JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

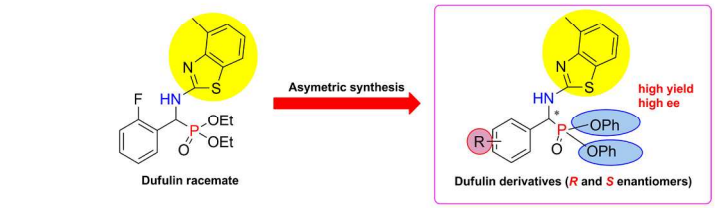
Asymmetric Synthesis and Bioselective Activities of #-Amino-phosphonates Based on Dufulin Motif

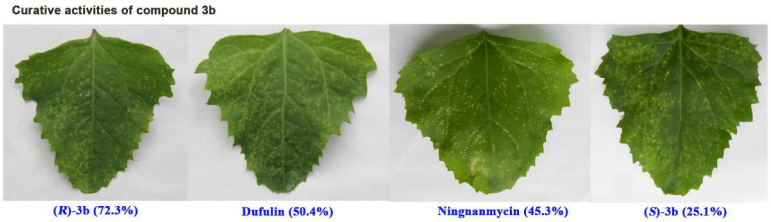
Guoping Zhang, Ge-Fei Hao, Jianke Pan, Jian Zhang, Deyu Hu, and Bao-An Song

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.6b01256 • Publication Date (Web): 10 May 2016

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036


Asymetric synthesis
high yield
high ee
Dufulin racemate
Dufulin derivatives (R and S enantiomers)


Curative activities of compound 3b
(R)-3b (72.3%)
Dufulin (50.4%)
Ningnanmycin (45.3%)
(S)-3b (25.1%)

Asymmetric Synthesis and Bioselective Activities of *α*-Amino-phosphonates Based on Dufulin Motif

Guoping Zhang,[†, ‡] Gefei Hao,[§] Jianke Pan,[†] Jian Zhang,[†] Deyu Hu,[†] and Baoan Song*[†]

[†]State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Huaxi District, Guiyang 550025, China

[‡]Department of Chemistry, Huaibei Normal University, Huaibei, Anhui 235000, China

[§]Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University, Wuhan430079, China

*Corresponding author (Tel.: 86-851-83620521; Fax: 86-851-83622211; E-mail: songbaoan22@yahoo.com)

ABSTRACT: The asymmetric synthesis of enantiomerically pure α-aminophosphonates with high and bioselective activities is a challenge. Here, we report that both enantiomers of α-aminophosphonates bearing *N*-benzothiazole motief can be prepared in high yields (up to 99%) and excellent enantioselectivities (up to 99% ee) by using chiral thiourea organocatalysts. Evaluation of the antiviral activities of our reaction products against cucumber mosaic virus (CMV) led to promising hits with high and selective biological activities, wherein (*R*)-enantiomers exhibit higher biological activities than the corresponding (*S*)-enantiomers. Especially, compound (*R*)-**3b** with excellent anti-CMV activity (curative activity, 72.3%; protection activity, 56.9%; inactivation activity, 96.9%) at 500 μg/mL emerged as a potential inhibitor of the plant virus. The difference in the selective bioactivity could be affected by the combination mode of the three-dimensional space between the enantiomers of α-aminophosphonate and cucumber mosaic virus coat protein (CMV-CP) via florescence spectroscopy and molecular docking.

KEYWORDS: asymmetric synthesis; α-aminophosphonate; bioselective activity; cucumber mosaic virus coat protein; florescence spectroscopy; molecular docking

INTRODUCTION

Chiral α-aminophosphonic derivatives show interesting bioactivities.[1-4] Therefore, the synthesis of enantiomerically enriched α-aminophosphonates has received considerable attention, with a number of catalytic enantioselective methods reported.[5-10] Among these methods, the most direct approach involves the addition of phosphites to imines. Unfortunately, in all these asymmetric methods, highly selectivities are generally restricted to imine substrates without *N*-heterocyclic moieties that are necessary for bioactivity.[11-14] In recent years, Dufulin (Figure 1) a pesticide product, which was developed by our group has been widely used to prevent and control rice, vegetable, and tobacco viral diseases in China.[15-16] To exploit highly effective antiviral chiral agents, asymmetric catalysis is seen as one of the economical strategies to satisfy the growing demand for enantiomerically pure novel α-aminophosphonates based on "dufulin" skeleton.

Chiral thioureas are privileged organic catalysts which were found effective in a large set of reactions such as Michael additions,[17-18] Mannich,[19-21] and Aldol,[22-23] Diels-Alder reactions,[24]etc. Thiourea derivatives can bind to the nitrogen or oxygen atom serving as catalysts. Notably, *N*-benzothiazole moiety in the design of synthetic compounds contains the nitrogen atom which easily forms hydrogen bonding of chiral thiourea. In addition, there are few studies in synthesizing highly enantiomerically pure α-aminphosphonates with *N*-benzothiazole that exhibit high selective activities.

In this study, chiral α-aminophosphonates based on the structure of Dufulin were synthesized by thiourea organic catalyst (Figure 1). The antiviral activities against cucumber mosaic virus (CMV) of both enantiomers from our catalytic reactions were

54 investigated. Meanwhile, The origins of bioselective activities were further investigated
55 via fluorescence spectroscopy analysis and molecular docking.

56 **MATERIALS AND METHODS**

57 **General Information.** All reactions were carried out in oven dried glassware with
58 magnetic stirring. Unless otherwise stated, all reagents were purchased from Aladdin
59 Chemicals Co. (Aladdin, Shanghai, China) and used without further purification. All
60 solvents used in the reactions were distilled from appropriate drying agents prior to use.
61 Silica gel GF_{254}-coated glass plates (Branch Qingdao Haiyang Chemical Co., Qingdao,
62 China) were used for thin layer chromatography under detection at 254 nm. Silica gel 200
63 ~ 300 mesh (Branch Qingdao Haiyang Chemical Co., Qingdao, China) was applied to
64 column chromatography. NMR spectra were recorded on a JEOL ECX-500 spectrometer
65 (JEOL, Tokyo, Japan). Infrared (IR) spectra were recorded on Bruker VECTOR 22
66 spectrometer (Bruker, Karlsruhe, Germany) using KBr disks. HRMS data were measured
67 on Thermo Scientific Q Exactive (Thermo, Missour, USA). HPLC analysis was
68 conducted by an Agilent Technologies 1200 Series system (Agilent, California, USA)
69 with a 250 mm × 4.6 mm i.d., 5 μm, Chiralpak IA (Daicel) column. Optical rotation
70 values were measured by a Wzz-2s polarimeter (Shanghai Yue Feng instrument and
71 Meter Co., Shanghai, China). Fluorescence spectra were performed on Horiba
72 FluoroMax- 4 spectrofluorometer (Horiba, Paris, France). The CMV-CP protein was
73 purified from the supernatant by an AKTA purifier protein liquid chromatography system
74 (GE Healthcare, Pittsburgh, USA) with the Ni-NTA column.

