An Efficient One-Pot Three-Component Synthesis of Novel Quinazoline-4 Carboxylic Acid and Its Ester and Amide Derivatives

Bir Etkili Tek Ortamda Üç Bileşenli Yeni Kinazolin-4-Karboksilik Asit ve Onun Ester ve Amit Türevlerinin Sentezi

Derviş Gök
Department of Chemistry and Chemical Processing Technologies, Kütahya Technical Sciences Vocational School, Kütahya Dumlupinar University, Kütahya, Turkey

ABSTRACT

A series of novel quinazoline derivatives, which may be drug candidates, were synthesized, and their structures were characterized by IR, $^1$H NMR, $^{13}$C NMR and Q-TOF LC/MS spectrometry. First, 2-(4-chloro-phenyl)-quinazoline-4-carboxylic acid (2) was synthesized from a one-pot three-component condensation reaction of (2-amino-phenyl)-oxo-acetic acid sodium salt obtained from alkaline hydrolysis of isatin (indole-2,3-dione) with 4-chlorobenzaldehyde and ammonium acetate. The carboxylic acid compound 2 allowed the synthesis of the ester, acid chloride and amide derivatives. New quinazoline ester derivatives (3-6, 8) were synthesized by the reactions of compound 2 and various alcohols. Quinazoline amide derivatives (9-13) were then obtained from the reaction of different aliphatic and aromatic amines and 2-(4-chloro-phenyl)-quinazoline-4-carbonyl chloride (7) formed from the reaction of SOCl$_2$ and compound 2.

Key Words
Isatin, quinazoline-4-carboxylic acid, amide, ester.

ÖZ

İlaç adayları olabilecek bir dizi yeni kinazolin türevleri sentezlendi ve yapıları IR, $^1$H NMR, $^{13}$C NMR ve Q-TOF LC/MS spektrometresi ile karakterize edildi. Önce amonyum asetat ve 4-klorobenzaldehit ile isatinin (indol-2,3-dione) hidrolizinden elde edilen (2-amino-fenil)-okso-asetik asit sodiyum tuzunun aynı ortamda üç bileşenli kondensasyon reaksiyonundan 2-(4-kloro-fenil)-kinazolin-4-karboksilik asit (2) sentezlendi. Karboksilik asit bileşğini 2, ester, asit klorür ve amit türevlerinin sentezine izin verdi. Yeni kinazolin ester türevleri (3-6, 8), 2 bileşğini ve çeşitli alkollerin reaksiyonlarından sentezlendi. Daha sonra kinazolin amit türevleri (9-13), çeşitli alifatik ve aromatik aminlerin 2 bileşğini ve SOCl$_2$'ün reaksiyonundan oluşan 2-(4-kloro-fenil)-kinazolin-4-carbonyl klorürünün (7) reaksiyonundan elde edildi.

Anahtar Kelimeler
İsatin, kinazolin-4-karboksilik asit, amit, ester.

Article History:
Received: Oct 5, 2021; Revised: Feb 18, 2022; Accepted: Mar 5, 2022; Available Online: Oct 5, 2022.
DOI: https://doi.org/10.15671/hjbc.1004758
Correspondence to: D. Gök, Department of Chemistry and Chemical Processing Technologies, Kütahya Technical Sciences Vocational School, Kütahya Dumlupinar University, Kütahya, Turkey.
E-Mail: dervis.gok@dpu.edu.tr
INTRODUCTION

Quinazolines are one of the important classes of organic chemistry due to their presence in the structure of natural products and drugs. Also, they have an important place in medical chemistry because of the variety of biological and pharmacological activities exhibited by their natural and synthetic derivatives. The literature studies show that quinazolines have a wide range of biological activities such as antifungal [1], antitubercular [2, 3], anticancer [4], antiplasmodial [5], antiviral [6], anti-HIV [7], antimalarial [8], anti-inflammatory [9], antihypertensive [10], anti-diabetic [11] etc. Furthermore, compounds containing quinazoline were found to inhibit dihydrofolate reductase [12], the epidermal growth factor receptor (EGFR) tyrosine kinase [13], and cellular phosphorylation [14]. It has also been reported that quinazolines are antagonists of some biological receptors [15].

