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Research Article

Chiral Resolution, Absolute Configuration Assignment, and Genotoxicity Evaluation of Racemic 3,4-Dihydroquinazoline as a Novel Anticancer Agent

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If a new drug candidate will be a mixture of enantiomers, both enantiomers should be separately studied for at least latent genotoxicity as early as possible since the thalidomide tragedy. Our group has recently reported that **KCP-10043F** (**OZ-001**) as a racemate (±)-3,4-dihydroquinazoline derivative strongly represses the proliferation of human A549 lung cancer cells by caspase-mediated apoptosis via STAT3 inactivation. To investigate the possible teratological effects of the two enantiomers of a racemic **KCP-10043F**, therefore chiral resolution of (±)-**KCP-10043F** was performed and subsequently followed by a series of chemical processes to afford the corresponding chiral diastereomers. By using ^{1}H NMR anisotropy method, the absolute configuration (+)-**KCP-10043F** and (−)-**KCP-10043F** could be assigned as *S* and *R* configuration, respectively. The bacterial reverse mutation test (Ames test) for racemate (±)-**KCP-10043F** and its two enantiomers exhibited that all three stereoisomers were found to be nongenotoxic against five bacterial strains with/without metabolic activation. In addition, (*R*)-(−)-**KCP-10043F** displayed almost equal anticancer activity to (*S*)-(+)-**KCP-10043F** against three cancer cell lines. Based on these overall results, racemate **KCP-10043F (OZ-001)** could be used for our ongoing preclinical and clinical studies without the expensive asymmetric process and/or chiral separation.

1. Introduction

The significance of chirality in drug design and development cannot be understated [1]. About more than half of the drugs currently in use are chiral compounds and near 90% of the last ones are marketed as racemates (or racemic mixtures) [2]. Although the enantiomers of chiral drugs have the same chemical connectivity of atoms, they would exhibit marked differences in their pharmacology, toxicology, pharmacokinetics, metabolism, etc.: while one enantiomer is beneficial to the body, the other enantiomer can be highly toxic to the body [3]. A well-known example of enantiomer-related toxicity is the *R*- and *S*-enantiomers of thalidomide [4]. The *R*-enantiomer is an effective sedative, but the *S*-enantiomer is known to cause teratogenic birth defects. During the late 1950s and early 1960s, thalidomide produced birth defects in children born to women who took the racemic mixture during pregnancy [5]. According to the United States Food and Drug Administration (US FDA) guidelines, it is mandatory to separate chiral drugs before they are marketed [6]. Rigorous justification is needed for the market approval of racemic drugs. FDA has approved the marketing of some

racemic drugs such as flecainide, ketorolac, and prilocaine [7]. In this case, both enantiomers have equal therapeutic potency without any toxicity or they are known to racemize in the body.

In our previous work, a family of 3,4-dihydroquinazoline derivatives was discovered as a promising anticancer agent [8–10], among which **KCP-10043F** (**OZ-001**) represses the proliferation of human A549 lung cancer cells by caspase-mediated apoptosis via STAT3 inactivation [11, 12]. This compound was a racemate (±)-3,4-dihydroquinazoline derivative with a single chiral center (Figure 1). In this work, we separated the racemate (±)-**KCP-10043F** into two optically pure enantiomers (+)-**KCP-10043F** and (−)-**KCP-10043F**, which were assigned for their absolute configuration by the formation of chiral diastereomers and ^{1}H NMR anisotropy method. The bacterial reverse mutation test (Ames test) for two enantiomers and a racemate of **KCP-10043F** was performed to investigate their latent genotoxicity as well as their anticancer activities.

2. Materials and Methods

2.1. Chiral Resolution. Two enantiomers of racemate (±)-**KCP-10043F** (**OZ-001**) were separated by a Shimadzu LC-20AD (CP-HPLC-08) with CHIRALPAK IA column (2.5 cm I.D. × 25 cm L, 10 μm particle size) using ethanol (100%) as a mobile phase with a flow rate of 25 mL/min and a detection wavelength of 214 nm at a temperature of 38°C. Each separated enantiomer was analyzed by a Shimadzu LC-20AD (CP-HPLC-08) with CHIRALPAK IA-3 (IA30CE-OB011) column (0.46 cm I.D. × 25 cm L.) using ethanol/diethylamine (100/0.1: v/v) as a mobile phase with a flow rate of 0.5 mL/min and a detection wavelength of 214 nm at a temperature of 35°C.

2.2. Chemistry. Analytical thin-layer chromatography (TLC) was performed on silica gel precoated on glass-backed plates (Fluka Kieselgel 60F_{254}, Merck). An UV light (λ = 254 nm) was used for the detection. Flash chromatography was performed on silica gel 60 (particle size 230–400 mesh, Merck). Commercially available reactants were supplied by Sigma-Aldrich and used without further purification.