75 **General Procedure for Preparation of Thiourea-Quinine Catalyst, Q-1**.[25, 26]

76 To a solution of 9-aminoquinine [27] (1.60 g, 5 mmol) in CH_2Cl_2 (10 mL) was slowly

added a solution of 3,5-bis(trifluoromethyl)phenyl isothiocyanate (1.39g, 5.1 mmol) in 10 mL of THF at ambient temperature. The mixture was stirred overnight, and the solvent was removed in vacuo. The residue was recrystallized with ethanol to afford the catalyst **Q-1** (1.5 g, 75% yield) as white solid (m.p. 121-123°C; lit.,[26] m.p. 121.0-121.5 °C). Catalyst **Q-2** was prepared under the same reaction condition using 9-aminoquinindium.

General Procedure for Preparation of Aldeimines, 1a~1r.[28] A solution of 2-amino-4-methylbenzothiazole (10 mmol) and aldehyde (10 mmol) in toluene (40 mL) was heated to reflux for 6 h in the presence of HOAc (25mol%). The mixture was cooled down to room temperature and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel with EtOAc/hexane (1:10, v/v) as eluant, affording the imine, **1a~1r**.

General Procedure for Asymmetric Synthesis of *α*-Aminophosphonates Containing *N*-benzothiazole, 3a~3r. Imine, **1** (0.1 mmol) was added to a dry tube containing a solution of catalyst, **Q** (0.01mmol), dry solvent (1 mL), and 4A activating molecular sieves (100 mg). The mixture was stirred at room temperature for 0.5 h. Then diphenylphosphite, **2** (0.10 mmol) was added in the mixture. The resulting reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then purified by column chromatography on silica gel with EtOAc/hexane (2/1, v/v) as eluant, affording the product **3**. Enantiomeric excess of the product was determined by chiral stationary phase HPLC analysis. The representative data for **3a** is shown below.

*(R)-Diphenyl-1-(4-methylbenzothiazole-2-amino)-1-(4-chlorophenyl)methylphosphonate **(3a)***. White solid, m.p. 147–149 °C, $[\alpha]_D^{20}$ + 80.30 (c 1.00, $CHCl_3$), the opposite configuration: $[\alpha]_D^{20}$ -78.64 (c 0.93, $CHCl_3$); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.42 (s,

3H), 6.17 (dd, J = 20.0, 10.0 Hz, 1H), 6.94 (t, J = 10.0 Hz, 1H), 6.98 (dd, J = 10.0 Hz, 2H), 7.04-7.06 (m, 3H), 7.14-7.18 (m, 2H), 7.28-7.33 (m, 4H), 7.48-7.50 (m, 3H), 7.66 (dd, J = 10.0, 5.0 Hz, 2H), 9.36 (dd, J = 10.0, 5.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.46 (s), 54.80 (d, J = 157.5 Hz), 119.11 (s), 120. 85 (d, J = 3.8 Hz), 120.92 (d, J = 3.8 Hz), 122.19 (s), 126.01 (s), 126.91 (s), 128.44 (s), 129.16 (s), 130.44 (s), 130.80 (d, J = 6.3 Hz), 130.95(s), 134.25 (s), 150.35 (dd, J = 18.9, 10.1 Hz), 150.66 (s), 164.87 (d, J = 10.1 Hz); ^{31}P NMR (202 MHz, DMSO-d_6) δ 14.60 (s); IR (KBr, cm^{-1}): 3265 (s), 3053 (s), 1587 (s), 1543 (s), 1489 (s), 1256 (s), 1209 (s), 1026 (s), 949 (s), 937 (s); HRMS (ESI) m/z for $C_{27}H_{22}ClN_2O_3PS$ $[M+H]^+$ cacld. 521.0856, found. 521.0848; HPLC analysis: Chiralpak IA, hexane: *i*PrOH = 75:25, 1.0 mL/min, t_R = 12.03 min (major, *R*), 17.03 min (minor, *S*).

Antiviral Biological Assay. The procedure of purifying CMV and the method to test the anti-CMV activity of both enantiomers of α-aminophosphonates bearing *N*-benzothiazole motief were the same as those reported previously in the literature.[29, 30]

Purification of CMV-CP.[31, 32] Genetically engineered CMV-CP was expressed, into which hexahistidine (His) tags were incorporated. To express the protein, we transformed each plasmid into *E. coli* strain BL21 (DE3) RIL. The *E. coli* strain BL21 (DE3) RIL harboring the recombinant plasmid was cultured in LB medium that contained 30 μg/mL kanamycin at 37 °C until the OD at 600 nm reached 0.65. Protein expression was induced at 16 °C by adding IPTG at 1 mM for 16 h. Bacteria were harvested by centrifugation at 12,000 rpm for 10 min at 4 °C. The pellets were suspended in a buffer that contained 20 mM PBS, 150 mM NaCl, 20 mM imidazole, and 1 mM β-mercaptoethanol. The suspension was sonicated for 35 min in an ice bath and then centrifuged at 12,000 rpm for

30 min at 4 °C to remove cell debris. The protein was purified from the supernatant by an AKTA purifier protein liquid chromatography system. The Ni-NTA column was washed with above buffer, and the proteins were eluted with buffer (50 mM PBS, 250 mM imidazole, 150 mM NaCl, pH 7.4). The protein was desalinated into a storage buffer (20 mM PBS, and 100 mM NaCl, pH 7.4). The expressed protein was initially assayed by coomassie brilliant blue method in a small-scale experiment, in which the final concentration was 10 μM.

RESULTS AND DISCUSSION

Chemistry. Chiral *α*-aminophosphonates based on the structure of Dufulin were prepared using our methodology.

