CHAPTER III

EXPERIMENTAL

3.1 General procedures

For the synthesis, all solvents were dried with molecular seives UOP type 4A (Fluka) and filtered by filter papers (Whatman No.1, 0.25 mm diameter). The progress of the reactions was followed by thin-layer chromatography (TLC, aluminium sheets precoated with silica gel, Merck Kieselgel 60 F_{254}) and detected under ultraviolet light at 254 nm. Purification was performed by column chromatography on silica gel (Merck Kieselgel 60, 0.063-0.200 mm of particle size). The identities of the synthesized esters were confirmed by ^{1}H-NMR ($CDCl_3$ 99.8%, Aldrich) with tetramethylsilane (TMS) as an internal reference on a 400 MHz NMR spectrometer (Varian Mercury Plus 400).

All chromatographic analyses were performed on a gas chromatograph (Agilent 6890) with a split injector and a flame ionization detector (FID). The capillary columns were prepared from deactivated fused-silica tubing (J&W Scientific). Analyte solutions in dichloromethane were manually injected with a 10μL microsyringe (SGE) through a microseal septum (Merlin).

3.2 Synthesis

3.2.1 Chemicals and reagents

Most of chemicals and solvents used in this research were purchased from Fluka, Aldrich, Merck and J.T. Baker and used without further purifications.

The racemic mixtures of phenoxy acid methyl esters were prepared by using methyl 2-bromopropionate and phenol derivatives as the substrates. All substrates and other chemicals used in this research are:

chiral reagent

- methyl 2-bromopropionate, 98% (Aldrich)

non chiral reagents

- 2-fluorophenol, 98% (Fluka)
- 3-fluorophenol, 97% (Fluka)
- 4-fluorophenol, 98% (Fluka)
- 2-chlorophenol, 98% (Fluka)
- 3-chlorophenol, 95% (Fluka)
- 4-chlorophenol, 99% (Fluka)
- 2-bromophenol, 97% (Fluka)
- 3-bromophenol, 97% (Fluka)
- 4-bromophenol, 98% (Fluka)
- 2-methoxyphenol, 98% (Fluka)
- 3-methoxyphenol, 97% (Fluka)
- 4-methoxyphenol, 97% (Fluka)
- 2-methylphenol, 99.5% (Fluka)
- 3-methylphenol, 99% (Merck)
- 4-methylphenol, 98% (Fluka)
- 2-cyanophenol, 97% (Fluka)
- 3-cyanophenol, 99% (Aldrich)
- 4-cyanophenol, 95% (Aldrich)
- 2-trifluoromethylphenol, 97% (Aldrich)
- 3-trifluoromethylphenol, 99% (Aldrich)
- 4-trifluoromethylphenol, 99% (Fluka)
- 2-nitrophenol, 98% (Fluka)
- 3-nitrophenol, 99% (Aldrich)
- 4-nitrophenol, 95% (Fluka)
- 2,3-dimethylphenol, 99% (Fluka)
- 2,4-dimethylphenol, 97% (Fluka)
- 2,5-dimethylphenol, 97% (Fluka)
- 2,6-dimethylphenol, 97% (Fluka)
- 3,4-dimethylphenol, 97% (Fluka)

- 3,5-dimethylphenol, 98% (Fluka)
- 2,3-difluorophenol, 98% (Aldrich)
- 2,4-difluorophenol, 99% (Aldrich)
- 2,5-difluorophenol, 95% (Aldrich)
- 2,6-difluorophenol, 98% (Aldrich)
- 3,4-difluorophenol, 99% (Aldrich)
- 3,5-difluorophenol, 99% (Aldrich)
- 2,3-dichlorophenol, 98% (Fluka)
- 2,4-dichlorophenol, 99% (Aldrich)
- 2,5-dichlorophenol, 98% (Fluka)
- 2,6-dichlorophenol, 99% (Aldrich)
- 3,4-dichlorophenol, 99% (Aldrich)
- 3,5-dichlorophenol, 97% (Aldrich)
- 2,4,6-trifluorophenol, 99% (Aldrich)
- 2,4,6-trichlorophenol, 97% (Aldrich)
- 2,3,4,5,6-pentafluorophenol, 99% (Fluka)