Optical rotations were obtained with an Autopol III Automatic Polarimeter (Rudolph Research Analytical). Experimental electronic circular dichroism (ECD) spectrum was recorded on a Jasco J-715 circular dichroism (CD) spectropolarimeter (Jasco). Melting points were measured on a MEL-TEMP® 3.0 capillary melting point apparatus (Sigma-Aldrich). ^{1}H NMR spectra on a Bruker Avance DXR 400 (400 MHz) spectrometer (Bruker) and Jeol JNM-ECZR 500 MHz spectrometer were recorded in $CDCl_3$. Chemical shifts were reported in δ (ppm) units relative to tetramethylsilane (TMS).

2.3. Preparation of Compound (±)-6. To a solution of (±)-**5** (1.86 g, 3.57 mmol) in THF/H_2O (1 : 1, v/v, 15 mL) LiOH (0.45 g, 10.71 mmol) was added. The reaction mixture was stirred at room temperature for 4 h and concentrated under reduced pressure. The reaction mixture was dissolved with a mixed solvent of DCM (20 mL) and water (20 mL). The extracted aqueous layer was acidified by 0.5 N HCl (pH = 4) and extracted with DCM (25 mL). The extracted organic layer was dried with $MgSO_4$ and evaporated under reduced pressure to give the desired product (±)-**6** (180 mg, >99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.42 (br s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.35–7.31 (m, 1H), 7.18–7.14 (m, 3H), 7.13–7.07 (m, 3H), 6.31 (br s, 1H), 5.18 (dd, J = 10.4 and 4.8 Hz, 1H), 3.59 (br s, 1H), 3.27 (br s, 2H), 2.96 (s, 6H), 2.93–2.89 (m, 3H), 2.68–2.63 (m, 1H), 2.47–2.44 (m, 1H), 2.06 (s, 1H), 1.86–1.81 (m, 5H), 1.76–1.72 (d, J = 13.2 Hz, 2H), 1.38–1.28 (m, 4H), 1.26–1.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 171.8, 159.4, 153.4, 147.6, 140.3, 132.4, 129.2, 128.6, 126.1, 125.4, 123.9, 118.8, 61.9, 50.6, 43.8, 40.6, 38.1, 36.5, 34.1, 34.1, 27.3, 26.6, 25.8.

*2.4. Preparation of Compound **8a-8b**.* To a solution of (±)-**6** (1.05 g, 2.08 mmol), **7** (0.75 mL, 6.23 mmol), and DMAP (0.13 g, 1.04 mmol) in dry dichloromethane (20 mL) a solution of EDC·HCl (0.48 g, 2.49 mmol) in dry dichloromethane (5 mL) was added dropwise at 0°C. The reaction mixture was stirred at room temperature for 3 h in argon atmosphere and concentrated under reduced pressure. The reaction mixture was dissolved with a mixed solvent of EtOAc (30 mL) and water (3 × 10 mL). The extracted combined organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. The crude product was then purified by flash column chromatography (EA:DCM: hexane:EtOH = 2:2:12:1) to afford (*S*, 4*R*)-**8a** and (*S*, 4*S*)-**8b** (1:1 ratio, 899 mg, 71%), for (*S*, 4*R*)-**8a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.35 (m, 1H), 7.35–7.33 (m, 2H), 7.32–7.31 (m, 1H), 7.29–7.27 (m, 1H), 7.15 (td, J = 7.9 and 1.3 Hz, 1H), 7.02–6.89 (m, 3H), 6.94–6.93 (m, 1H), 6.88–6.86 (m, 3H), 5.99 (q, J = 6.6 Hz, 1H), 5.04 (dd, J = 11.2 and 4.4 Hz, 1H), 4.28 (br s, 1H), 3.66 (br s, 1H), 2.97 (s, 6H), 2.81 (d, J = 13.2 Hz, 1H), 2.72–2.67 (m, 2H), 2.54–2.50 (m, 1H), 2.41–2.36 (m, 1H), 2.07 (s, 3H), 1.81–1.78 (m, 4H), 1.72–1.70 (m, 1H), 1.57 (d, J = 6.6 Hz, 4H), 1.35–1.28 (m, 4H), 1.25–1.23 (m, 2H), 1.21–1.15 (m, 1H).

2.5. Preparation of Compound (R)-6. To a solution of (*S*, 4*R*)-**8a** (0.17 g, 0.28 mmol) in THF/H_2O (1 : 1, v/v, 8 mL) LiOH (70 mg, 1.67 mmol) was added. The reaction mixture was stirred at room temperature for 72 h and concentrated under reduced pressure. The reaction mixture was dissolved with a mixed solvent of DCM (10 mL) and water (10 mL). The extracted aqueous layer was acidified with 0.5 N HCl until the pH of the aqueous layer was 2, extracted with DCM (15 mL). The extracted organic layer was dried with $MgSO_4$ and evaporated under reduced pressure to give desired product (*R*)-**6** (140 mg, >99%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.86 (br s, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.35–7.28 (m, 1H), 7.18–7.12 (m, 3H), 7.11–7.08 (m, 3H), 6.27 (br s, 1H), 5.18 (dd, J = 10.8 and 5.0 Hz, 1H), 3.52 (br s, 1H), 3.28 (br s, 2H), 2.96 (s, 6H), 2.92–2.87 (m, 3H), 2.64–2.60 (m, 1H), 2.48–2.42