Optimization of Asymmetric Reaction Conditions. The enantioselective hydrophosphonylation reaction of *N*-(4-methyl-benzothiazol-2-yl)imine, **1a**, with diphenylphosphite, **2**, was chosen as a model reaction to optimize reaction conditions (Figure 2, Table 1). Without any catalyst, the desired product **3a** could not be obtained (entry 1) (Table 1). In initial studies, different catalysts were screened in toluene as the solvent (1 mL) at room temperature for 12 h (entry 2 and 3) (Table 1). It was found that the use of the catalyst **Q-1** afforded the product **3a** in good enantioselectivity with moderate yield (entry 3) (Table 1). The effects of solvents were then evaluated by using **Q-1** as the catalyst (entry 3~8) (Table 1). CH_2Cl_2 (entry 7) (Table 1) was found to be an optimal solvent, giving **3a** in a good yield (up to 84%) and high enantioselectivity (up to 96%). When using two equivalents of diphenylphosphite (entry 9) (Table 1), the reaction yield decreased from 84% to 66%, and ee value decreased from 96% to 88%. When 4A molecular sieves (100 mg) was added to the reaction mixture, the reaction proceeded

cleanly (97% yield) with 99% ee (entry 10) (Table 1). It should be noted that the other enantiomer of the reaction could be obtained by using **Q-2** as the catalyst (entry 11) (Table 1). Diethyl phosphite (instead of diphenyl phosphite) could also be used, but with a lower yield and ee under the current condition without further optimizations (entry 12) (Table 1). Finally, the optimal condition was set as: **1a** (1.0 equ.), diphenylphosphite (1.0 equ.), catalyst **Q** (0.1 equ.), 4A molecular sieves (100 mg), CH_2Cl_2 (1mL), room temperature, 12 h.

Asymmetric synthesis of α-aminophosphonates, ***3a~3r****.* With the optimized reaction conditions in hand, the scope of imines were examined to demonstrate the generality of this enantioselective hydrophosphonylation reaction (Table 2). All substituted imines, **1a~1r**, using the catalyst **Q-1** or **Q-2** underwent the reaction smoothly, leading to the desired enantiomerically pure *α*-aminophosphonates, **3a~3r**, in good to excellent isolated yields of 93 – 99% and with high levels of enantioselectivity ranging from 91 – 99% *ee*. Significantly, the electronic properties of substituted imines do not obviously influence the reaction outcomes. Quantitative yields and high enantioselectivities were obtained for aromatic imines bearing a substitutent at the *para*-, *ortho*-, and *meta*- positions of either electron-donating groups, such as CH_3- (product **3b**), CH_3O- (product **3d, 3i, 3l**) and electron-withdrawing groups, such as F-(product **3f**), and CF_3- (product **3h**), and imines with aromatic substituent also proved to be good substrates (product **3o~3r**) with a high yield and *ee* values.

Determination of absolute configuration of chiral α-aminophosphonates. To determine the absolute configuration of our product, the crystal of the product **3f** obtained by **Q-1** catalyst was generated and analyzed by X-ray crystallography (Figure 3). The structure **3f**

is given in Figure 3. The absolute configuration of the molecule was determined to be *R*. Accordingly the absolute configuration of the product **3f** obtained by **Q-2** catalyst was *S* by analogy. The absolute configuration of all other products, **3**, was assigned by analogy.

Antiviral Activity. The antiviral activities of the enantiomers of *α*-aminophosphonates, **3a~3r**, against cucumber mosaic virus (CMV) were tested. Dufulin and Ningnanmycin[33] were used as the positive controls respectively.

In Vivo Anti-TMV Activity. As shown in Table 3, (*R*)-compounds generally exhibited higher antiviral activity than the corresponding (*S*)-enantiomer. Among them, some compounds display higher in *vivo* activity than controls. Compounds *(R)*-**3b** possesses the best anti-CMV activity at 500 μg/mL (curative activity, 72.3%; protection activity, 56.9%; inactivation activity, 96.9%), which is significantly higher than that of Ningnanmycin (curative activity, 45.3%; protection activity, 47.9%; inactivation activity, 71.3%) and Dufulin (curative activity, 50.4%; protection activity, 54.1%; inactivation activity, 78.2%) at 500 μg/mL. Compound (*S*)**-3b** had lower in *vivo* activity (curative activity, 25.1%; protection activity, 37.4%; inactivation activity, 57.2%) at 500 μg/mL. In addition, the phenyl substitution pattern greatly affected the in *vivo* anti-CMV activity of (*R*)-*α*-aminophosphonates. The F-and NO_2- moieties at the 2 position of phenyl ring also showed good anti-CMV activity in *vivo* (compounds (*R*)**-3f** and (*R*)**-3j**). Introduction of the thiophene hetercycle at the position of phenyl ring also possessed good activity (compound (*R*)**-3p**). In a short, the *R*-configuration of title compounds is the preferred antiviral configuration. Among them, compound *(R)*-**3b** could have great potential for further development as chiral antivirus agents.

The Origins of Bioselective Acticity. CMV-CP is an important protein involved in the

plant virus infections,[34-37] and is being studied as potential protein target to develop effective antivirus agents.[38] To determine the cause of the better activity of the (*R*)- versus the (*S*)- compounds, the interactions between chiral compounds **3b** and cucumber mosaic virus coat protein (CMV-CP) were performed via fluorescence spectroscopy (Figure 4). Dufulin and Ningnanmycin were used as the positive controls respectively. The effects of drugs on CMV-CP have been evaluated through binding constants. The binding constant (K_a) between (*R*)**-3b** and CMV-CP is 2.19×10^5 L/mol, indicating a strong ligand- protein interactions. In contrast, the corresponding binding constants of (*S*)-**3b** (1.17×10^4 L/mol), Dufulin (5.25×10^4 L/mol) and Ningnanmycin (2.40×10^4 L/mol) with CMV-CP are much lower. Results of the binding studies via fluorescence spectroscopy are consistent with our experimental observations that (*R*)**-3b** shows high antivirus activities. To further understand the origins of the experimental results showing that (*R*)-enantiomers exhibited better activity than (*S*)-enantiomers, molecular docking with AutoDock 4.0[39] between chiral compound **3b** and CMV-CP was performed (Figure 5). The X-ray crystal structure of CMV-CP used for computation was downloaded from RCSB.[40] Most of parameters for the docking calculation were set to the default values. Each docked structure was scored by the built-in scoring function and was clustered by 1 Å of RMSD criteria. Finally, the enzyme ligand complex structures were selected according to the criteria for Autodock Score. The interactions between both enantiomers of **3b** and CMV-CP can likely occur in the binding pocket defined by five residues (Phe58, Thr57, Val218, His55 and Ala147). The binding energy of (*R*)**-3b** with CMV-CP (-8.74 Kcal/mol) is lower than that of (*S*)**-3b** (-6.57 Kcal/mol) and the interaction between (*R*)**-3b** and CMV-CP is therefore stronger than that of (*S*)**-3b**. The results from molecular docking are in agreement with our in vivo

experimental observations on the antiviral activities and the results from fluorescence spectroscopy.

