 65.87; H, 5.93; N, 2.81.

*2.2.2.13. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(o-tolyl)propyl)malonate (**5m**).* Yield, 50%; white solid; m.p. 52–54 °C; IR (KBr, cm^{-1}) ν: 2849–3065 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–1616 (C═C and benzene and Py-ring), 1319 (C—N), 1244 (Ar—C—O), 1179 (—CH_2—O—C═O), 828 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.41 (d, 1H, J = 2.25 Hz, Py-2-H), 7.70 (dd, 1H, $J_1 = J_2$ = 2.40 Hz, Py-4-H), 7.54 (d, 1H, J = 7.80 Hz, Ar′-6-H), 7.33–7.30 (m, 2H, Ar′-4-H, Py-5-H), 7.22 (t, 1H, J_1 = 7.55 Hz, J_2 = 7.45 Hz, Ar′-5-H), 7.15–7.12 (m, 3H, Ar′-3-H, Ar-2,6-H), 6.82 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.26–4.17 (m, 2H, —CO_2—CH_2—), 4.05–4.00 (m, 1H, Ar—CH—), 3.95 (q, 2H, —CO_2—CH_2—), 3.73 (d, 1H, J = 9.90 Hz, —CO_2—CH—), 3.48 (dd, 1H, J_1 = 4.35 Hz, J_2 = 4.30 Hz, —CO—CH—), 3.26 (dd, 1H, $J_1 = J_2$ = 10.20 Hz, —CO—CH—), 2.15 (s, 3H, Ar′—CH_3), 1.26 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.10 Hz, —CO_2—CH_2—CH_3), 1.01 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.10 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 202.1, 168.3, 167.7, 157.2, 151.1, 148.8, 138.2, 138.0, 137.9, 133.1, 131.8, 131.7, 131.2, 129.7, 128.3, 125.7, 124.3, 114.8, 66.7, 61.7, 61.4, 57.8, 45.8, 40.6, 20.7, 14.1, 13.9; MS (ESI) m/z: 546.4 ($[M + Na]^+$). Anal. Calcd for $C_{29}H_{30}ClNO_6$: C, 66.47; H, 5.77; N, 2.67. Found: C, 66.39; H, 6.08; N, 2.83.

*2.2.2.14. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(m-tolyl)propyl)malonate (**5n**).* Yield, 52%; white solid; m.p. 61–63 °C; IR (KBr, cm^{-1}): ν 2928–3065 (C—H), 1740 (—O—C═O), 1674 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1265 (C—N), 1242 (Ar—C—O), 1150 (—CH_2—O—C═O), 822 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.40 (d, 1H, J = 2.05 Hz, Py-2-H), 7.71–7.70 (m, 3H, Py-4-H, Ar′-2,6-H), 7.34–7.28 (m, 3H, Ar′-4,5-H, Py-5-H), 7.22 (d, 2H, J = 8.60 Hz, Ar-2,6-H), 6.83 (d, 2H, J = 8.70 Hz, Ar-3,5-H), 4.97 (s, 2H, Py—CH_2—), 4.26–4.13 (m, 3H, —CO_2—CH_2—, Ar—CH—), 3.97 (q, 2H, —CO_2—CH_2—), 3.80 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.50 (dd, 1H, J_1 = 4.45 Hz, J_2 = 4.35 Hz, —CO—CH—), 3.43 (dd, 1H, $J_1 = J_2$ = 9.35 Hz, —CO—CH—), 2.36 (s, 3H, Ar′—CH_3), 1.25 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.10 Hz, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.25 Hz, J_2 = 7.00 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.8, 168.4, 167.9, 157.1, 151.1, 148.8, 138.4, 138.2, 136.8, 134.0, 133.6, 131.7, 129.6, 128.7, 128.5, 125.4, 124.3, 114.7, 66.6, 61.8, 61.4, 57.7, 42.9, 40.1, 21.4, 14.1, 13.9; MS (ESI) m/z: 546.4 ($[M + Na]^+$). Anal. Calcd for $C_{29}H_{30}ClNO_6$: C, 66.47; H, 5.77; N, 2.67. Found: C, 66.26; H, 6.10; N, 2.72.

*2.2.2.15. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxo-3-(p-tolyl)propyl)malonate (**5o**).* Yield, 44%; white solid; m.p. 87–89 °C; IR (KBr, cm^{-1}): ν 2851–3055 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1289 (C—N), 1244 (Ar—C—O), 1179 (—CH_2—O—C═O), 829 (C—Cl); ^{1}H NMR

(500 MHz, $CDCl_3$, ppm): δ 8.38 (d, 1H, J = 2.20 Hz, Py-2-H), 7.79 (d, 2H, J = 8.15 Hz, Ar′-2,6-H), 7.68 (dd, 1H, $J_1 = J_2$ = 2.35 Hz, Py-4-H), 7.29 (d, 1H, J = 8.15 Hz, Py-5-H), 7.21–7.20 (m, 4H, Ar-2,6-H, Ar′-3,5-H), 6.82 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.95 (s, 2H, Py—CH_2—), 4.25–4.12 (m, 3H, —CO_2—CH_2—, Ar—CH—), 3.95 (q, 2H, —CO_2—CH_2—), 3.80 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.49 (dd, 1H, $J_1 = J_2$ = 4.40 Hz, —CO—CH—), 3.39 (dd, 1H, $J_1 = J_2$ = 9.50 Hz, —CO—CH—), 2.36 (s, 3H, Ar′—CH_3), 1.24 (t, 3H, J_1 = 7.05 Hz, J_2 = 7.15 Hz, —CO_2—CH_2—CH_3), 1.02 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.05 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.3, 168.4, 167.8, 157.2, 151.0, 148.7, 143.9, 138.2, 134.4, 133.6, 131.8, 129.6, 129.3, 128.3, 124.2, 114.7, 66.7, 61.7, 61.4, 57.8, 42.7, 40.3, 21.6, 14.1, 13.9; MS (ESI) m/z: 546.4 ($[M + Na]^+$). Anal. Calcd for $C_{29}H_{30}ClNO_6$: C, 66.47; H, 5.77; N, 2.67. Found: C, 66.18; H, 5.84; N, 2.74.