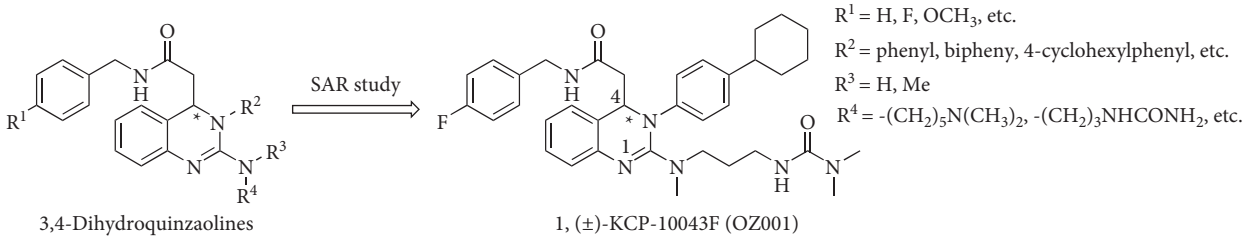


Figure 1: Chemical structures of 3,4-dihydroquinazolines and **KCP-10043F** (**OZ-001**).

(m, 1H), 1.96 (br s, 1H), 1.86–1.78 (m, 5H), 1.73 (d, $J = 12.7$ Hz, 2H), 1.38–1.28 (m, 4H), 1.25–1.19 (m, 2H).

*2.6. Preparation of Compound **1** [(R)-(-)-KCP-10043F (OZ-001)].* To a solution of (*R*)-**6** (0.26 g, 0.50 mmol) and TEA (0.09 mL, 0.64 mmol) in DMF (20 mL) pivaloyl chloride (0.073 mL, 0.60 mmol) was added dropwise. The reaction mixture was stirred for 3 h at room temperature. The reaction mixture was treated with 4-fluorobenzylamine (0.053 mL, 0.46 mmol) and TEA (0.07 mL, 0.50 mmol), further stirred for 2 h at room temperature. The reaction mixture was dissolved with a mixed solvent of DCM (20 mL) and water (20 mL). The extracted combined organic layer was dried with $MgSO_4$ and evaporated under reduced pressure. The crude product was then purified by flash column chromatography (EA:DCM: hexane:EtOH = 2:2:12:1) to provide the final product **1** [(*R*)-(-)-**KCP-10043F** (**OZ-001**)] as a white solid (245 mg, 80%): mp 107–116°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.26 (br m, 2H), 7.16–7.14 (m, 2H), 7.07–7.03 (m, 3H), 6.98–6.93 (m, 5H), 6.90–6.88 (m, 1H), 5.21 (dd, $J = 10.4$ and 4.8 Hz, 1H), 4.45–4.37 (m, 2H), 4.16 (br, 1H), 3.41 (br s, 1H), 3.02–2.88 (m, 2H), 2.85 (s, 6H), 2.58 (dd, $J = 14.4$ and 10.4 Hz, 1H), 2.43–2.38 (m, 5H), 1.83–1.81 (m, 4H), 1.75–1.73 (m, 3H), 1.40–1.32 (m, 4H), 1.28–1.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 158.9, 154.5, 144.0, 143.7, 143.4, 134.7, 129.9, 129.8, 127.9, 127.6, 126.6, 125.1, 121.9, 121.8, 115.2, 115.0, 61.2, 43.8, 42.8, 41.4, 39.6, 37.2, 36.3, 34.5, 34.4, 27.1, 26.9, 26.1; low MS (ES): m/z 613.4 [M+H]+; HRMS (FAB): m/z calcd for $C_{36}H_{46}N_6O_2F$ $[M+H]^+$ 613.3661, found 613.3660; $[\alpha]_D^{26}$ of −261° (*c* 0.003, $CHCl_3$).

2.7. Time-Dependent Density Functional Theory (TDDFT) Calculation. The simulated ECD spectrum of (*R*)-(−)-**KCP-10043F** was calculated with TDDFT approach at B3LYP/TZVP level based on the structure optimized at the same level in Gaussian09 program package [13].

2.8. Ames Test. The Ames test for mutagenicity evaluation was performed by using Ames MPF™ Penta 1 Mutagenicity Assay Kit (Xenometrix; B10-513-S1-P) [14, 15]. Each compound was studied at concentrations of 20~5,000 μg/mL in the presence or absence of metabolic activation (S9 fraction).

2.9. MTT Assay (Anticancer Activity). Human lung adenocarcinoma A549 cells, human colorectal adenocarcinoma HT-29 cells, and human pancreatic cancer PANC-1 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin (100 U/mL), and streptomycin sulfate (100 μg/mL). Cells were cultured at 37°C in an atmosphere of 5% CO_2.

The cancer cells (5×10^4/mL) were seeded in each well containing 100 μL of RPMI medium supplemented with 10% FBS in a 96-well plate. Various concentrations of each compound were added and incubated for 24 h. MTT (5 mg/mL stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and formazan blue, which was formed in the cells, was dissolved with 100 μL DMSO. The optical density was measured at 540 nm by an automatic microplate reader (Molecular Devices Corp).