In summary, we have developed a highly efficient and selective catalytic method for the synthesis of *α*-aminophosphonates with *N*-benzothiazole. The products from our reaction showed potent antiviral activities against CMV, with *R*-enantiomer performing much better than the *S*-enantiomer. The difference in the selective bioactivity could be affected by the combination mode of the three-dimensional space between the enantiomers of *α*-aminophosphonate and CMV-CP via fluorescence spectroscopy and molecular docking. Given the promising bioactivity of this class of molecules and the simplicity of the catalysts and substrates, our results are expected to find important applications in developing future generations of plant protective agents.

ASSOCIATED CONTENT

Supporting Information

Physical and analytical data, ^{1}H NMR, ^{13}C NMR, HPLC spectra of intermediates, **1a~1r**, target compounds, **3a~3r**, this material is available free of charge via the Internet at http://pubs.acs.org; crystallographic data of compound **3f** (CCDC1402656) for this paper could be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

ACKNOWLEDGMENTS

The authors gratefully acknowledge the National Natural Science Foundation of China (Nos. 21132003, 21362004)

Notes

The authors declare no competing financial interest.

ABRREVIATIONS

CMV, cucumber mosaic virus; CMV−CP, cucumber mosaic virus coat protein; PBS, phosphonic acid buffer solution; RCSB, research collaboratory for structural bioinformatics; RMSD, root mean square deviation.

REFERENCES

(1) Allen, J. G.; Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. Phosphonopeptides, a new class of synthetic antibacterial agents. *Nature* **1978**, *272*, 56 − 58.

(2) Atherton, F. R.; Hassall, C. H.; Lambert, R. W. Synthesis and structure-activity relationships of antibacterial phosphonopeptides incorporating (1-aminoethyl)phosphonic acid and (aminomethyl)phosphonic acid. *J. Med. Chem*. **1986**, *29*, 29 − 40.

(3) Hirschmann, R.; Smith, A. B., III; Taylor, C. M.; Benkovic, P. A.; Taylor, S. D.; Yager, K. M.; Sprengeler, P. A.; Benkovic, S. J. Peptide synthesis catalyzed by an antibody containing a binding site for variable amino acids. *Science* **1994**, *265*, 234 − 237.

(4) Smith, W. W.; Bartlett, P. A. Macrocyclic inhibitors of penicillopepsin. 3. Design, synthesis, and evaluation of an inhibitor bridged between P2 and P1'. *J. Am. Chem. Soc*. **1998**, *120*, 4622 − 4628.

(5) Kobayashi, S.; Ishitani, H. Catalytic enantioselective addition to imines. *Chem. Rev.*, **1999**, *99*, 1069 – 1094.

(6) Gröger, H.; Hammer, B. Catalytic concepts for the enantioselective synthesis of α-amino and α-hydroxy phosphonates. *Chem. Eur. J.* **2000**, *6*, 943 – 948.

(7) Merino, P.; Marques-Lopez, E.; Herrera, R. P. Catalytic enantioselective hydrophosphonylation of aldehydes and imines. *Adv. Synth. Catal*. **2008**, *350*, 1195 – 1208.

(8) Ordonez, M.; Rojas-cabrera, H.; Cativiela, C. An overview of stereoselective synthesis of α-aminophosphonic acids and derivatives. *Tetrahedron* **2009**, *65*, 17 –

49.

(9) Albrecht, L.; Albrecht, A.; Krawczyk, H.; Jorgensen, K. A. Organocatalytic asymmetric synthesis of organophosphorus compounds. *Chem. Eur. J.* **2010**, *16*, 28 – 48.

(10) Ordonez, M.; Viveros-Ceballos, J. L.; Cativiela, C.; Sayago, F. J. An update on the stereoselective synthesis of *α*-aminophosphonic acids and derivatives. *Tetrahedron* **2015**, *71*, 1745 – 1784.

(11) Dieltiens, N.; Moonen, K.; Stevens, C. V. Enyne metathesis oxidation sequence for the synthesis of 2-phosphono pyrroles: proof of the "yne-then-ene" pathway. *Chem. Eur. J.* **2006**, *13*, 203 – 214.

(12) Boukallaba, K.; Elachqar, A.; El Hallaoui, A.; Alami, A.; ElHajji, S.; Labriti, B.; Martinez, J.; Rolland, V. Synthesis of new heterocyclic-*α*-aminophosphonates. *Phosphorus, Sulfur Silicon Relat. Elem.* **2006**, *181*, 819 – 823.

(13) Jin, L. H.; Song, B. A.; Zhang, G. P.; Xu, R. Q.; Zhang, S. M.; Gao, X. W.; Hu, D. Y.; Yang, S. Synthesis, X-ray crystallographic analysis, and antitumor activity of N-(benzothiazole-2-yl)-1-(fluorophenyl)-*O,O*-dialkyl--aminophosphonates. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 1537 – 1543.

(14) Zhou, J.; Fan, H. T.; Song, B. A.; Jin, L. H.; Bhadury, P. S.; Hu, D. Y.; Yang, S. Synthesis and antiviral activities of *α*-aminophosphonate derivatives containing a pyridazine moiety. *Phosphorus, Sulfur Silicon Relat. Elem*. **2011**, *186*, 81 – 87.

(15) Song, B. A.; Zhang, G. P.; Hu, D. Y.; Pang, L.; Yang, S.; Liu, G.; Wang, H. N-substituted benzothiazolyl-1-substitutedphenyl-*O,O*-dialkyl-*α*-aminophosphonate ester derivatives preparation and application. China Patent. CN 1687088, 2005.

(16) Chen, Z.; Li, G. J.; Fan, H. T.; Liu, J. J; Bi, L.; Yang, S.; Song, B. A.; Hu, D. Y. The study of efficiency of dufulin against southern rice black-streaked dwarf virus. *Chin. Agr. Sci. Bull*. **2011**, *27*, 250 – 254.

(17) Rout, S.; Ray, S. K.; Unhale, R. A.; Singh, V. K. Asymmetric direct vinylogous Michael addition to 2-enoylpyridine *N*-oxides catalyzed by bifunctional thio-urea. *Org. Lett.* **2014**, *16*, 5568 − 5571.

(18) Saha, P.; Biswas, A.; Molleti, N. Singh, V. K. Enantioselective synthesis of highly substituted chromans via the oxa-Michael−Michael cascade reaction with a bifunctional organocatalyst. *J. Org. Chem*. **2015**, *80*, 11115 − 11122.

(19) Neuvonen, A. J.; Pihko, P. M. Enantioselective Mannich reaction of β-keto esters with aromatic and aliphatic imines using a cooperatively assisted bifunctional catalyst. *Org. Lett*. **2014**, *16*, 5152 − 5155.