In the structure of some drug molecules such as icotinib [16], prazosin [17] and lapatinib [18], the quinazoline ring forms the basic core structure (Fig. 1).

Dacomitinib and afatinib containing quinazoline scaffold are drugs confirmed for the treatment of patients with non-small-cell lung cancer (NSCLC) [19, 20] (Fig. 2).

As explained above, the fact that quinazolines have very important biological activities has led to the synthesis of their different derivatives. For this reason, it is very important to develop new, efficient and practical methods for the synthesis of N-heterocycles in organic chemistry. When literature sources are considered, it is seen that quinazoline derivatives are obtained by different methods such as condensation of aldehydes with 2-aminobenzylamines [21], copper-catalyzed condensation of amidine hydrochlorides with o-halobenzaldehydes [22], dehydrogenative coupling reaction of (2-aminophenyl)methanols with ammonia and aldehyde [23], tandem reactions from benzylic amines and 2-aminobenzophenones [24] and copper-catalyzed Ullmann N-arylation coupling reaction [25]. Most of these methods have certain limitations, such as difficult reaction conditions, long reaction times, use of expensive and toxic catalysts and reagents, low product yields and use of volatile organic solvents. Today, more stringent legislation and restrictions are applied to reduce the environmental impact of synthetic chemicals. Therefore, it becomes very important to meet environmental sustainability requirements when creating new reaction conditions. One of the synthesis methods of quinazolines is the condensation reaction of 2-aminobenzophenones with ammonia and various aldehydes [26]. In this study, we...
synthesized quinazoline derivatives according to this method. We used 4-chlorobenzaldehyde as the aldehyde. We added ammonium acetate as the ammonia source in the reaction. We used hydrolysis isatin as an alternative to the 2-aminobenzophenone compound. When isatin is hydrolysed with NaOH\(_{(aq)}\) in alkaline medium, it turns into (2-amino-phenyl)-oxo-acetic acid sodium salt (Scheme 1).

While the aromatic primary amine group on the hydrolysis isatin compound reacts with 4-chlorobenzaldehyde, the ketone group reacts with ammonia. Thus, the synthesis of 2-(4-chloro-phenyl)-quinazoline-4-carboxylic acid sodium salt (1), a quinazoline derivative, was carried out by a one-pot three-component condensation reaction of hydrolysis isatin, 4-chlorobenzaldehyde and ammonium acetate. The quinazoline ring with a carboxyl group in the 4-position was synthesized for the first time by us in our previous study [27].

Two routes have been proposed for the mechanism of the synthesis of dihydroquinazoline and quinazoline by condensation reactions between aldehyde, 2-aminobenzophenone and ammonium acetate. In one of these routes (path a), firstly, the aldime compound is obtained from the reaction of the 2-aminobenzenophenone and aldehyde compounds, and the reaction of the obtained aldime and ammonium acetate compounds is converted to the diimine compound. Finally, it is converted to the 1,2-dihydroquinazoline compound by intramolecular cyclization of the diimine compound, which is then converted to the quinazoline compound upon aromatization by interacting with air. In the second route (path b), the reaction of ammonium acetate and aldehyde compounds is first converted to aldime, the reaction of the obtained aldime compound with 2-aminobenzophenone is then converted to 1,2-dihydroquinazoline and quinazoline as in the first route (Scheme 2) [28].
Compound 1, which is the acid salt, was converted to the quinazoline carboxylic acid derivative 2 by dissolving in water and acidifying the medium with HCl. The carboxyl group attached to the 4-position of the quinazoline ring allowed the synthesis of ester and amide derivatives. For this, new ester derivatives 3–6 of 2-(4-chloro-phenyl)-quinazoline-4-carboxylic acid (2) in various alcohols were easily synthesized under the catalysis of sulfuric acid. The carbonyl group of compound 2 was then activated by its interaction with SOCl₂ to give the 2-(4-chloro-phenyl)-quinazoline-4-carbonyl chloride (7) compound. Finally, new amide derivatives 9–13 were synthesized from the reaction of compound 7 with various aliphatic and aromatic amines. The synthesis of compounds is shown in scheme 3.