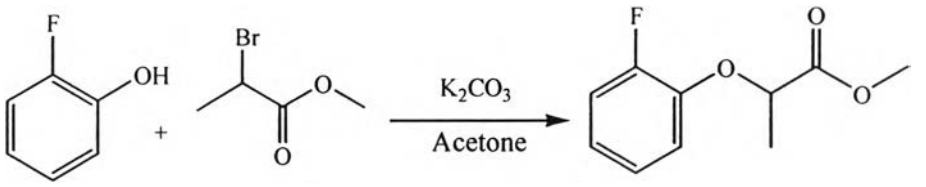
organic solvents

- acetone (J.T. Baker)
- dichloromethane (J.T. Baker)
- ethyl acetate (Merck)
- hexane (Merck)
- pentane (J.T. Baker)

other chemicals

- potassium carbonate (Fluka)
- anhydrous sodium sulfate (Fluka)
- sodium chloride (Fluka)

3.2.2 Synthesis of methyl 2-(2′-fluorophenoxy)propanoate

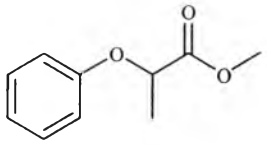
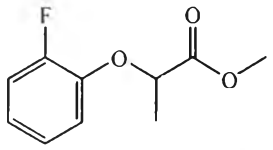
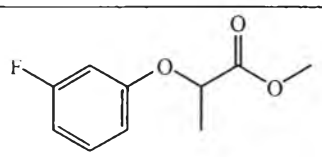
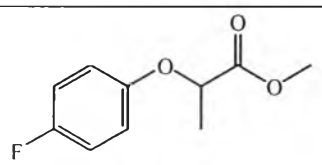
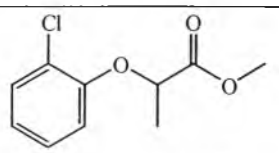
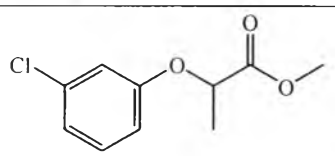
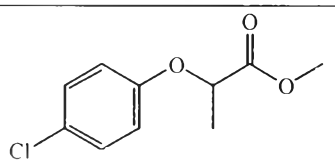
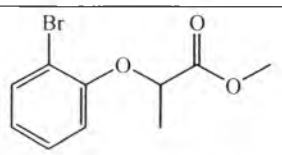
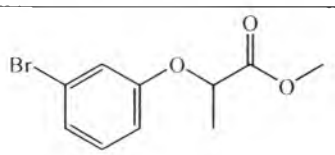


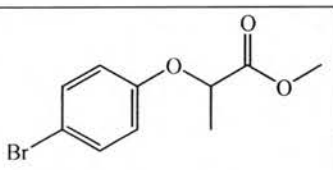
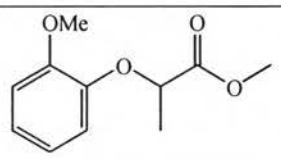
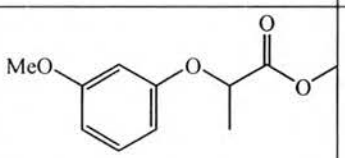
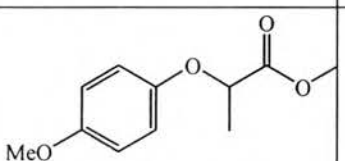
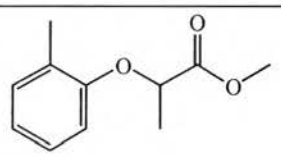
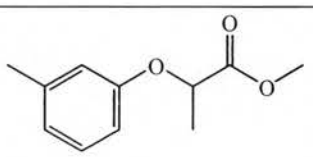
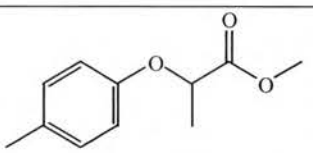
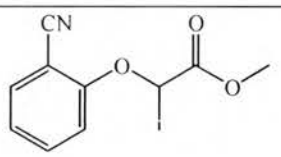
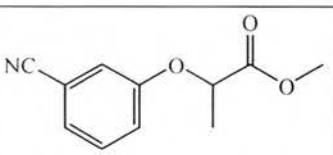
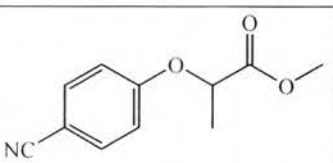
Methyl 2-(2′-fluorophenoxy)propanoate **(2F)**: 2-Fluorophenol (3 mmol, 0.34 g) was dissolved in acetone (5 mL) in a round bottom flask. An excess amount of potassium carbonate (4 mmol, 0.55 g) was gradually added into the solution of 2-fluorophenol and the reaction was stirred at room temperature for 15 minutes. Then, methyl 2-bromopropionate (3 mmol, 0.33 mL) was added dropwise thereto. This solution was refluxed in an oil bath for approximately 7 hours and stirring was continued. The progress of the reaction was followed by TLC (hexane:CH_2Cl_2 1:2). The reaction solution was cooled down, and then the precipitate was filtered off. The filtrate was evaporated in order to remove an excess solvent.
The residue was dissolved in water and extracted twice with dichloromethane.
The combined extracts were dried over anhydrous sodium sulfate, filtered and concentrated by evaporation. Methyl 2-(2′-fluorophenoxy)propanoate was obtained with 70 % yield. The purity of the desired product was examined by TLC (hexane:CH_2Cl_2 1:2); R_f = 0.57 and identified by ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.68 (3H, d, CH_3), 3.79 (3H, s, OCH_3), 4.82 (1H, q, CH-O), 7.04 (4H, m, ArH).