*2.2.2.16. Diethyl 2-(3-(2-chlorophenyl)-1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxopropyl)malonate (**5p**).* Yield, 60%; white solid; m.p. 63–65 °C; IR (KBr, cm^{-1}): ν 2911–3050 (C—H), 1726 (—O—C═O), 1680 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1294 (C—N), 1236 (Ar—C—O), 1155 (–CH_2–O—C═O), 829 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.43 (d, 1H, J = 2.15 Hz, Py-2-H), 7.73 (dd, 1H, J_1 = 2.30 Hz, J_2 = 2.35 Hz, Py-4-H), 7.36–7.31 (m, 3H, Ar′-4,6-H, Py-5-H), 7.24–7.21 (m, 1H, Ar′-5-H), 7.18–7.15 (m, 3H, Ar′-3-H, Ar-2,6-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 5.01 (s, 2H, Py—CH_2–), 4.24–4.18 (m, 2H, —CO_2—CH_2—), 4.06–4.01 (m, 1H, Ar—CH—), 3.96 (q, 2H, —CO_2—CH_2—), 3.72 (d, 1H, J = 9.85 Hz, —CO_2—CH—), 3.50 (dd, 1H, J_1 = 4.45 Hz, J_2 = 4.40 Hz, —CO—CH—), 3.41 (dd, 1H, $J_1 = J_2$ = 9.90 Hz, —CO—CH—), 1.26 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.05 Hz, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.10 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 200.9, 168.2, 167.7, 157.2, 151.2, 148.8, 139.2, 138.2, 133.0, 131.8, 131.6, 130.8, 130.5, 129.8, 129.0, 126.9, 124.3, 114.7, 66.7, 61.8, 61.5, 57.7, 46.9, 40.2, 14.2, 13.9; MS (ESI) m/z: 566.4 ($[M + Na]^+$). Anal. Calcd for $C_{28}H_{27}Cl_2NO_6$: C, 61.77; H, 5.00; N, 2.57. Found: C, 61.36; H, 5.36; N, 2.69.

*2.2.2.17. Diethyl 2-(3-(3-chlorophenyl)-1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxopropyl)malonate (**5q**).* Yield, 53%; white solid; m.p. 59–61 °C; IR (KBr, cm^{-1}): ν 2905–3065 (C—H), 1740 (—O—C═O), 1684 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1294 (C—N), 1256 (Ar—C—O), 1150 (—CH_2—O—C═O), 824 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.42 (d, 1H, J = 2.20 Hz, Py-2-H), 7.84 (t, 1H, J_1 = 1.65 Hz, J_2 = 1.70 Hz, Ar′-2-H), 7.79 (d, 1H, J = 7.90 Hz, Ar′-6-H), 7.71 (dd, 1H, J_1 = 2.40 Hz, J_2 = 2.35 Hz, Py-4-H), 7.52–7.50 (m, 1H, Ar′-4-H), 7.39–7.34 (m, 2H, Ar′-5-H, Py-5-H), 7.19 (d, 2H, J = 8.65 Hz, Ar-2,6-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.99 (s, 2H, Py—CH_2—), 4.25–4.17 (m, 2H, —CO_2—CH_2—), 4.13–4.09 (m, 1H, Ar—CH—), 3.97 (q, 2H, —CO_2—CH_2—), 3.77 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.50 (dd, 1H, J_1 = 4.25 Hz, J_2 = 4.30 Hz, —CO—CH—), 3.38 (dd, 1H, J_1 = 9.55 Hz, J_2 = 9.45 Hz, —CO—CH—), 1.26 (t, 3H, J_1 = 7.25 Hz, J_2 = 7.00 Hz, —CO_2—CH_2—CH_3), 1.04 (t, 3H, $J_1 = J_2$ = 7.10 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.5, 168.4, 167.8, 157.2, 151.0, 148.7, 138.3, 138.3, 134.9, 133.2, 133.1, 131.7, 130.1, 129.5, 128.2, 126.3, 124.4, 114.7, 66.6, 61.9, 61.5, 57.6, 43.0, 40.1, 14.1, 13.9; MS (ESI) m/z: 566.3 ($[M + Na]^+$). Anal. Calcd for $C_{28}H_{27}Cl_2NO_6$: C, 61.77; H, 5.00; N, 2.57. Found: C, 61.39; H, 5.16; N, 2.73.