(20) George, J.; Sridharb, B.; Reddy, B. V. S. First example of quinine-squaramide catalyzed enantioselective addition of diphenyl phosphite to ketimines derived from isatins. *Org. Biomol. Chem.* **2014**, *12*, 1595 – 1602.

(21) Wang, C. J.; Dong, X. Q.; Zhang, Z. H.; Xue, Z. Y.; Teng, H. L. Highly anti-selective asymmetric nitro-Mannich reactions catalyzed by bifunctional amine-thiourea-bearing multiple hydrogen-bonding donors. *J. Am. Chem. Soc*. **2008**, *130*, 8606 – 8607.

(22) Vizcaíno-Milla, P.; Sansano, J. M.; Najera, C.; Fiser, B.; Gómez-Bengoa, E. Pyrimidine-derived prolinamides as recoverable bifunctional organocatalysts for enantioselective inter- and intramolecular aldol reactions under solvent-free conditions. *Eur. J. Org. Chem.* **2015**, 2614 – 2621.

312 (23) Lu, H. F.; Bai, J. T., Xu, J. Y.; Yang, T.; Lin, X. C.; Li, J.; Ren, F. Chiral 2-
313 aminopyrimidin-4(*1H*)-one derivative as the novel bifunctional organocatalyst:
314 enantioselective aldol reaction of isatins with ketones. *Tetrahedron* **2015**, *71*, 2610 –
315 2615.
316 (24) Wang, Y.; Li, H. M.; Wang, Y. Q.; Liu, Y.; Foxman, B. M.; Deng, L. Asymmetric
317 Diels−Alder reactions of 2-pyrones with a bifunctional organic catalyst. *J. Am.*
318 *Chem. Soc.* **2007**, *129*, 6364 – 6365.
319 (25) Vakulya, B.; Varga, S.; Csampai A.; Soos, T. Highly enantioselective conjugate
320 addition of nitromethane to chalcones using bifunctional cinchona organocatalysts.
321 *Org. Lett*. **2005**, *7*, 1967 − 1969.
322 (26) Fukata, Y.; Asano, K.; Matsubara, S. Procedure-controlled enantioselectivity switch
323 in organocatalytic 2‐oxazolidinone synthesis. *J. Am. Chem. Soc.* **2013**, *135*, 12160 –
324 12163.
325 (27) Brunner, H.; Bügler, J.; Nuber, B. Enantioselective catalysis. 98. Preparation of 9-
326 amino(9-deoxy)cinchona alkaloids. *Tetrahedron: Asymmetry* **1995**, *6*, 1699 − 1702.
327 (28) Li, L.; Song, B. A.; Bhadury, P. S.; Zhang, Y. P.; Hu, D. Y.; Yang, S.
328 Enantioselective synthesis of *β*-amino esters bearing a benzothiazole moiety via a
329 Mannich-type reaction catalyzed by a cinchona alkaloid derivative. *Eur. J. Org.*
330 *Chem*. **2011**, 4743 – 4746.
331 (29) Scott, H. Purification of cucumber mosaic virus. *Virology* 1963, **20,** 103 − 106.
332 (30) Song, B. A.; Zhang, H. P.; Wang, H.; Yang, S.; Jin, L. H.; Hu, D. Y.; Pang, L. L.;
333 Xue, W. Synthesis and antiviral activity of novel chiral cyanoacrylate derivatives. *J.*
334 *Agric. Food Chem.* **2005**, *53*, 7886 – 7891.

(31) Sambrook, J; Lassel, D. W. Molecular cloning: a laboratory manual, (the third edition). The first volume of the book. 2008.

(32) Choi, J. M.; Phat, C.; Kim, E.; Kim, M.; Lee, G. P.; Ryu, K. H.; Lee, C. Improved detection of cucumber mosaic virus coat protein (CMV-CP) in genetically modified pepper (*Capsicum annuum*) using a polyclonal antibody to a synthetic CP peptide. *Hort. Environ. Biotechnol*. **2015**, *56*, 316 − 323.

(33) Hu, H. Z.; Xiang, G. X.; Chen, J. R. A new antibiotic pesticides-ningnan meisu. China Patent. CN 1093869, 1994.

(34) Canto, T.; Prior, D. A. M.; Hellwald, K. H.; Oparka, K. J.; Palukaitis, P. Characterization of cucumber mosaic virus IV. Movement protein and coat protein are both essential for cell-to-cell movement of cucumber mosaic virus. *Virology* **1997**, *237*, 237 – 248.

(35) Smith, T. J.; Chase, E.; Schmidt, T.; Perry, K. L. The structure of cucumber mosaic virus and comparison to cowpea chlorotic mottle virus. *J. Virol*. **2000**, *74*, 7578 – 7586.

(36) Schmitz, Isabelle; Rao, A. L. N. Deletions in the conserved amino-terminal basic arm of cucumber mosaic virus coat protein disrupt virion assembly but do not abolish infectivity and cell-to-cell movement. *Virology* **1998**, *248*, 323 – 331.

(37) Jan, G.; Tineke, U.; Sylvain, B.; Wouter, L.; Alie, V. S.; Annemarie, W.; Peter, D. H.; Mart, V. G. Coat protein-mediated protection to cucumber mosaic virus infections in cultivated tomato. *Euphytica* **1996**, *88*, 139 – 149.

(38) Chen, M. H.; Li, P.; Hu, D. Y.; Zeng, S.; Li, T. X.; Jin, L. H.; Xue, W.; Song, B. A. Synthesis, antiviral activity, 3D-QSAR, and interaction mechanisms study of novel

malonate derivatives containing quinazolin-4(*3H*)-one moiety. *Bioorg. Med. Chem. Lett*. **2015**, *26*, 168 – 173.

(39) Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. A semiempirical free energy force field with charge-based desolvation. *J. Comput. Chem.* **2007**, *28*, 1145 – 1152.

(40) Smith, T. J.; Chase, E.; Schmidt, T.; Perry, K. The structure of cucumber mosaic virus and comparison to cowpea chlorotic mottle virus. *J. Virol*. **2000**, *74*, 7578 – 7586.

366 FIGURE CAPTIONS

367 Figure 1 Synthesis of chiral α-aminophosphonates based on Dufulin motif.

368 Figure 2 Asymmetric synthetic route of condition optimization.

369 Figure 3 Absolute configuration of compound **3f** obtained by thiourea-quinine catalyst **Q-**

370 **1**.

371 Figure 4 Fluorescence emission spectra of CMV-CP in the presence of testing compounds

372 with different concentrations (λ_{ex} 280 nm). Inset: the linear relationship for quenching

373 CMV-CP.

374 Figure 5 Molecular docking models of CMV-CP in a complex with (*R*)**-3b** and (*S*)**-3b**

375 respectively.