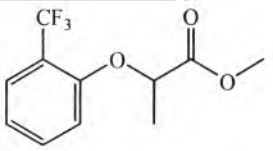
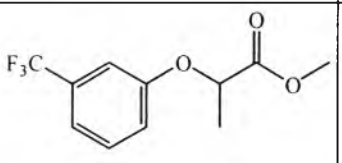
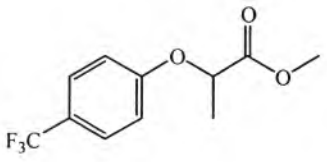
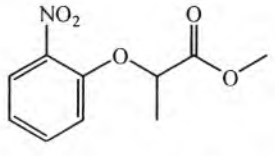
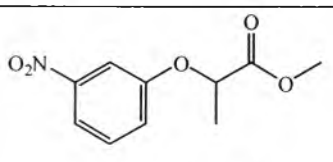
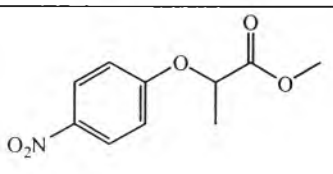
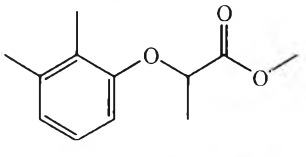
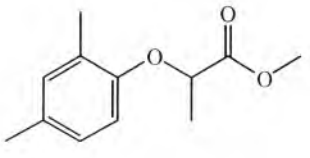
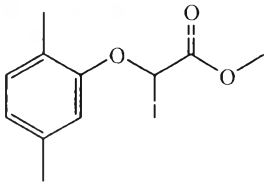
The synthetic scheme of other compounds is the same as that of methyl 2-(2′-fluorophenoxy)propanoate. Most of synthetic products were obtained in approximately 30-70 % yield, except for methyl 2-(3′,4′-difluorophenoxy)propanoate and methyl 2-(3′,5′-difluorophenoxy)propanoate.

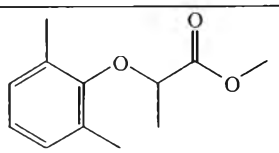
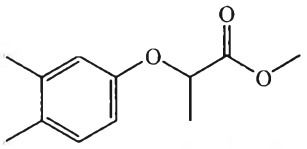
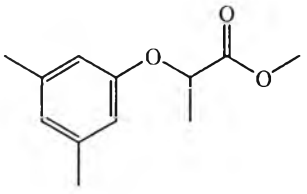
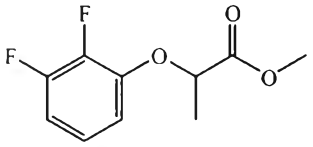
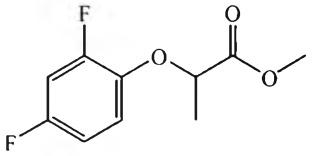
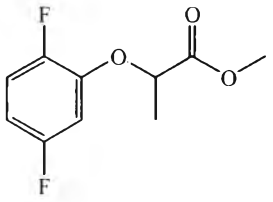
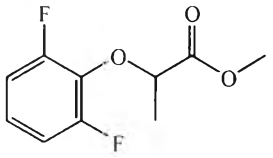
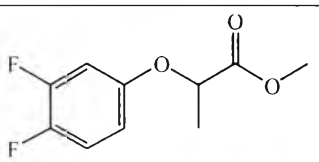
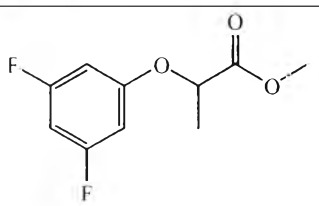
Fourty-six phenoxy acid methyl ester derivatives for this research are shown in table 3.1.