*2.2.2.18. Diethyl 2-(3-(4-chlorophenyl)-1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-oxopropyl)malonate (**5r**).* Yield, 50%; white solid; m.p. 67–69 °C; IR (KBr, cm^{-1}): ν 2911–3050 (C—H), 1726 (—O—C═O), 1680 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1294 (C—N), 1236 (Ar—C—O), 1159 (—CH_2—O—C═O), 824 (C-Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.40 (d, 1H, J = 2.20 Hz, Py-2-H), 7.84 (d, 2H, J = 8.55 Hz, Ar′-2,6-H),7.71 (dd, 1H, J_1 = 2.45 Hz, J_2 = 2.30 Hz, Py-4-H), 7.40 (d, 2H, J = 8.50 Hz, Ar′-3,5-H), 7.34 (d, 1H, J = 8.25 Hz, Py-5-H), 7.18 (d, 2H, J = 8.70 Hz, Ar-2,6-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.26–4.15 (m, 2H, —CO_2—CH_2—), 4.12–4.07 (m, 1H, Ar—CH—), 3.97 (q, 2H, —CO_2—CH_2—), 3.77 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.51 (dd, 1H, J_1 = 4.20 Hz, J_2 = 4.25 Hz, —CO—CH—), 3.35 (dd, 1H, J_1 = 9.75 Hz, J_2 = 9.65 Hz, —CO—CH—), 1.25 (t, 3H, J_1 = 7.25 Hz, J_2 = 7.00 Hz, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.05 Hz, J_2 = 7.15 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.5, 168.3, 167.7, 157.1, 151.1, 148.7, 139.5, 138.1, 135.0, 133.1, 131.5, 129.6, 129.4, 128.9, 124.2, 114.6, 66.6, 61.7, 61.4, 57.6, 42.8, 40.2, 14.0, 13.8; MS (ESI) m/z: 566.3 ($[M + Na]^+$). Anal. Calcd for $C_{28}H_{27}Cl_2NO_6$: C, 61.77; H, 5.00; N, 2.57. Found: C, 61.52; H, 5.12; N, 2.64.

*2.2.2.19. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(2,4-dichlorophenyl)-3-oxopropyl)malonate (**5s**).* Yield, 41%; white solid; m.p. 71–73 °C; IR (KBr, cm^{-1}): ν 2849–3088 (C—H), 1734 (—O—C═O), 1684 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1300 (C—N), 1244 (Ar—C—O), 1153 (—CH_2—O—C═O), 826 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.43 (d, 1H, J = 2.25 Hz, Py-2-H), 7.73 (dd, 1H, J_1 = 2.40 Hz, J_2 = 2.50 Hz, Py-4-H), 7.37–7.34 (m, 2H, Ar′-6-H, Py-5-H), 7.22 (dd, 1H, J_1 = 2.05 Hz, J_2 = 1.75 Hz, Ar′-3-H), 7.17–7.14 (m, 3H, Ar′-5-H, Ar-2,6-H), 6.83 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 5.00 (s, 2H, Py—CH_2—), 4.23–4.19 (m, 2H, —CO_2—CH_2—), 4.03–3.94 (m, 3H, Ar—CH—, —CO_2—CH_2—), 3.71 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.49 (dd, 1H, $J_1 = J_2$ = 4.40 Hz, —CO—CH—), 3.38 (dd, 1H, J_1 = 10.05 Hz, J_2 = 9.95 Hz, —CO—CH—), 1.26 (t, 3H, J_1 = 7.05 Hz, J_2 = 7.20 Hz, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.00 Hz, J_2 = 7.25 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 199.6, 168.2, 167.6, 157.3, 151.2, 148.8, 138.1, 137.3, 132.9, 132.0, 131.6, 130.3, 129.7, 127.3, 124.3, 114.8, 66.7, 61.8, 61.5, 57.6, 46.8, 40.3, 14.1, 13.9; MS (ESI) m/z: 600.3 ($[M + Na]^+$). Anal. Calcd for $C_{28}H_{26}Cl_3NO_6$: C, 58.10; H, 4.53; N, 2.42. Found: C, 57.86; H, 4.84; N, 2.33.

*2.2.2.20. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(4-ethoxyphenyl)-3-oxopropyl)malonate (**5t**).* Yield, 40%; white solid; m.p. 88–90 °C; IR (KBr, cm^{-1}): ν 2913–2986 (C—H), 1738 (—O—C═O), 1667 (Ar′—C═O), 1458–1603 (C═C and benzene and Py-ring), 1308 (C—N), 1254 (Ar—C—O), 1179 (—CH_2—O—Ar′), 1150 (—CH_2—O—C═O), 822 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.41 (d,

1H, J = 2.00 Hz, Py-2-H), 7.88 (d, 2H, J = 8.80 Hz, Ar′-2,6-H), 7.71 (dd, 1H, J_1 = 2.30 Hz, J_2 = 2.50 Hz, Py-4-H), 7.34 (d, 1H, J = 8.20 Hz, Py-5-H), 7.19 (d, 2H, J = 8.60 Hz, Ar-2,6-H), 6.88 (d, 2H, J = 8.95 Hz, Ar′-3,5-H), 6.82 (d, 2H, J = 8.65 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.25–4.06 (m, 5H, —CO_2—CH_2—, Ar—CH—, Ar′—OCH_2—), 3.96 (q, 2H, —CO_2—CH_2—), 3.77 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.45 (dd, 1H, J_1 = 4.40 Hz, J_2 = 4.25 Hz, —CO—CH—), 3.33 (dd, 1H, J_1 = 9.65 Hz, J_2 = 9.70 Hz, —CO—CH—), 1.43 (t, 3H, J_1 = 6.90 Hz, J_2 = 6.95 Hz, Ar′—OCH_2—CH_3), 1.25 (t, 3H, J_1 = 7.00 Hz, J_2 = 7.20 Hz, —CO_2—CH_2—CH_3), 1.02 (t, 3H, J_1 = 7.20 Hz, J_2 = 7.10 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.1, 168.4, 167.8, 162.9, 157.0, 151.1, 148.7, 138.1, 133.4, 131.6, 130.4, 129.6, 129.5, 124.2, 114.5, 114.1, 66.6, 63.7, 61.7, 61.3, 57.7, 42.4, 40.3, 14.7, 14.1, 13.8; MS (ESI) m/z: 576.4 ([M + Na]$^+$). Anal. Calcd for $C_{30}H_{32}ClNO_7$: C, 65.04; H, 5.82; N, 2.53. Found: C, 65.24; H, 5.50; N, 3.00.