376

377 **Table 1** Condition Optimization for The Enantioselective Addition of Phosphite to Imine

Entry[a]	Catalyst	Solvent	Yield (%)[e]	*ee* (%)[f]
1	-	Toluene	0	0
2	Quinine	Toluene	32	10 (*S*)
3	Q-1	Toluene	45	85 (*R*)
4	Q-1	THF	17	20 (*R*)
5	Q-1	Et_2O	21	49 (*R*)
6	Q-1	Hexane	78	89 (*R*)
7	Q-1	CH_2Cl_2	84	96 (*R*)
8	Q-1	Xylene	80	94 (*R*)
9[b]	Q-1	CH_2Cl_2	66	88 (*R*)
10[c]	Q-1	CH_2Cl_2	97	99 (*R*)
11	Q-2	CH_2Cl_2	96	94 (*S*)
12[d]	Q-2	CH_2Cl_2	43	73 (*R*)

378 [a] Reaction Conditions:**1a** (0.10 mmol), diphenylphosphite (0.10 mmol), catalyst (0.01 mmol), solvent
379 (1 mL), r.t, 12 h; [b]2.0 equiv of diphenylphosphite was employed; [c]100 mg 4Å molecular sieve was
380 employed; [d]using diethylphosphite; [e]isolated yields; [f]the *ee* was determined by chiral HPLC analysis.
381

382 **Table 2** Enantioselective Hydrophosphonylation of Imine

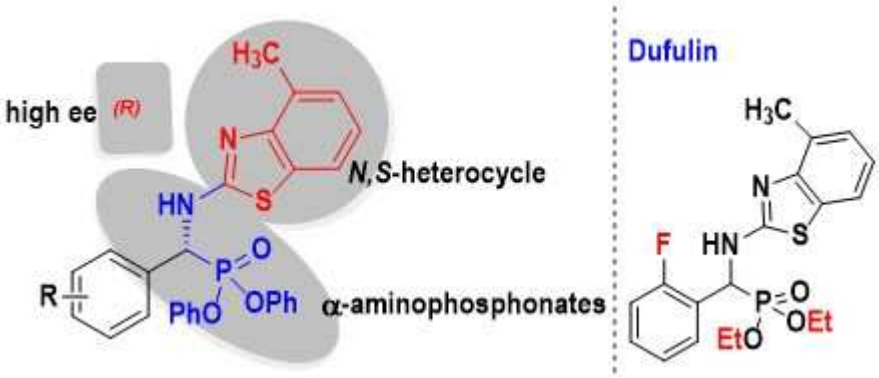
Entry	R	Product	Yield (%)[a]	*ee* (%)[b]
1	*p*-ClC_6H_4	**3a** (*R*)/(*S*)	99/94	97/96
2	*p*-$CH_3C_6H_4$	**3b** (*R*)/(*S*)	95/96	97/96
3	*p*-BrC_6H_4	**3c** (*R*)/(*S*)	93/95	91/94
4	*p*-$OMeC_6H_4$	**3d** (*R*)/(*S*)	94/93	96/95
5	Ph	**3e** (*R*)/(*S*)	99/97	97/96
6	*o*-FC_6H_4	**3f** (*R*)/(*S*)	98/96	97/95
7	*o*-ClC_6H_4	**3g** (*R*)/(*S*)	98/96	97/93
8	*o*-$CF_3C_6H_4$	**3h** (*R*)/(*S*)	98/97	98/94
9	*o*-$OMeC_6H_4$	**3i** (*R*)/(*S*)	98/97	98/96
10	*o*-$NO_2C_6H_4$	**3j** (*R*)/(*S*)	95/95	95/94
11	2,4-$Cl_2C_6H_4$	**3k** (*R*)/(*S*)	95/95	97/93
12	*m*-$OMeC_6H_4$	**3l** (*R*)/(*S*)	97/98	97/97
13	*m*-BrC_6H_4	**3m** (*R*)/(*S*)	96/96	98/97
14	*m*-$NO_2C_6H_4$	**3n** (*R*)/(*S*)	94/96	94/97
15	2-furanyl	**3o** (*R*)/(*S*)	97/95	98/97
16	2-thienyl	**3p** (*R*)/(*S*)	95/95	98/99
17	2-benzofuranyl	**3q** (*R*)/(*S*)	95/96	92/94
18	2-benzothienyl	**3r** (*R*)/(*S*)	96/97	98/96

383 [a]Isolated yield after silica gel chromatography; [b]the *ee* was determined by chiral HPLC analysis.
384

385 **Table 3** Inhibitory Effect of The Title Compounds against CMV *in vivo* at 500 μg/mL

Compd.	Curative effect (%)[a]	Protective effect (%)[a]	Inactivation effect (%)[a]	Compd.	Curative effect (%)[a]	Protective effect (%)[a]	Inactivation effect (%)[a]
(*R*)-**3a**	53.4±1.8	51.1±2.5	79.6±1.9	(*S*)-**3a**	36.8±2.3	37.4±3.3	67.5±2.4
(*R*)-**3b**	72.3±1.2	56.9±2.6	96.3±1.2	(*S*)-**3b**	25.1±2.5	37.4±1.7	57.2±1.3
(*R*)-**3c**	34.4±2.2	47.2±1.6	63.5±1.9	(*R*)-**3c**	21.5±2.3	24.6±3.2	51.1±1.7
(*R*)-**3d**	47.8±2.5	52.2±1.7	72.5±2.6	(*S*)-**3d**	27.3±3.1	43.4±2.5	50.7±2.8
(*R*)-**3e**	70.2±0.4	50.6±1.2	93.4±1.7	(*S*)-**3e**	42.1±1.6	39.8±2.3	60.4±2.8
(*R*)-**3f**	71.4±2.1	57.3±1.4	94.1±1.3	(*S*)-**3f**	54.5±1.9	44.4±1.7	77.8±2.3
(*R*)-**3g**	58.6±1.8	42.1±2.5	88.3±2.3	(*S*)-**3g**	45.2±2.7	34.3±3.1	67.3±1.4
(*R*)-**3h**	43.1±2.2	48.2±2.7	75.6±1.8	(*S*)-**3h**	20.3±3.3	39.5±2.6	51.4±2.1
(*R*)-**3i**	57.5±1.7	45.8±2.9	82.2±1.0	(*S*)-**3i**	34.8±2.6	22.4±1.9	66.3±2.8
(*R*)-**3j**	64.2±1.5	67.4±2.1	90.7±1.6	(*S*)-**3j**	47.3±2.2	43.1±2.8	71.1±1.9
(*R*)-**3k**	46.1±1.8	46.4±2.2	82.7±1.6	(*S*)-**3k**	17.4±2.7	23.1±1.4	47.9±1.7
(*R*)-**3l**	36.2±2.5	46.3±1.8	70.8±2.2	(*S*)-**3l**	15.5±3.1	34.1±2.3	47.3±1.9
(*R*)-**3m**	22.9±2.3	35.1±1.6	55.4±2.2	(*S*)-**3m**	10.6±2.8	30.2±2.1	47.2±2.6
(*R*)-**3n**	34.7±3.4	44.4±1.9	71.6±1.7	(*S*)-**3n**	23.8±1.5	32.7±2.6	57.5±1.9
(*R*)-**3o**	55.1±1.3	51.4±2.1	88.6±2.7	(*S*)-**3o**	44.2±1.7	42.5±2.3	73.1±2.6
(*R*)-**3p**	62.4±1.8	53.6±2.4	89.3±1.7	(*S*)-**3p**	49.7±1.9	47.3±2.4	71.6±2.1
(*R*)-**3q**	43.3±2.1	27.4±3.3	55.8±1.9	(*S*)-**3q**	35.2±2.4	17.6±3.1	41.4±2.6
(*R*)-**3r**	41.2±2.3	29.8±1.8	53.3±2.4	(*S*)-**3r**	19.6±3.1	18.2±2.7	36.5±2.4
Control[b]	45.3±1.7	47.9±2.1	71.3±1.6	Dufulin[c]	50.4±1.2	54.1±1.7	78.2±2.3