3. Results and Discussion

3.1. Chiral Resolution. Firstly, we separated racemate (±)-**KCP-10043F** with the aid of the chiral technique of supercritical fluid chromatography (SFC) to get optically pure enantiomers (+)-**KCP-10043F** and (−)-**KCP-10043F** with >99% enantiomeric excess (*ee*) and purity >99% (Figure 2) [16, 17]. The specific optical rotations $[\alpha]_D^{22}$ of (+)-**KCP-10043F** (Figure 2(b)) and (−)-**KCP-1-170043F** (Figure 2(c)) were determined to be +273° (*c* 0.01, $CHCl_3$) and −281° (*c* 0.01, $CHCl_3$), respectively.

3.2. Absolute Configuration Assignment. Our method for absolute configuration assignment of each enantiomer was based on the direct covalent diastereomer formation between a racemic compound and a chiral selector [18]. For this matter, racemic ester (±)-**5** was routinely prepared according to our previously reported method (Scheme 1) [11]. Treatment of (±)-**5** with LiOH in THF-H_2O (1 : 1) solvent at room temperature produced racemic acid (±)-**6** in quantitative yield. Subsequent esterification of (±)-**6** with chiral (*S*)-(−)-1-phenylethanol **7** (purity ≥99.0%) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (DMAP) coupling condition yielded a mixture of diastereomeric esters **8a** and **8b** with a ratio of 1 : 1 (^{1}H NMR) in 71% yield (Supplemental data: Figure S1) [19]. The mixture was easily separated by column chromatography on normal silica gel