*2.2.2.21. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(4-fluorophenyl)-3-oxopropyl)malonate (**5u**).* Yield, 52%; white solid; m.p. 65–67 °C; IR (KBr, cm^{-1}): ν 2932–3065 (C—H), 1740 (—O—C═O), 1684 (Ar′—C═O), 1458–1599 (C═C and benzene and Py-ring), 1314 (C—N), 1260 (C—F), 1236 (Ar—C—O), 1159 (—CH_2—O—C═O), 824 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.40 (d, 1H, J = 2.00 Hz, Py-2-H), 7.94–7.91 (m, 2H, Ar′-2,6-H), 7.70 (dd, 1H, J_1 = 2.30 Hz, J_2 = 2.35 Hz, Py-4-H), 7.32 (d, 1H, J = 8.20 Hz, Py-5-H), 7.19 (d, 2H, J = 8.60 Hz, Ar-2,6-H), 7.09 (t, 2H, J_1 = 8.55 Hz, J_2 = 8.60 Hz, Ar′-3,5-H), 6.83 (d, 2H, J = 8.60 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.26–4.15 (m, 2H, —CO_2—CH_2—), 4.13–4.09 (m, 1H, Ar—CH—), 3.97 (q, 2H, —CO_2—CH_2—), 3.78 (d, 1H, J = 9.65 Hz, —CO_2—CH—), 3.51 (dd, 1H, J_1 = 4.35 Hz, J_2 = 4.20 Hz, —CO—CH—), 3.37 (dd, 1H, J = 9.6 Hz, —CO—CH—), 1.25 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.05 Hz, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.05 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 196.2, 168.4, 167.8, 166.8, 164.8, 157.2, 151.2, 148.8, 138.2, 135.1, 133.3, 131.7, 130.9, 130.8, 129.5, 124.3, 115.8, 115.6, 114.8, 66.7, 61.8, 61.4, 57.7, 42.8, 40.3, 14.1, 13.9; ^{19}F NMR (470 MHz, $CDCl_3$, ppm): δ −105.10; MS (ESI) m/z: 550.3 ([M + Na]$^+$). Anal. Calcd for $C_{28}H_{27}ClFNO_6$: C, 63.70; H, 5.15; N, 2.65. Found: C, 63.33; H, 5.34; N, 2.70.

*2.2.2.22. Diethyl 2-(1-(4-((6-chloropyridin-3-yl)methoxy)phenyl)-3-(4-isopropylphenyl)-3-oxopropyl)malonate (**5v**).* Yield, 45%; white solid; m.p. 80–82 °C; IR (KBr, cm^{-1}): ν 2874–2974 (C—H), 1728 (—O—C═O), 1676 (Ar′—C═O), 1458–1609 (C═C and benzene and Py-ring), 1292 (C—N), 1238 (Ar—C—O), 1157 (—CH_2—O—C═O), 824 (C—Cl); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 8.41 (d, 1H, J = 2.30 Hz, Py-2-H), 7.84 (d, 2H, J = 8.25 Hz, Ar′-2,6-H), 7.71 (dd, 1H, J_1 = 2.50 Hz, J_2 = 2.40 Hz, Py-4-H), 7.34 (d, 1H, J = 8.25 Hz, Py-5-H), 7.28 (d, 2H, J = 8.40 Hz, Ar-2,6-H), 7.20 (d, 2H, J = 8.65 Hz, Ar′-3,5-H), 6.82 (d, 2H, J = 8.75 Hz, Ar-3,5-H), 4.98 (s, 2H, Py—CH_2—), 4.24–4.11 (m, 3H, —CO_2—CH_2—, Ar—CH—), 3.96 (q, 2H, —CO_2—CH_2—), 3.78 (d, 1H, J = 9.75 Hz, —CO_2—CH—), 3.48 (dd, 1H, J_1 = J_2 = 4.30 Hz, —CO—CH—), 3.39 (dd, 1H, J_1 = J_2 = 9.60 Hz, —CO—CH—), 2.97–2.92 (m, 1H, Ar′—CH—), 1.26–1.24 (m, 9H, Ar′—CH—$(CH_3)_2$, —CO_2—CH_2—CH_3), 1.03 (t, 3H, J_1 = 7.15 Hz, J_2 = 7.05 Hz, —CO_2—CH_2—CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 197.2, 168.4, 167.8, 157.0, 154.6, 151.1, 148.7, 138.1, 134.6, 133.5, 131.6, 129.5, 128.4, 126.7, 124.2, 114.6, 66.6, 61.7, 61.4, 57.7, 42.6, 40.1, 34.2, 23.7, 14.1, 13.8; MS (ESI) m/z: 574.4 ([M + Na]$^+$). Anal. Calcd for $C_{31}H_{34}ClNO_6$: C, 67.44; H, 6.21; N, 2.54. Found: C, 67.25; H, 6.10; N, 2.61.