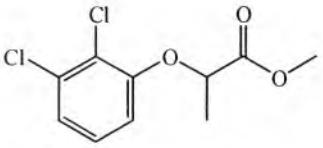
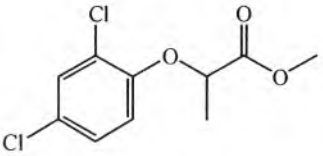
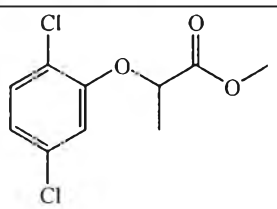
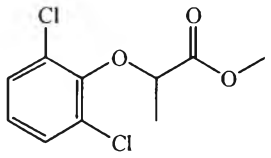
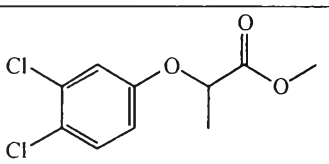
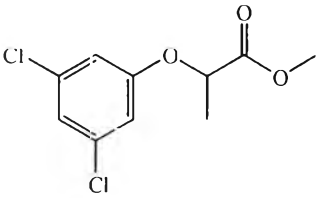
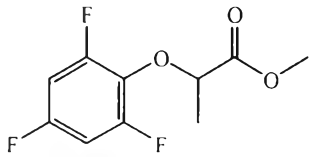
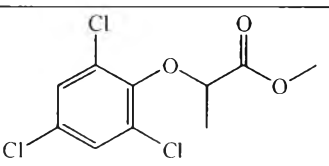
Table 3.1 Chemical structures and abbreviations of phenoxy acid methyl esters.

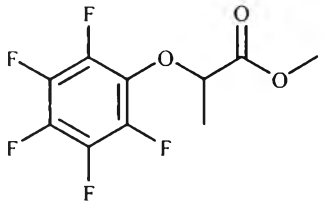
chemical structure	abbreviation	MW (g/mol)	compound
	1	180.20	methyl 2-phenoxypropanoate
I. Monosubstituted phenoxy acid methyl esters			
	2F	198.19	methyl 2-(2′-fluorophenoxy) propanoate
	3F	198.19	methyl 2-(3′-fluorophenoxy) propanoate
	4F	198.19	methyl 2-(4′-fluorophenoxy) propanoate
	2Cl	214.65	methyl 2-(2′-chlorophenoxy) propanoate
	3Cl	214.65	methyl 2-(3′-chlorophenoxy) propanoate
	4Cl	214.65	methyl 2-(4′-chlorophenoxy) propanoate
	2Br	259.10	methyl 2-(2′-bromophenoxy) propanoate
	3Br	259.10	methyl 2-(3′-bromophenoxy) propanoate

chemical structure	abbreviation	MW (g/mol)	compound
	4Br	259.10	methyl 2-(4′-bromophenoxy) propanoate
	2OMe	210.23	methyl 2-(2′-methoxyphenoxy) propanoate
	3OMe	210.23	methyl 2-(3′-methoxyphenoxy) propanoate
	4OMe	210.23	methyl 2-(4′-methoxyphenoxy) propanoate
	2Me	194.23	methyl 2-(2′-methylphenoxy) propanoate
	3Me	194.23	methyl 2-(3′-methylphenoxy) propanoate
	4Me	194.23	methyl 2-(4′-methylphenoxy) propanoate
	2CN	205.21	methyl 2-(2′-cyanophenoxy) propanoate
	3CN	205.21	methyl 2-(3′-cyanophenoxy) propanoate
	4CN	205.21	methyl 2-(4′-cyanophenoxy) propanoate

chemical structure	abbreviation	MW (g/mol)	compound
	$2CF_3$	248.20	methyl 2-(2′-(trifluoromethyl)phenoxy) propanoate
	$3CF_3$	248.20	methyl 2-(3′-(trifluoromethyl)phenoxy) propanoate
	$4CF_3$	248.20	methyl 2-(4′-(trifluoromethyl)phenoxy) propanoate
	$2NO_2$	225.20	methyl 2-(2′-nitrophenoxy) propanoate
	$3NO_2$	225.20	methyl 2-(3′-nitrophenoxy) propanoate
	$4NO_2$	225.20	methyl 2-(4′-nitrophenoxy) propanoate
II. Disubstituted phenoxy acid methyl esters			
	2,3Me	208.25	methyl 2-(2′,3′dimethylphenoxy) propanoate
	2,4Me	208.25	methyl 2-(2′,4′-dimethylphenoxy) propanoate
	2,5Me	208.25	methyl 2-(2′,5′-dimethylphenoxy) propanoate