**MATERIALS and METHODS**

**Chemicals and Instruments**

The chemicals used in the synthesis and solvents were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (Steinheim, Germany) Chemical Company. Solvents were used without further purification. Reaction progress was monitored using thin layer chromatography (TLC) with an aluminium plate coated with silica gel 60F₂₅₄ (Merck Millipore, Billerica, MA, USA). The Melting points were determined on a Stuart SMP30 apparatus. IR spectra were recorded as KBr pellets on a Bruker Vertex 70 Sample compartment spectrometer. ¹H-NMR and ¹³C-NMR were recorded on a Bruker AVANCE 300 MHz spectrometer with tetramethylsilane.
(TMS) as internal standard. ¹H-NMR data are reported as follows chemical shift (s=singlet, d=doublet, t=triplet, and m=multiplet). The mass analyses were performed on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. High resolution mass spectra were recorded on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS using ESI mode.

**Synthesis**

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid sodium salt (1). A mixture of ammonium acetate (0.154 g, 2 mmol) and 4-chlorobenzaldehyde (0.140 g, 1 mmol) were added to a solution of (2-amino-phenyl)-oxo-acetic acid sodium salt (0.187 g, 1 mmol) in ethanol (10 mL) at room temperature. The mixture was stirred and heated to reflux for 24 h. The formed precipitate was filtered while it was still hot. The product was crystallized from toluene. Yield: 0.10 g (33%); M.P.: above 350 °C; IR (v, cm⁻¹): 3074 (Ar CH), 1611 (C=O), 1566-1450 (C=C and C=N), 778 (C-Cl); ¹H NMR (300 MHz, DMSO-d₆) δ (ppm): 8.54 (d, J=8.5 Hz, 2H, Ar-H), 8.14 (d, J=8.2 Hz, 1H, Ar-H), 7.98-7.89 (m, 2H, Ar-H), 7.65-7.60 (m, 3H, other Ar-H); ¹³C NMR (75 MHz, DMSO-d₆) δ (ppm): 170.79 (COONa), 169.17, 158.82, 150.73, 145.79, 134.36, 130.24, 129.11, 128.29, 127.41, 119.65; HRMS (QTOF-ESI): m/z calcd for C₁₁H₈ClₑN₂O₂: 306.0172; found: 306.0172 [M+Na⁺].

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid (2). Compound 1 (0.306 g, 1 mmol) was added to distilled water (10 mL). The obtained solution was heated to 90 °C and slowly acidified with 2N HCl (aq) till reaching pH 1. The resulting mixture was kept at 5 °C overnight. Then, the precipitate was filtered off and washed with water (3 x 15 mL). The product was crystallized from toluene. Yield: 0.26 g (91%); M.P.: 172-173 °C; IR (v, cm⁻¹): 3500-2500 (COOH), 3119 (Ar CH), 1776 (C=O, acid), 1614-1455 (C=C and C=N), 778 (C-Cl); ¹H NMR (300 MHz, DMSO-d₆) δ (ppm): 14.55 (br, s, 1H, COOH), 8.58 (d, J=8.6 Hz, 2H, Ar-H), 8.43 (d, J=8.4 Hz, 1H, Ar-H), 8.18-8.09 (m, 2H, Ar-H), 7.83 (t, J=8.2, 1H, Ar-H), 7.67 (d, J=8.6 Hz, 2H, other Ar-H); ¹³C NMR (75 MHz, DMSO-d₆) δ (ppm): 166.64 (C=O, acid), 159.85, 158.45, 157.18, 136.53, 136.01, 135.82, 130.35, 129.37, 129.07, 126.49, 119.83; HRMS (QTOF-ESI): m/z calcd for C₁₅H₉ClN₂O₂: 284.0353; found: 284.0357 [M⁺].