2.3. Antiviral activities against CMV

2.3.1. Purification of CMV

Nicotiana tabacum L. leaves inoculated with CMV were selected, ground in phosphate butter, and then filtered through a double-layer pledget by using the method of Zhou et al. (1995). The filtrate was centrifuged at 8000g, and the supernatant liquid was collected as the crude extract of the virus. All of the experiments were performed at 4 °C.

The virus concentration was calculated as

$$\text{Virus concentration (mg/mL)} = (A_{260} \times \text{dilution ratio})/E_{1\text{ cm}}^{0.1\%\ 260\text{ nm}}$$

where E represents the extinction coefficient of TMV and $E_{1\text{ cm}}^{0.1\%\ 260\text{ nm}}$ is 3.1.

2.3.2. Curative activities of the title compounds against CMV in vivo

Leaves of the same age were gathered from *Chenopodium amaranticolor*. Crude CMV (6×10^{-3} mg/mL) was inoculated using a brush on whole leaves on which silicon carbide had previously been scattered. After 0.5–1 h, the leaves were washed with water and dried. The compound solution was then smeared on the right side of leaves, and solvent was smeared on the left side as a control. The number of local lesions that developed was recorded 3–4 d after inoculation. All compounds were tested with three repetitions to ensure accuracy in the results.

2.3.3. Protection activities of the title compounds against CMV in vivo

Leaves of *C. amaranticolor* of the same age were selected. The compound solution was smeared on the right side of the leaves. Meanwhile, the solvent was smeared on the left side as control. After 12 h, crude CMV, at the concentration of 6×10^{-3} mg/mL, was inoculated with a brush on whole leaves, which had previously been scattered with silicon carbide. After 0.5–1 h, the leaves were washed with water and dried. The number of local lesions was then recorded 3–4 d after inoculation. All compounds were tested with three repetitions to ensure veracity of the results.

2.3.4. Inactivation activities of the title compounds against CMV in vivo

The virus was inhibited by mixing with the compound solution at the same volume for 0.5 h. The right side of *C. amaranticolor* leaves was then inoculated with the mixture and CMV diluted to suitable concentration (6×10^{-3} mg/mL) was inoculated on the left side as control, of leaves that had previously been scattered with silicon carbide. After 0.5–1 h, the leaves were washed with water and dried. The number of local lesions was recorded 3–4 d after inoculation. All compounds were tested with three repetitions to ensure veracity of the results.

Table 1 Selection of conditions for the reaction of the target compound **5l**.

Entry	Solvent	Catalyst	Molar equivalent (**4a**:diethyl malonate:catalyst)	T (°C)	t (h)	Yield (%)
1	EtOH	KOH	1:1:0.5	80	4	37.4
2	EtOH	KOH	1:1:1	80	2	44.9
3	EtOH	KOH	1:2:1	80	1	61.0
4	EtOH	KOH	1:5:1	80	0.5	71.2
5	EtOH	KOH	1:5:1	20	12	43.4
6	EtOH	KOH	1:5:1	40	8	56.7
7	EtOH	KOH	1:5:1	60	3	62.2
8	THF	KOH	1:5:1	65	3	–[a]
9	EtOH	NaOH	1:5:1	80	1	68.0
10	EtOH	KOH	1:5:1	80	3	10.6

[a] No reaction.

The inhibitory rates of the target compounds were calculated according to the following formula ("av" means average):

Inhibition rate (%) = [(av local lesion no of control (not treated with compound solution) − av local lesion no smeared with drugs)/av local lesion no of control (not treated with compound solution)] × 100%.

2.4. 3D-QSAR analysis

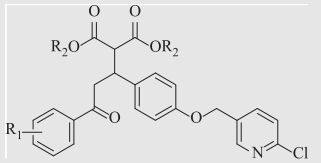
2.4.1. Datasets for 3D-QSAR analysis

The data of 22 target compounds were obtained, and biological activity was expressed as pEC_{50}. A total of 16 compounds (labeled with asterisks) were randomly chosen as the training set for CoMFA (Cramer et al., 1988), and the remaining 6 compounds were used as the testing set.

2.4.2. Molecular modeling and alignment

Molecular modeling and CoMFA analysis were performed using SYBYL 7.3 (Tripos Inc., St. Louis, MO, USA) software. The 3D structures of all molecules were built using the Sketch Molecule function in SYBYL. Initial optimization of the structures was conducted using the Gasteiger–Hückel charge, Tripos force field, and Powell conjugate gradient algorithm with a convergence criterion of 0.005 kcal/mol Å

Table 2 Antiviral activities of the target compounds against CMV *in vivo* at 500 μg/mL.