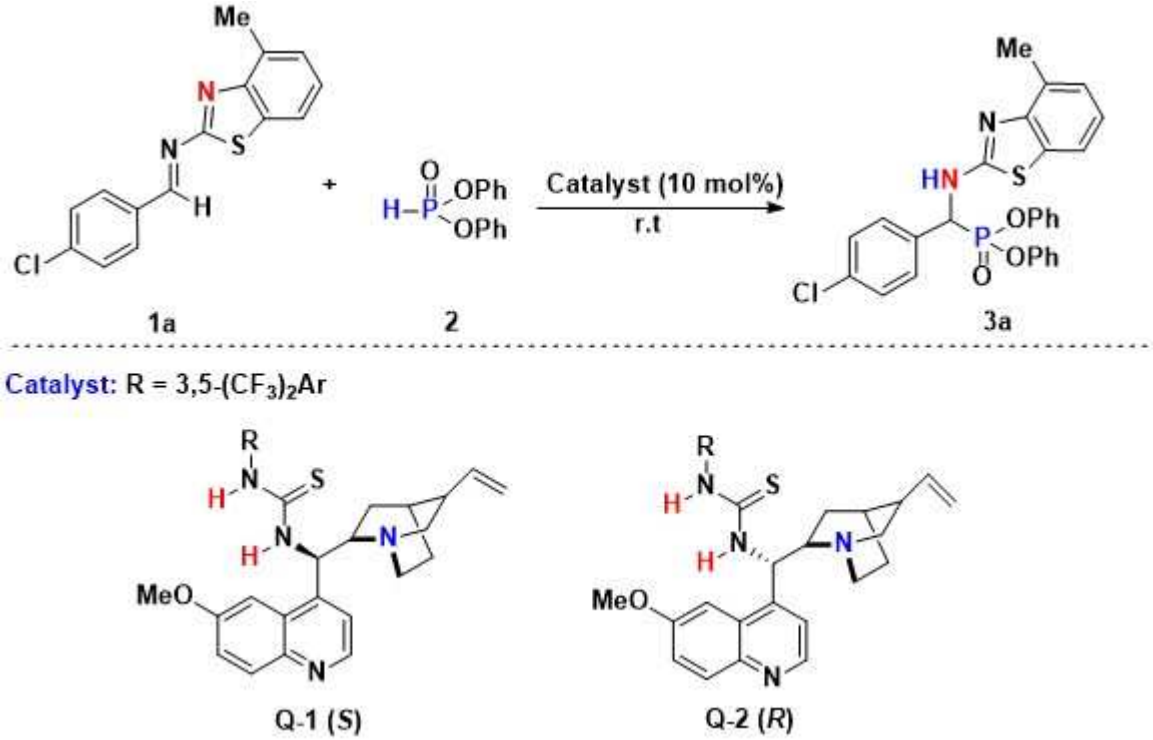
386 [a] Average of three replicates; [b] Ningnanmycin was used as the control; [c] Dufulin was also used as the
387 control.
388