chemical structure	abbreviation	MW (g/mol)	compound
	2,6Me	208.25	methyl 2-(2′,6′-dimethylphenoxy) propanoate
	3,4Me	208.25	methyl 2-(3′,4′-dimethylphenoxy) propanoate
	3,5Me	208.25	methyl 2-(3′,5′-dimethylphenoxy) propanoate
	2,3F	216.18	methyl 2-(2′,3′-difluorophenoxy) propanoate
	2,4F	216.18	methyl 2-(2′,4′-difluorophenoxy) propanoate
	2,5F	216.18	methyl 2-(2′,5′-difluorophenoxy) propanoate
	2,6F	216.18	methyl 2-(2′,6′-difluorophenoxy) propanoate
	3,4F	216.18	methyl 2-(3′,4′-difluorophenoxy) propanoate
	3,5F	216.18	methyl 2-(3′,5′-difluorophenoxy) propanoate

chemical structure	abbreviation	MW (g/mol)	compound
	2,3Cl	249.09	methyl 2-(2′,3′-dichlorophenoxy) propanoate
	2,4Cl	249.09	methyl 2-(2′,4′-dichlorophenoxy) propanoate
	2,5Cl	249.09	methyl 2-(2′,5′-dichlorophenoxy) propanoate
	2,6Cl	249.09	methyl 2-(2′,6′-dichlorophenoxy) propanoate
	3,4Cl	249.09	methyl 2-(3′,4′-dichlorophenoxy) propanoate
	3,5Cl	249.09	methyl 2-(3′,5′-dichlorophenoxy) propanoate
III. Other phenoxy acid methyl esters			
	2,4,6F	234.17	methyl 2-(2′,4′,6′-trifluorophenoxy) propanoate
	2,4,6Cl	283.54	methyl2-(2′,4′,6′-trichlorophenoxy) propanoate

chemical structure	abbreviation	MW (g/mol)	compound
	pentaF	270.15	methyl 2-(2′,3′,4′,5′,6′-pentafluorophenoxy) propanoate

3.3 Preparation of capillary gas chromatographic columns

The deactivated fused silica tubings (15 m × 0.25 mm i.d., Agilent Technologies) were statically coated with the stationary phase solution (0.4% w/v) to obtain 0.25 μm film thickness [41].

Three types of stationary phases were used in this research:

achiral reference column :

- polysiloxane OV-1701 (7% phenyl, 7% cyanopropyl, 86% dimethyl polysiloxane, Supelco) was used as a reference stationary phase and diluent for solid cyclodextrin derivatives in two chiral columns

chiral columns :

- 30.0% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl) cyclomaltoheptaose (or BSiMe) in OV-1701
- 33.5% heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl) cyclomaltoheptaose (or BSiAc) in OV-1701

Two chiral columns contained identical molality of derivatized cyclodextrin in polysiloxane. After coating, the column was mounted on a GC oven and conditioned at 200 °C until a stable baseline was observed. The overall column performance was determined by means of Grob test [42]. Efficiency for all columns was determined at 200°C with *n*-eicosane which gave the plate number (N) above 3000 plates/m for all columns.

Additionally, the enantioselectivity of two chiral columns were regularly checked with a represented compound to verify column performance for analytes.

3.4 Gas chromatographic analyses

All GC separations were performed on an Agilent 6890 gas chromatograph with split injection and FID detection. The detector and injector temperature were maintained at 250 °C. Hydrogen was used as a carrier gas at an average linear velocity of 50 cm/s. A split ratio of 100:1 was used for all columns and the injection volume was 0.4 μL. Before injection, phenoxy acid methyl esters were dissolved in dichloromethane at a concentration range of 10-20 mg/mL. Each solution of analyte was injected at least in duplicate. Retention times of the same analyte under the same condition from two consecutive runs were within ± 0.005 minutes. Thermodynamic measurements were performed isothermally between 90 and 240 °C at 10 °C increment. From GC chromatograms, retention factors and enantioselectivities of all analytes were calculated and used for thermodynamic studies. Finally, the thermodynamic parameters were determined by means of van't Hoff approach in order to explain the interactions between the analytes and stationary phase [43].