Compd.			Curative activity (%)[a]	Protection activity (%)[a]	Inactivation activity (%)[a]
	R_1	R_2			
5a	H	$-CH_3$	31.0 ± 2.4	28.6 ± 2.1	78.5 ± 1.7
5b	2-CH_3	$-CH_3$	29.1 ± 1.7	40.0 ± 2.1	82.3 ± 1.7
5c	3-CH_3	$-CH_3$	39.1 ± 2.0	25.8 ± 1.9	79.1 ± 1.7
5d	4-CH_3	$-CH_3$	53.8 ± 1.6	26.0 ± 2.1	78.3 ± 1.8
5e	2-Cl	$-CH_3$	57.5 ± 1.1	12.7 ± 3.3	75.8 ± 1.1
5f	3-Cl	$-CH_3$	48.0 ± 2.1	37.7 ± 2.1	81.1 ± 1.6
5g	4-Cl	$-CH_3$	29.2 ± 1.4	24.6 ± 1.5	69.1 ± 2.1
5h	2,4-di-Cl	$-CH_3$	26.7 ± 2.0	19.5 ± 1.3	67.2 ± 2.4
5i	4-OC_2H_5	$-CH_3$	28.9 ± 0.5	42.7 ± 2.6	74.9 ± 1.7
5j	4-F	$-CH_3$	25.4 ± 0.9	38.2 ± 2.0	68.9 ± 1.5
5k	4-*i*-Pr	$-CH_3$	38.9 ± 0.8	43.7 ± 2.1	67.2 ± 1.6
5l	H	$-C_2H_5$	69.8 ± 1.8	39.1 ± 2.6	88.1 ± 1.5
5m	2-CH_3	$-C_2H_5$	55.5 ± 1.6	37.1 ± 2.6	84.4 ± 1.9
5n	3-CH_3	$-C_2H_5$	65.0 ± 3.9	54.9 ± 1.4	88.6 ± 1.2
5o	4-CH_3	$-C_2H_5$	54.1 ± 0.7	24.2 ± 1.5	83.3 ± 1.5
5p	2-Cl	$-C_2H_5$	46.6 ± 1.3	50.1 ± 1.2	73.0 ± 2.4
5q	3-Cl	$-C_2H_5$	56.4 ± 1.8	41.2 ± 2.7	86.2 ± 1.8
5r	4-Cl	$-C_2H_5$	31.4 ± 1.8	29.5 ± 1.0	75.2 ± 2.1
5s	2,4-di-Cl	$-C_2H_5$	33.3 ± 0.9	22.1 ± 1.0	72.3 ± 2.1
5t	4-OC_2H_5	$-C_2H_5$	33.3 ± 0.9	46.9 ± 1.7	70.0 ± 2.3
5u	4-F	$-C_2H_5$	34.6 ± 1.4	39.3 ± 0.8	78.6 ± 1.2
5v	4-*i*-Pr	$-C_2H_5$	50.4 ± 2.2	28.0 ± 2.1	64.8 ± 2.5
Ningnanmycin[b]			56.9 ± 0.9	58.3 ± 0.8	92.2 ± 0.9

[a] Average of three replicates.
[b] The commercial antiviral product ningnanmycin was used for activity comparison.

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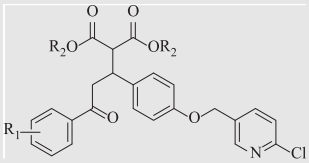
Table 3 EC_{50} and pEC_{50} values of the target compounds against CMV *in vivo*.

Compd.			EC_{50} (μg/mL)[a]	pEC_{50} (μM)
	R_1	R_2		
5a	H	CH_3	1262.79 ± 4.7	2.581
5b	2-CH_3	CH_3	1282.19 ± 2.9	2.587
5c	3-CH_3	CH_3	792.25 ± 3.2	2.796
5d	4-CH_3	CH_3	430.46 ± 1.9	3.061
5e	2-Cl	CH_3	328.97 ± 2.1	3.196
5f	3-Cl	CH_3	495.48 ± 1.3	3.017
5g	4-Cl	CH_3	1251.87 ± 2.5	2.614
5h	2,4-di-Cl	CH_3	1728.63 ± 3.1	2.502
5i	4-OC_2H_5	CH_3	1301.58 ± 4.3	2.606
5j	4-F	CH_3	2066.61 ± 2.6	2.383
5k	4-*i*-Pr	CH_3	803.08 ± 3.4	2.814
5l	H	C_2H_5	186.17 ± 3.2	3.437
5m	2-CH_3	C_2H_5	400.68 ± 2.5	3.116
5n	3-CH_3	C_2H_5	211.47 ± 3.5	3.393
5o	4-CH_3	C_2H_5	426.93 ± 3.1	3.088
5p	2-Cl	C_2H_5	548.26 ± 2.8	2.996
5q	3-Cl	C_2H_5	338.09 ± 2.7	3.206
5r	4-Cl	C_2H_5	1213.43 ± 3.8	2.651
5s	2,4-di-Cl	C_2H_5	1132.99 ± 2.3	2.707
5t	4-OC_2H_5	C_2H_5	1053.92 ± 3.6	2.720
5u	4-F	C_2H_5	942.08 ± 3.7	2.748
5v	4-*i*-Pr	C_2H_5	466.88 ± 4.6	3.072
Ningnanmycin[b]			330.52 ± 1.7	

[a] Average of three replicates.
[b] Commercial, agricultural, and antiviral ningnanmycin products were used for activity comparison.

(Huang et al., 2011). The 3D structures of the 22 molecules were aligned to the common template molecule of **5l** to reveal the best curative activity against CMV in the CoMFA model study.