389

390 **Figure 1**

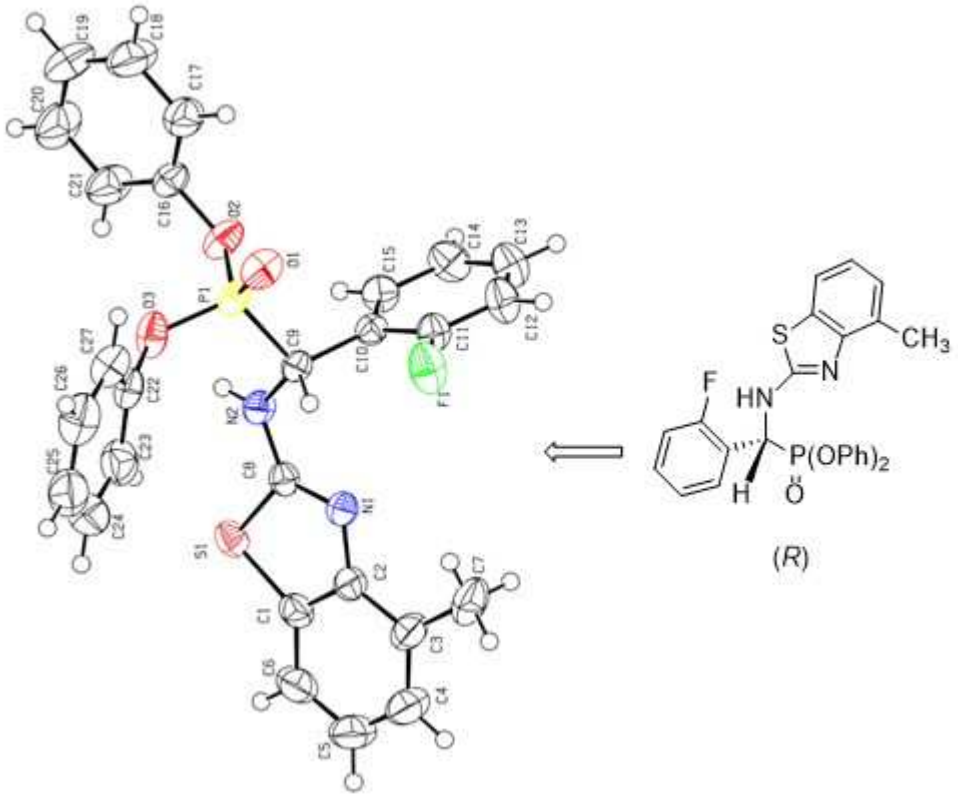
391



392

393 **Figure 2**

394



395

396 **Figure 3**

397

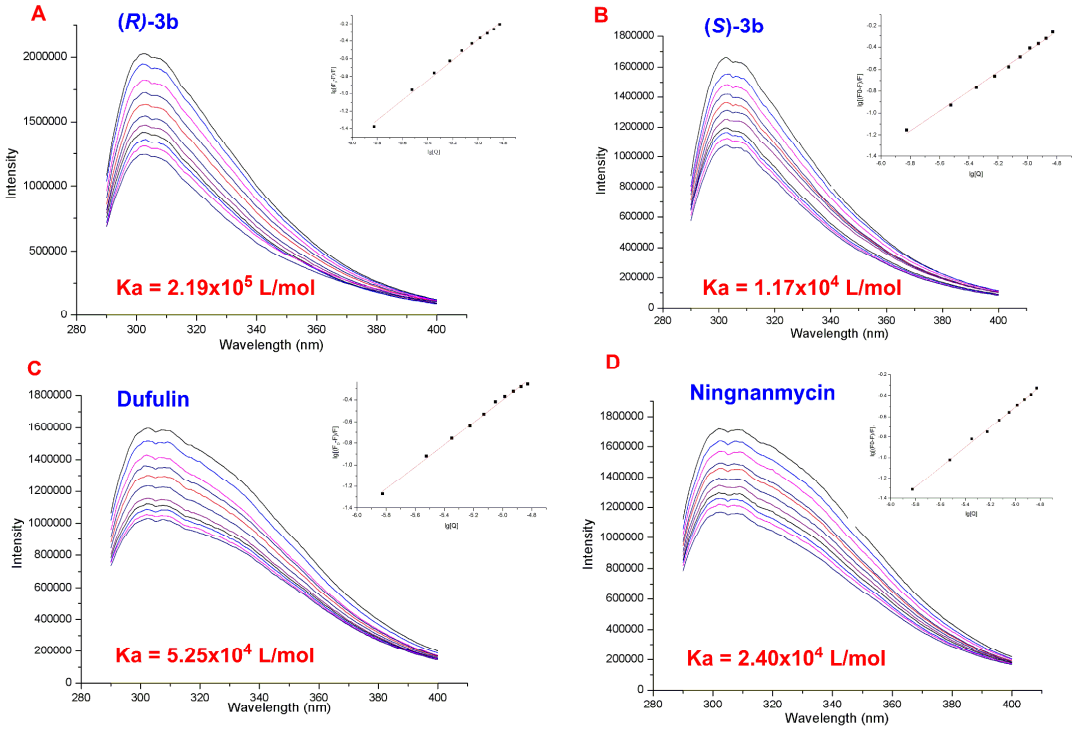
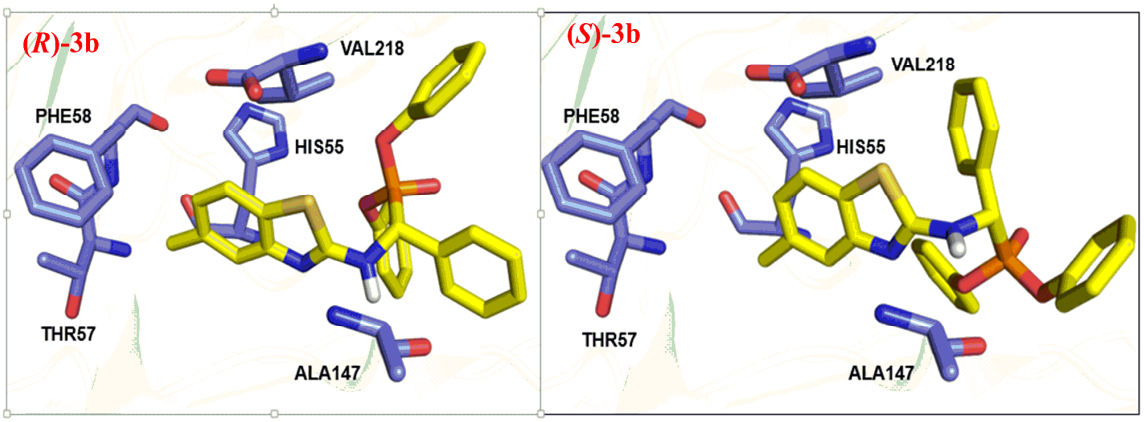

A
(R)-3b
Ka = 2.19x10^5 L/mol
B
(S)-3b
Ka = 1.17x10^4 L/mol
C
Dufulin
Ka = 5.25x10^4 L/mol
D
Ningnanmycin
Ka = 2.40x10^4 L/mol
Intensity
Wavelength (nm)

Figure 4

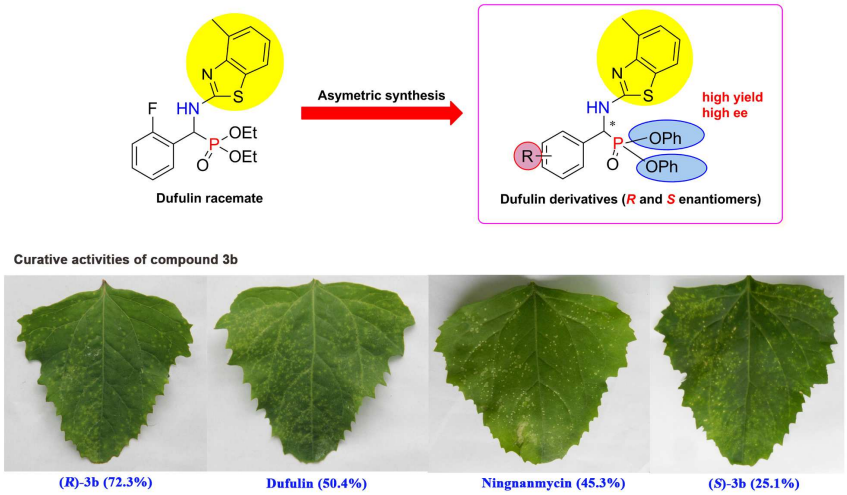


Binding Energy = -8.74 Kcal/mol

Binding Energy = -6.57 Kcal/mol

Figure 5

404 **Table of Contents Graphic**



405