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid methyl ester (3). H₂SO₄ (95-97%, 0.3 mL) was added to a solution of 2 (0.284 g, 1 mmol) in methanol (20 mL) at room temperature, and the mixture was stirred and heated to reflux for 6 h. The resulting mixture was filtered while it was still hot. The solution was kept at 5 °C overnight. Then, the obtained crystals were filtered. The product was recrystallized from methanol. Yield:
0.23 g (77%); M.P.: 154-155 °C; IR (v, cm\(^{-1}\)): 2999 (Ar CH), 2952 (aliphatic CH), 1728 (C=O, ester), 1611-1445 (C=C and C=N), 1222 (C-O, ester), 781 (C-Cl); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) (ppm): 8.60 (d, \(J=8.7\) Hz, 2H, Ar-H); 8.44 (d, \(J=8.1\) Hz, 1H, Ar-H); 8.14 (d, \(J=8.5\) Hz, 1H, Ar-H), 7.95 (t, \(J=7.7\), 1H, Ar-H), 7.67 (t, \(J=7.7\), 1H, Ar-H), 7.50 (d, \(J=8.7\) Hz, 2H, other Ar-H), 4.57 (t, \(J=6.7\) Hz, 2H, CH\(_2\)). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) (ppm): 165.21 (C=O, car-bonyl), 159.13, 155.67, 152.93, 137.94, 136.14, 135.26, 121.6 (C-O, ester), 121.6, 119.30; HRMS (QTOF-ESI): m/z calcd for C\(_{18}\)H\(_{17}\)ClN\(_2\): 326.0822; found: 326.0825 [M \(^+\)].

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid butyl ester (6). H\(_2\)SO\(_4\): 302.0458 [M]; \(\delta\) (ppm): 8.63 (d, \(J=8.7\) Hz, 2H, Ar-H), 8.55 (d, \(J=8.1\) Hz, 1H, Ar-H), 8.19 (d, \(J=8.5\) Hz, 1H, Ar-H), 8.00 (t, \(J=7.7\), 1H, Ar-H), 7.74 (t, \(J=7.7\), 1H, Ar-H), 7.52 (d, \(J=8.7\) Hz, 2H, other Ar-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) (ppm): 164.72 (C=O, ester), 159.07, 158.28, 152.15, 137.07, 135.84, 134.39, 130.30, 129.13, 128.76, 128.22, 125.75, 120.22, 70.68 (OCH\(_3\)), 21.93 (CH\(_3\)); HRMS (QTOF-ESI): m/z calcd for C\(_{18}\)H\(_{17}\)ClN\(_2\): 326.0822; found: 326.0825 [M \(^+\)].
reflux for 7 h. The reaction mixture was filtered while it was still hot. The solution was kept at 5 °C overnight. Then, the obtained crystals were filtered. The product was recrystallized from toluene. Yield: 0.20 g (55%); M.P.: 172-173 °C; IR (ν, cm⁻¹): 3093 (Ar CH), 1749 (C=O, ester), 1612-1450 (C=C and C=N), 1199 (C-O, ester), 782 (C-Cl); 1H NMR (300 MHz, CDCl₃) δ (ppm): 8.67-8.61 (m, 3H, Ar-H), 8.17 (d, J=8.5 Hz, 1H, Ar-H), 7.97 (t, J=7.7 Hz, 1H, Ar-H), 7.69 (t, J=7.8, 1H, Ar-H), 7.54-7.49 (m, 4H, Ar-H), 7.41-7.33 (m, 3H, other Ar-H); 13C NMR (75 MHz, CDCl₃) δ (ppm): 163.50 (C=O, ester), 159.14, 156.24, 152.57, 141.15 (NH), 3068 (Ar CH), 2929 (aliphatic CH), 1653 (C=O, amide), 157.75, 155.90, 153.07, 135.68, 134.51, 129.62, 128.87, 128.70, 128.50, 127.79, 121.07, 41.35 (NHCH₃), 22.90 (CH₂CH₃), 11.54 (CH₃CH₃); HRMS (QTOF-ESI): m/z calcd for C₁₇H₁₆ClN₃O: 325.0982; found: 325.0981 [M⁺].