2.4.3. Partial least-squares analysis

3D-QSAR was derived using partial least square (PLS) analysis wherein molecules were placed in a rectangular grid, and interaction energies between a probe atom and all compounds were computed at surrounding points by using a volume-dependent lattice with a 2.0 grid spacing (default in SYBYL) to improve the signal-to-noise ratio (Elizabeth and William, 2006). CoMFA descriptors were used as independent variables, and experimental pEC_{50} values were presented as the dependent variables. 3D-QSAR analysis was then conducted in two steps by using the PLS technique. First, the performance of the models was evaluated by leave-one-out cross-validation, and the optimal number of components (ONC) was determined with the highest cross-validated q^2 (Baroni et al., 1992). The non-cross-validated correlation coefficient r^2, standard error of estimate (SEE), and F were subsequently calculated according to the definitions in the SYBYL 7.3 package. Contour maps and standard deviations of CoMFA were generated by using PLS coefficients.

3. Results and discussion

3.1. Chemistry

High product yields, good molar equivalence, suitable reaction temperatures, and short reaction times encouraged us to apply previously reported methods under optimized reaction conditions with the aim of synthesizing title compounds. Thus, the reactions were carried out using the key intermediate **4a** and diethyl malonate as the starting materials and the synthesis of the target compound **5l** as the model reaction. The effects of different reaction solvents, catalysts, molar equivalents, and reaction temperatures on the yields and reaction times of the target compounds were determined. As shown in Table 1, the highest yield (71.2%) and shortest reaction time (0.5 h) were obtained when the reaction solvent, catalyst, molar equivalent, and reaction temperature were C_2H_5OH, KOH, 1:5:1, and 80 °C, respectively. Therefore, the target compounds **5a**–**5v** were synthesized under the following optimal conditions: solvent, C_2H_5OH (CH_3OH); catalyst, KOH; temperature, 80 °C; and molar equivalent of **4**: diethyl (dimethyl) malonate:catalyst, 1:5:1. The IR spectra of compounds **5a**–**5v** showed characteristic absorption bands at 2837–3088 cm^{-1}, which reveals the presence of C—H. The

Table 4 Experimental and predicted pEC_{50} results against CMV.

Compd.	Experimental[a]	Predicted[b]	Residual[c]
*5a	2.581	2.597	0.016
5b	2.587	2.584	−0.003
*5c	2.796	2.800	0.004
*5d	3.061	3.046	−0.015
*5e	3.196	3.161	−0.035
*5f	3.017	3.043	0.026
*5g	2.614	2.599	−0.015
5h	2.502	2.481	−0.021
*5i	2.606	2.611	0.005
*5j	2.383	2.399	0.016
*5k	2.814	2.796	−0.018
*5l	3.437	3.427	−0.010
*5m	3.116	3.124	0.008
5n	3.393	3.465	0.072
5o	3.088	3.338	0.250
*5p	2.996	3.053	0.057
5q	3.206	3.353	0.153
*5r	2.651	2.680	0.029
*5s	2.707	2.642	−0.065
*5t	2.720	2.723	0.003
*5u	2.748	2.741	−0.007
5x	3.072	3.185	0.113

[a] Experimental pEC_{50}.
[b] Predicted by CoMFA.
[c] Relative error of the experimentally predicted pEC_{50} (*b*–*a*).
* Samples of the training set.

stretching frequency at 1663–1749 cm^{-1} was assigned to C═O vibrations. Bands at 1150–1262 and 818–833 cm^{-1} revealed the respective stretching vibrations of C—O and C—Cl groups of the chalcone skeleton. ^1H NMR spectra showed one sharp peak appearing at 4.95–5.01 ppm, which indicates the presence of Py—CH_2—, and a doublet appearing at 3.72–3.84 ppm, which indicates the presence of —CH—$(CO_2R_2)_2$. Chemical shifts at approximately 197.6, 168.7, and 168.2 ppm in ^{13}C NMR spectra confirmed the presence of C═O; a chemical shift at approximately 66.6 ppm confirmed the presence of Py—CH_2—.

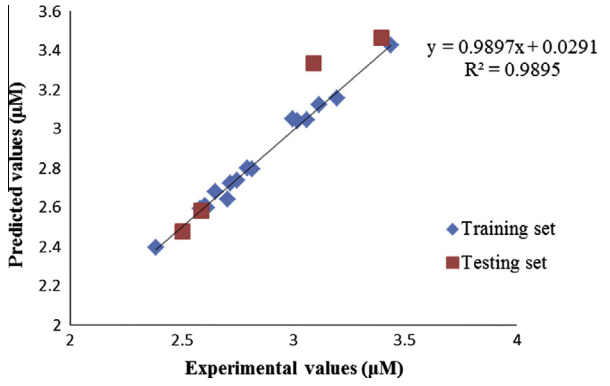


Figure 1 Graph of predicted pEC_{50} versus experimental pEC_{50} for the CoMFA model.

3.2. Antiviral activity

The antiviral activities of the target compounds **5a**–**5v** against CMV were assayed with ningnanmycin as the control. Table 2 shows that most of the title compounds present moderate to good antiviral activity against CMV *in vivo*. In particular, among the compounds tested, **5l** and **5n** exhibited excellent curative activities with inhibition rates as high as 69.8% and 65.0% at 500 μg/mL; these rates are better than that reported for ningnanmycin (56.9%). Compounds **5l** and **5n** showed increased CMV inactivation rates of 88.1% and 88.6%, respectively, which are nearly similar to that of ningnanmycin (92.2%). Compound **5n** also possessed good protective activity against CMV, with an inhibition rate of 54.9% at 500 μg/mL; this rate is only slightly lower than that of ningnanmycin (58.3%).