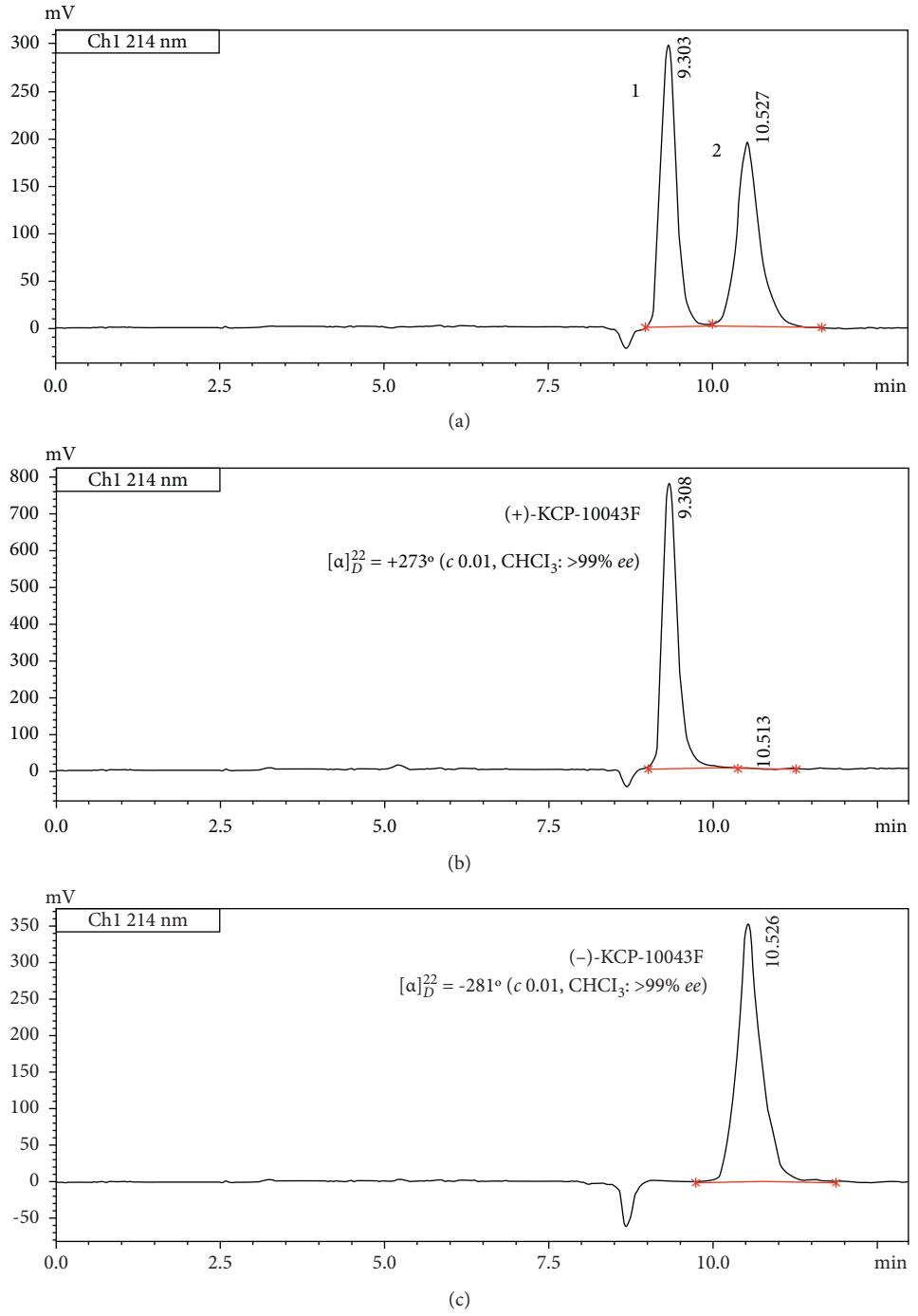
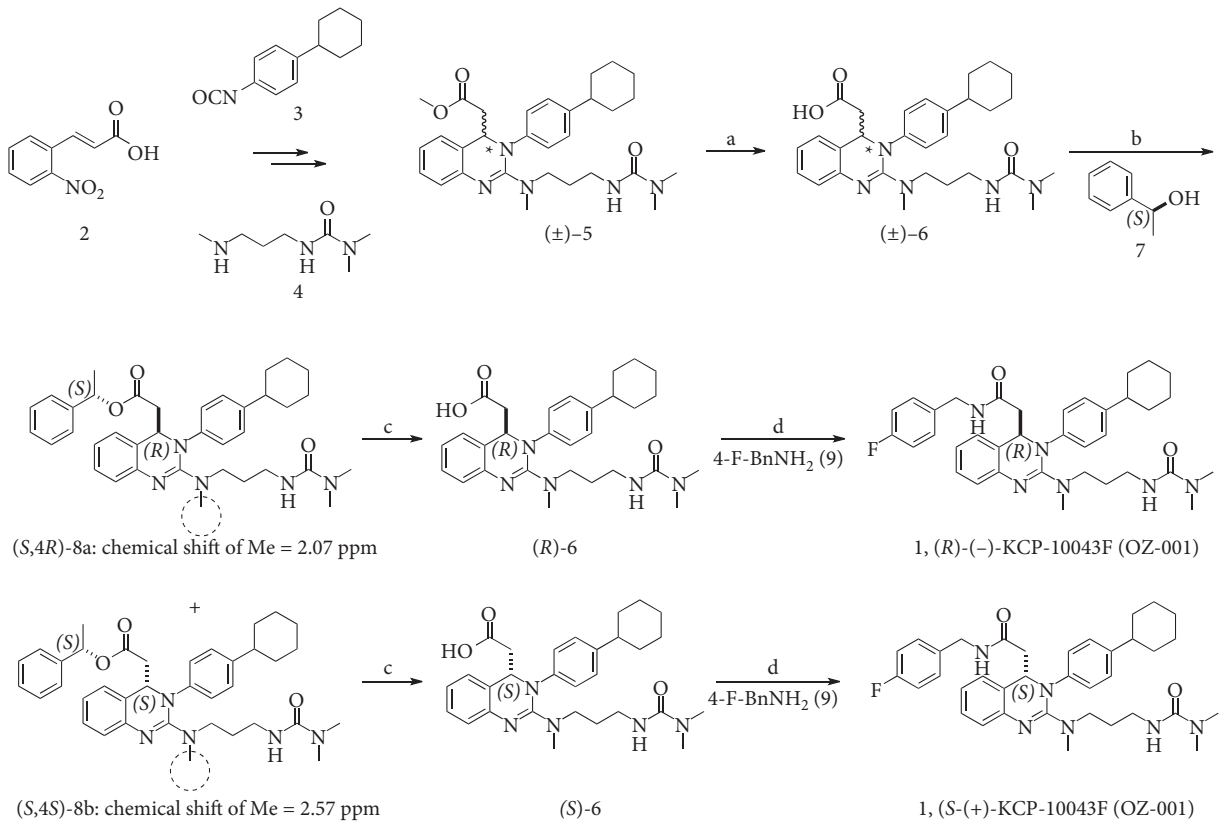


Figure 2: Chiral HPLC spectra, specific optical rotations, and enantiomeric excess (*ee*) of the racemate (±)-**KCP-10043F** (**OZ-001**) and its two enantiomers: (a) racemate (±)-**KCP-10043F** (**OZ-001**); (b) (+)-**KCP-10043F**; (c) (−)-**KCP-10043F**.

(EtOAc : CH_2Cl_2:hexane:EtOH = 2 : 2:12 : 1) to provide the first eluted ester **8a** (Supplemental data: Figure S2) and the second one **8b** (Supplemental data: Figure S3) as shown in Scheme 1. To prepare the enantiopure amide **1** (**KCP-10043F** : **OZ-001**), the first eluted ester **8a** was treated with LiOH in THF-H_2O (1 : 1) to afford chirally pure acid **5** in quantitative yield. This acid **5** was treated with pivaloyl chloride and triethylamine (TEA) in DMF to provide a mixed carboxylic acid anhydride *in situ* [20], which was directly coupled with 4-fluorobenzylamine **9** in the presence of TEA to afford the enantiopure amide **1**, (−)-**KCP-10043F** (**OZ-001**) in 80% yield: $[\alpha]_D^{26}$ of -261° (*c* 0.003, $CHCl_3$) and 95.57 ± 0.02% *ee* based on the same chiral HPLC analysis (Supplemental data: Figure S4). The (+)-**KCP-10043F** (**OZ-001**) could be also prepared in 78% yield starting from the second eluted ester **8b** by using the same procedure.