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid isopropylamide (11). Isopropylamine (0.118 g, 2 mmol) was added to a solution of 4 (0.302 g, 1 mmol) in toluene (30 mL) at room temperature. The mixture was stirred and heated to reflux for 6 h. The reaction mixture was filtered while it was still hot. The solution was kept at 5 °C overnight. Then, the obtained crystals were filtered. The product was recrystallized from toluene. Yield: 0.20 g (61%); M.P.: 191-192 °C; IR (ν, cm⁻¹): 3307 (NH), 3065 (Ar CH), 2933 (aliphatic CH), 1648 (C=O, amide), 1614-1491 (C=C and C=N), 787 (C-Cl); 1H NMR (300 MHz, CDCl₃) δ (ppm): 9.43 (d, J=8.6 Hz, 1H, Ar-H), 8.52 (d, J=8.5 Hz, 2H, Ar-H), 8.09 (d, J=8.5 Hz, 1H, Ar-H), 7.52 (d, J=8.5 Hz, 2H, other Ar-H), 4.37 (octet, J=7.7, 1H, Ar-H), 3.55 (q, J=7.7 Hz, 2H, CH₂CH₃), 1.39 (d, J=6.5 Hz, 6H, 2CH₃); 13C NMR (75 MHz, CDCl₃) δ (ppm): 164.70 (C=O, amide), 157.74, 155.99, 153.04, 137.08, 135.68, 129.63, 128.87, 128.70, 128.47, 127.79, 121.08, 41.76 (CH), 22.71 (CH₃); HRMS (QTOF-ESI): m/z calcd for C₁₈H₁₆ClN₃O: 325.0982; found: 325.0981 [M⁺].

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid phenylamide (12). Aniline (0.186 g, 2 mmol) was added to a solution of 4 (0.302 g, 1 mmol) in toluene (30 mL) at room temperature. The mixture was stirred and heated to reflux for 6 h. The reaction mixture was filtered while it was still hot. The solution was kept at 5 °C overnight. Then, the obtained crystals were filtered. The product was recrystallized from toluene. Yield: 0.21 g (58%); M.P.: 181-182 °C; IR (ν, cm⁻¹): 3305 (NH), 3064 (Ar CH), 1658 (C=O, amide), 1597-1493 (C=C and C=N), 780 (C=Cl); 1H NMR (300 MHz, CDCl₃) δ (ppm): 166.77 (C=O, amide), 165.59, 157.57, 151.49, 135.87, 135.53, 134.94, 130.61, 130.02, 129.27, 128.68, 126.76, 119.82; HRMS (QTOF-ESI): m/z calcd for C₁₈H₁₆ClN₃O: 325.0982; found: 325.0981 [M⁺].

2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid propylamide (10). n-Propylamine (0.118 g, 2 mmol) was added to a solution of 4 (0.302 g, 1 mmol) in toluene (30 mL) at room temperature. The mixture was stirred and heated to reflux for 6 h. The reaction mixture was filtered while it was still hot. The solution was kept at 5 °C overnight. Then, the obtained crystals were filtered. The product was recrystallized from toluene. Yield: 0.21 g (58%); M.P.: 165-166 °C; IR (ν, cm⁻¹): 3321 (NH), 3068 (Ar CH), 2929 (aliphatic CH), 1653 (C=O, amide), 1616-1491 (C=C and C=N), 786 (C-Cl); 1H NMR (300 MHz, CDCl₃) δ (ppm): 9.43 (d, J=8.0 Hz, 1H, Ar-H), 8.52 (d, J=8.6 Hz, 2H, Ar-H), 8.23 (br, s, 1H, NH), 8.09 (d, J=8.5 Hz, 1H, Ar-H), 7.93 (t, J=7.0, 1H, Ar-H), 7.67 (t, J=7.7, 1H, Ar-H), 7.52 (d, J=8.6 Hz, 2H, other Ar-H), 3.55 (q, J=7.4, 2H, NHCH₃), 1.77 (heptet, J=7.2 Hz, 2H, CH₂CH₃), 1.08 (t, J=7.3 Hz, 3H, CH₃); 13C NMR (75 MHz, CDCl₃) δ (ppm): 162.32 (C=O, amide), 157.66, 155.22, 153.44, 137.32, 137.21, 135.50, 134.82, 129.66, 129.26, 129.04, 128.92, 128.89, 127.69, 125.09, 121.22,
2-(4-Chloro-phenyl)-quinazoline-4-carboxylic acid (4-sulfamoyl-phenyl)-amide (13). 4-aminobenzenesulfonamide (0.344 g, 2 mmol) was added to a solution of 4 (0.302 g, 1 mmol) in THF (30 mL) at room temperature. The mixture was stirred and heated to reflux for 6 h. The reaction mixture was filtered while it was still hot. The resulting precipitate was washed with hot water and ether (3 x 5 mL). The product was crystallized from DMF-water mixture. Yield: 0.24 g (55%); M.P.: 323-324°C; IR (ν, cm⁻¹): 3303 (NH), 3122 (Ar CH), 1675 (C=O, amide), 1614-1448 (C=C and C=N), 1336 (SO₂ asym.), 1156 (SO₂ sym.), 778 (C-Cl); ¹H NMR (300 MHz, DMSO-d₆) δ (ppm): 11.35 (s, 1H, NH), 8.71-8.63 (m, 3H, Ar-H), 8.21-8.05 (m, 4H, Ar-H), 7.92-7.81 (m, 3H, Ar-H), 7.69 (d, J=8.5 Hz, 2H, other Ar-H), 7.37 (s, 2H, SO₂NH₂); ¹³C NMR (75 MHz, DMSO-d₆) δ (ppm): 164.08 (C=O, amide), 159.91, 158.19, 152.07, 141.52, 140.16, 136.63, 135.92, 135.82, 130.65, 129.41, 129.31, 129.07, 127.23, 126.82, 120.73, 120.19; HRMS (QTOF-ESI): m/z calcd for C₂₁H₁₄ClN₃O₃S: 438.0553; found: 438.0546 [M⁺].