Using the results of preliminary bioassays as bases, the EC_{50} values of the title compounds were evaluated and are summarized in Table 3. Compounds **5l** and **5n** exhibited notable curative activities against CMV with EC_{50} values of 186.2 and 211.5 μg/mL, respectively; these values are even better than that of ningnanmycin (330.5 μg/mL).

3.3. Performance of the CoMFA model

A CoMFA model based on the experimental EC_{50} values of the training set was developed for the 3D-QSAR study. The predicted and experimental pEC_{50} values of compounds in both the training and testing sets are presented in Table 4, and correlations between the predicted and experimental pEC_{50} values in the CoMFA model are presented in Fig. 1. Overall, the predicted pEC_{50} values were very similar to the corresponding experimental values among compounds in both the training and testing sets (Table 4). The mostly linear correlation in Fig. 1 demonstrated the high predictive power of the developed model. The calculated statistical parameters of the CoMFA model are shown in Table 5. The cross-validated coefficient q^2, non-cross-validated correlation coefficient r^2, SEE, and F of the model were 0.517 (>0.5) with 6 ONC, 0.990, 0.036, and 143.396, respectively. The relative contributions of steric and electrostatic to the CoMFA model were 0.658 and 0.342, respectively; these values suggest that bioactivity is mainly determined by steric interactions.

Table 5 Statistical parameters for the CoMFA model.

Statistical parameter	CoMFA
q^2[a]	0.517
ONC[b]	6
r^2[c]	0.990
SEE[d]	0.036
F[e]	143.396
Fraction of field contributions[f]	
Steric	0.658
Electrostatic	0.342

[a] Cross-validated correlation.
[b] Optimum number of components.
[c] Noncross-validated correlation.
[d] Standard error of estimate.
[e] *F*-test value.
[f] Field contributions: steric and electrostatic.

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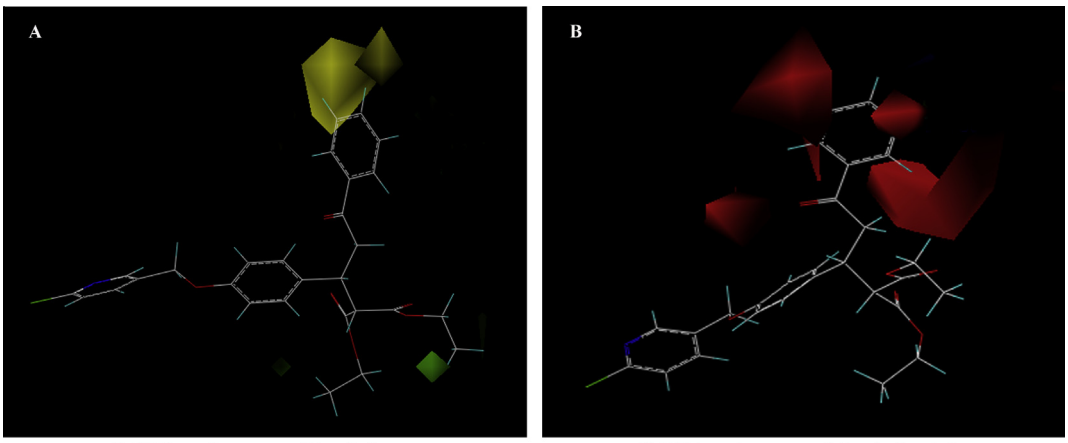

Figure 2 CoMFA contour maps of the steric (A) and electrostatic (B) fields.

CoMFA contour maps of the steric and electrostatic fields for antiviral activity against CMV are shown in Fig. 2. Green contours in the CoMFA steric field indicate regions where bulky groups increase activity, whereas yellow contours indicate regions where bulky groups decrease activity. Fig. 2A shows two large yellow contours around the benzene ring; this result indicates that bulky substituent groups at the position of R_1 are unfavorable. For example, a molecule with a Ph (i.e., **5l**) substituent showed better activity than that with a 4-OC_2H_5-Ph (**5t**) moiety at the R_1 position. A green contour was found near the R_2 substituent position, which suggests that bulky groups are favorable at this position. The results of the contour map agreed well with experimental data showing that replacement of methyl with ethyl at the R_2 substituent position results in increases in activity following the order of **5l** > **5a**, **5m** > **5b**, and **5n** > **5c**. An electrostatic contour map of the CoMFA model is displayed in Fig. 2B; here, blue contours indicate regions where electron-withdrawing groups would increase activity and red contours indicate regions where electron-donating groups would increase activity. As shown in Fig. 2B, several large regions of the red contours were observed near the phenyl ring, which indicates that electron-donating substituent groups at the position of the phenyl ring help increase the activity of the compounds. The contour maps agreed well with experimental data showing that replacement of —F with —H or —CH_3 at the R_1 substituent position on the phenyl ring results in increases in activity following the order of **5l** > **5u** and **5o** > **5u**.

4. Conclusion

In summary, novel chalcone derivatives containing malonate and pyridine moieties were prepared and evaluated in terms of their antiviral activities against CMV. The structures of all of the compounds were identified using spectral data (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, MS, and IR) and elemental analyses. Bioassay results showed that most of the compounds possess good antiviral activity against CMV *in vivo*; in particular, compounds **5l** and **5n** exhibited higher curative activities than ningnanmycin. The CoMFA model established based on the anti-CMV activities of the target compounds showed high predictive ability. The present work successfully demonstrated that chalcone derivatives containing malonate and pyridine moieties can effectively control CMV. Further studies on the structural optimization and modes of action of these compounds are ongoing in our laboratory.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2015.05.003.

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