To assign the absolute configuration at C-4 position of the quinazoline scaffold, we decided to investigate the ^{1}H NMR



Scheme 1: Reagents and conditions: (a) LiOH, THF/H_2O (1 : 1), rt, 4 h > 99% (b) EDC·HCl, DMAP (cat.), CH_2Cl_2, 0°C to rt, 3 h 71% (c) LiOH, THF/H_2O (1 : 1), rt, 72 h > 99% for (*R*)-**6** and (*S*)-**6**, respectively (d) pivaloyl chloride, TEA, DMF, rt, 3 h; TEA, DMF, rt, 2 h 80 and 78% for (*R*)-(-)-**1** and (*S*)-(+)-**1**, respectively.

data of the separated esters **8a** and **8b**, respectively. Before the analysis of the chemical shift data of ^{1}H NMR data, molecular mechanics (MM2) calculation and 3D-conformation analysis showed the considerable energy difference ($\Delta E = 1.02$ kcal/mol) between (*S*, 4*R*)-**8a** and (*S*, 4*S*)-**8b** as shown in Figure 3. *N*-methyl group inside the white-colored dotted circle in (*S*, 4*R*)-**8a** was completely located above the diamagnetic (shielding) zone of benzene ring (4 Å: yellow-colored dotted circle) as shown in Figure 3(a), while the corresponding methyl group of (*S*, 4*S*)-**8b** was located within the paramagnetic (deshielding) zone (Figure 3(b)). According to the calculated anisotropic effect zone of benzene ring (Supplemental data: Figure S5) [21], the protons of the methyl groups of (*S*, 4*R*)-**8a** were anticipated to be largely upfield-shifted by the diamagnetic anisotropy effect of the phenyl moiety compared to those of (*S*, 4*S*)-**8b**. In experimental ^{1}H NMR data, the chemical shifts of *N*-methyl groups in (*S*, 4*R*)-**8a** and (*S*, 4*S*)-**8b** exhibited 2.07 and 2.57 ppm, respectively (Figures 4(b) and 4(c)). Based on the consistency between the theoretical data and experimental data, the chiral center of (−)-**KCP-10043F** can be designated *R* configuration and thus (+)-**KCP-10043F** can be readily assigned as *S* configuration. Additionally, the ECD spectrum of (−)-**KCP-10043F** was further measured to afford a positive band at 268 nm and a negative band at 303 nm, which was consistent with Gaussian TDDFT-calculated *R* configuration of **KCP-10043F** as shown in Figure 5 [22].

3.3. Genotoxicity Evaluation. The Ames test was performed for mutagenicity evaluation of racemate (±)-KCP-10043F and its two enantiomers by using Ames MPF™ Penta 1 Mutagenicity Assay Kit (Xenometrix; B10-513-S1-P) [14, 15]. The pre-incubation method, system metabolic activation (rat liver S9 fraction), seven positive controls (mutagens; refer to Figures S6–S10 of supplemental data), negative control (DMSO), and five bacterial strains {*Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2 uvrA[pKM101]} were employed for the Ames test, in compliance with OECD Guideline 471 [23].

Each compound was studied at concentrations of 20~5,000 μg/mL in the presence or absence of metabolic activation (S9 fraction). As shown in Table 1, a revertant analysis showed no significant differences between the treatment doses (20~5,000 μg/mL) and the negative control (DMSO) regardless of +S9 and −S9, when compared to the positive control and included all strains of S. *typhimurium* and *E. coli* EP2 (Supplemental data: Figures S6–S10). All of these Ames test results showed that none of all stereoisomers of **KCP10043F** were genotoxic regarding frameshift mutations (TA98 and TA1537) and base pair substitutions {TA100, TA1535 and *E. coli* WP2 uvrA[pKM101]} regardless of the treatment dose [24].