RESULTS and DISCUSSION

In this study, new quinazoline derivative compounds carrying carboxyl group in 4-position, which were synthesized by us for the first time, were synthesized. The hydrolysis isatin compound we used as the starting material was easily obtained from the alkaline hydrolysis of isatin. Quinazoline derivative, which is a salt of carboxylic acid, was synthesized from the one-pot three-component reaction of hydrolysis isatin, ammonium acetate and 4-chlorobenzaldehyde. Ammonium acetate, one of the starting materials, creates a nitrogen source with ammonia, while acetic acid makes the medium weakly acidic. This shows the importance of the role of ammonium acetate in the reactions.

In the reaction mechanism shown above, an aromatic amine compound (DMAP) is used as a catalyst in the synthesis of quinazoline. Because we do not use a catalyst in the reaction to obtain the quinazoline derivative, the catalytic function is presumed to be the hydrolysis isatin compound containing the aromatic primary amine group such as DMAP. Therefore, while the hydrolysis isatin compound is present as a reactant in the reaction, it also acts as a catalyst.

The quinazoline carboxylic acid salt 1, insoluble in hot ethanol, was easily separated from the reaction medium by filtration. In this way, the main product was easily separated from the reaction medium, where there were both starting materials and by-products such as dihydroquinazoline derivative. The quinazoline carboxylic acid salt containing the aromatic ring was dissolved in water and turned into a water-insoluble carboxylic acid derivative by acidifying the medium with HCl. Then, quinazoline carboxylic acid derivative 2, which is insoluble in acidic medium, was easily isolated from the medium by filtration. The fact that 1 and 2 products are easily obtained by the filtration method has facilitated and accelerated the chemical process.

The carboxyl group attached to the 4-position of the obtained quinazoline ring was easily converted to its esters by the Fischer esterification method with various alcohols under the catalysis of sulfuric acid. Quinazoline carboxylic acid compound (2) was activated to quinazoline acid chloride compound (7) with high yield (89%) by reacting with SOCl₂. The synthesis of amide derivatives was also carried out from the reaction of compound 7 with various aromatic and aliphatic amines.

As a result, we successfully obtained the ester and amide derivatives of the quinazoline, which we synthesized with a one pot three component reaction. Because quinazoline derivatives show a wide variety of biological activities in the literature, we hope that the compounds obtained from this study will contribute to medicinal chemistry.

Acknowledgements
The author thank the Kütahya Dumlupınar University Technology Research Fund for supporting this study with a scientific research project (No, 2015/24).
References