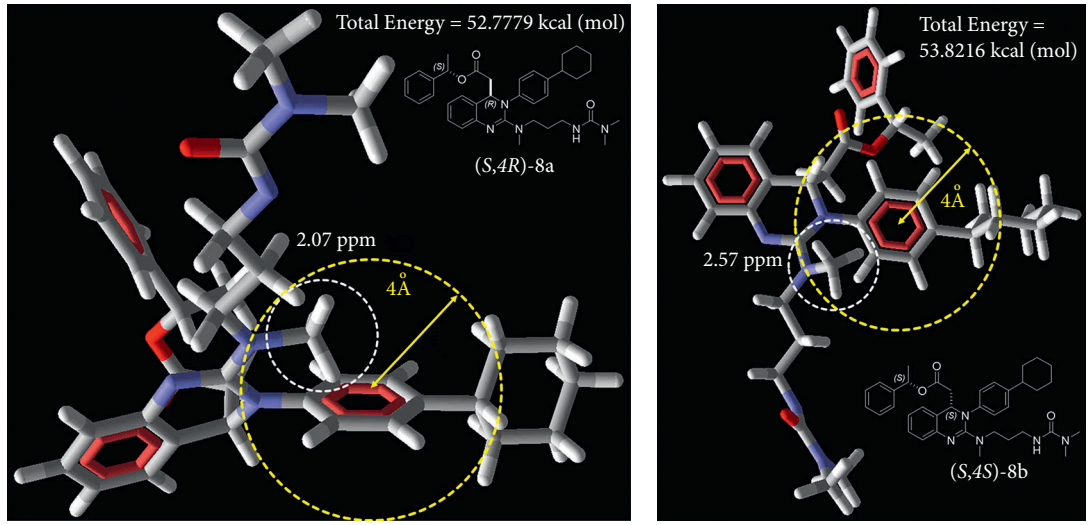


Figure 3: The most stable 3D-conformations of (*S*, 4*R*)-**8a** and (*S*, 4*S*)-**8b** based on molecular mechanics (MM2) calculation. The calculated anisotropic effect zone of the benzene ring was represented by yellow-colored dotted circle (radius 4 Å: $\Delta\delta = 0.5$ ppm) according to the reported literature [21]. The chemical shifts of *N*-methyl groups inside white yellow-colored dotted circles in (*S*, 4*R*)-**8a** (a) and (*S*, 4*S*)-**8b** (b) exhibited 2.07 and 2.57 ppm.

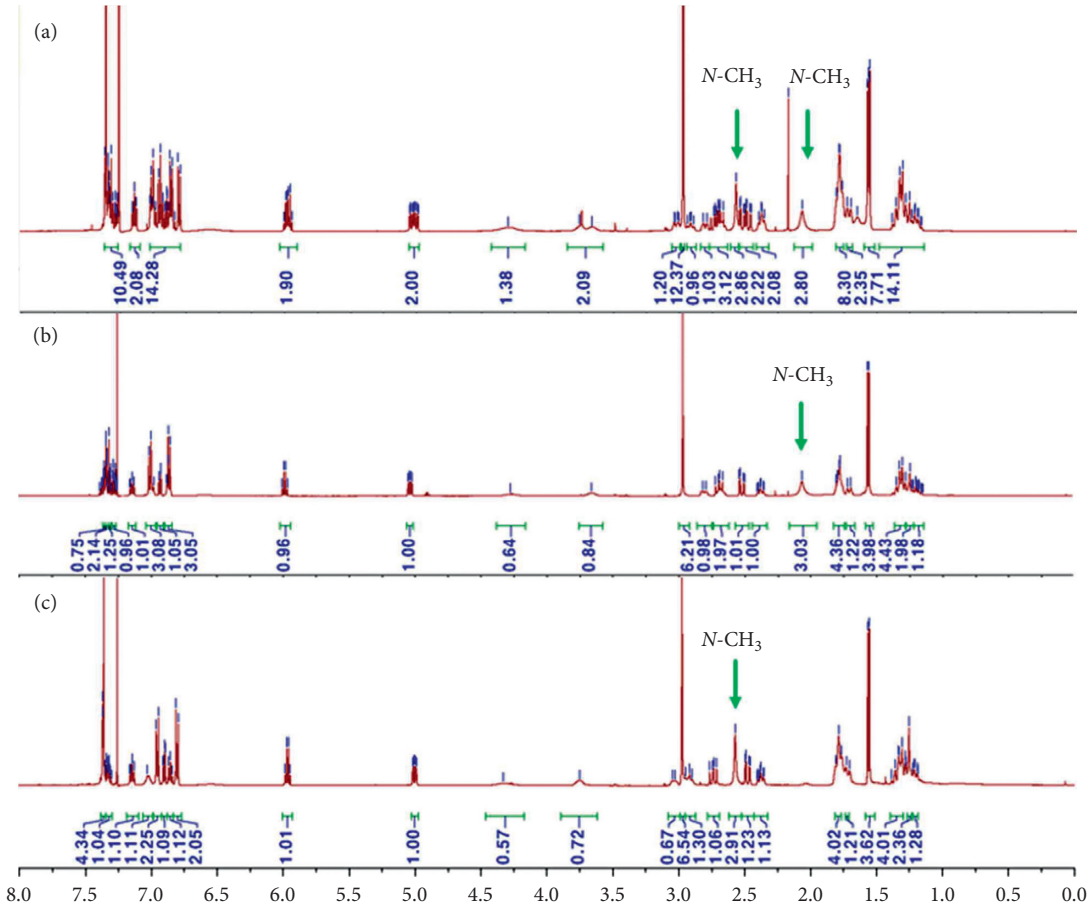


Figure 4: ^{1}H NMR spectra of (a) a mixture of diastereomeric esters **8a** and **8b**; (b) (*S*, 4*R*)-**8a**; (c) (*S*, 4*S*)-**8b**.

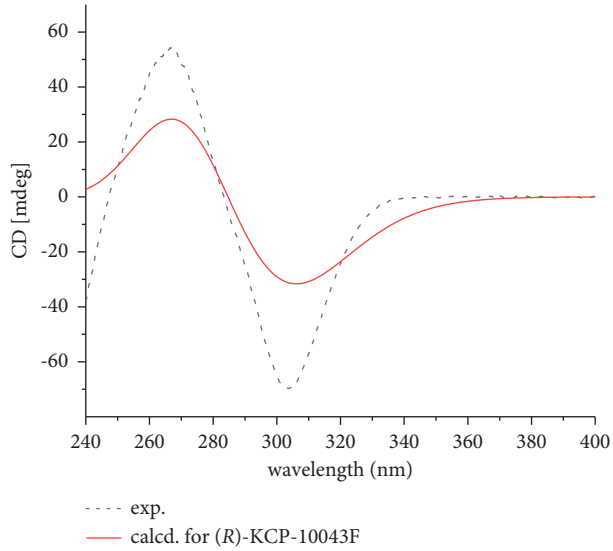


Figure 5: Experimental and TDDFT-calculated ECD spectrum of *R*-(−)-**KCP-10043F**.

Table 1: Non-mutagenic effects of racemic **(±)-KCP10043F** and its two enantiomers in *Salmonella typhimurium (TA)* and *Escherichia coli (E. coli)* strains with S9 and without S9[a].

Strain	Racemate (±)-KCP-10043F		(*R*)-(−)-KCP10043F		(S)-(+)-KCP10043F	
	+S9	−S9	+S9	−S9	+S9	−S9
Salmonella typhimurium (TA)						
TA98	x	x	x	x	x	x
TA100	x	x	x	x	x	x
TA1535	x	x	x	x	x	x
TA1537	x	x	x	x	x	x
Escherichia coli (E. Coli)						
WP2 uvrA[pKM101]	x	x	x	x	x	x

[a]The results were obtained on the baselines by using the calculation of Xenometrix (Xenometric AG, Swiss); After calculating the baseline of DMSO (negative control), the compound was regarded as a mutagen when the induction is more than 2-fold over the baseline and binomial *P* value ≥0.99; x means non-genotoxicity (negative response).

Table 2: Cytotoxicity of racemate (±)-KCP-10043F and its two enantiomers against human cancer cell lines.

Compound	Cytotoxicity (IC_{50}: μM)		
	A549[a,b]	HT-29[c]	PANC-1[d]
(*R*)-(−)-**KCP10043F**	9.35 ± 0.29	5.52 ± 0.46	12.96 ± 0.82
(*S*)-(+)-**KCP10043F**	9.78 ± 0.11	5.37 ± 0.31	10.60 ± 0.13

[a]Human lung adenocarcinoma cell line; [b]IC_{50} of racemate (±)-**KCP-10043F** = 7.0 ± 0.1 μM; [c]human colorectal adenocarcinoma cell line; [d]human pancreatic cancer cell line.

3.4. Anticancer Evaluation. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) method was used to evaluate the cytotoxic effects of (*R*)-(−)-**KCP-10043F** and (*S*)-(+)-**KCP-10043F** on three human cancer cell lines: lung cancer (A549), colon cancer (HT-29) and pancreatic cancer (PANC-1) [25]. As shown in Table 2, both enantiomers displayed almost equal cytotoxicity toward three cancer cell lines with micromolar IC_{50} values of PANC-1 (10–12 μM) > A549 (9 μM) > HT-29 (5 μM) together with the reported data (IC_{50} = 7 μM) of racemate (±)-**KCP-10043F** against A549 [11]. Therefore, it could be concluded that the anticancer activity of racemate (±)-**KCP-10043F** was equally derived from both enantiomers.

4. Conclusions

(*R*)-(−)-**KCP10043F** and (*S*)-(+)-**KCP10043F** were successfully separated from racemate (±)-KCP-10043F (OZ-001) by the chiral technique of supercritical fluid chromatography and assigned to their absolute configuration by the preparation of chiral diastereomers and ^{1}H NMR anisotropy method as well as the experimental ECD. The bacterial reverse mutation test (Ames test) for racemate **KCP-10043F** and its two enantiomers was performed to investigate their latent genotoxicities. All stereoisomers were fortunately found to be non-genotoxic against five bacterial strains with/without metabolic activation. In addition, (*R*)-(−)-**KCP-10043F** displayed almost equal cytotoxic activity to (*S*)-(+)-**KCP-10043F** against three cancer cell lines. Based on these overall results, racemate (±)-**KCP-10043F (OZ-001)** could be used for our ongoing preclinical and clinical studies without the expensive asymmetric process and/or chiral separation.

Data Availability

The supplementary materials (NMR spectrum, HPLC data, and Ames test data) used to support the findings of this study are included within the supplemental file.

Conflicts of Interest

The authors declare that there is no conflicts of interest regarding the publication of this paper.

Authors' Contributions

JunseongAhn, Dohyeong Ko. The contribution of the authors is equal.

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Supplementary Materials

The supplementary materials (NMR spectrum, HPLC data, and Ames test data) used to support the findings of this study are included within the supplemental file. (*Supplementary Materials*)

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