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Diastereomeric Separation of a Novel Chalcone Derivative by Chiral HPLC

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Abstract

In this study, a facile and versatile procedure for the synthesis of a chalcone derivate that a functionalized (*E*,*Z*)-2-(4-aminobenzylidene)acenaphthylen-1(2H)-one, **4** was synthesized and also characterized by using FT-IR, NMR and MS. A new HPLC method for the separation of its diastereomers was developed by using a chiral column that contains the amylose tris(3,5-dimethylphenylcarbamate) as a chiral selector since it is effective, reproducible and can be used for preparative purposes. High percentage recovery claims that the presented method was not affected by the impurities in the biological media. RSD% values for the repeatability studies suggest that the optimized method was highly precise and reproducible which is very important for these promising advances, chalcone-based science is expected to be applied in drug discovery.

Keywords: Chalcone; Diastereomeric separation; Acenaphtenone; Chiral column; HPLC.

Kiral HPLC ile Yeni Bir Kalkon Bileşiğinin Diastereomerik Ayrılması

Öz

Bu çalışmada (*E*,*Z*)-2-(4-aminobenziliden)asenaftilen-1(2H)-on, **4** bileşiği kolay ve hızlı bir metodla sentezlenip FT-IR, NMR ve MS kullanılarak karakterize edilmiştir. Bileşiğin diastereomerlerinin ayrılması için etkili, tekrarlanabilir, preparatif amaçlar için uygun amiloz

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tris(3,5-dimetilfenilkarbamat) kiral seçici kolon kullanıldığı bir HPLC metodu geliştirilmiştir. Yüksek geri kazanım oranı bileşiğin biyolojik ortamdaki safsızlıklardan etkilenmediğini göstermektedir. Tekrarlanabilirlik ile ilgili çalışmalarda % BSS değerleri, optimize edilen metodun kalkon temelli bileşiklerin yer aldığı ilaç geliştirme çalışmalarında yüksek hassasiyet ve tekrarlanabilirlik değerleri ile kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Kalkon; Diastereomerik ayrım; Asenaftenon; Kiral kolon; HPLC.

1. Introduction

Chalcones are biologically important compounds bearing in the scaffold 1,3-diarylpropenones. They have attracted much interest due to the several biological activities including antiproliferative, antioxidant, anti-inflammatory, and anticancer effects on the important areas of ongoing research in medicine and molecular biology [1-3]. Apart from the biological activities of chalcones, chalcone-types compounds such as bichalcones, dihydrochalcones, and fused chalcones are being used concerning nonlinear optics, photorefractive polymers, holographic recording materials, and fluorescent probes for the sensing of any chemical species in media [4-5]. The chalcone type compounds may exist in two diastereomeric forms, *Z* and *E*, the *E* isomer is the most thermodynamically stable and lower in energy because diaryl groups are found as far away from one another as possible and are arranged on the opposite side of the double bond.

HPLC with a chiral stationary phase (CSP) is very useful tool for the separation of the enantiomers of the chiral molecules since it is effective, reproducible, and can be used as preparative purposes [6-7]. Most analytical separations for enantiomers are performed by using chiral columns. However, there are fewer attempts to separate the diastereomers by using these type of columns [8]. There is a diastereomer separation method available either by using the silica gel on a column or diastereomeric ionic crystals, e.g., acid/amine, or inclusion complex crystals are fractionally recrystallized. Therefore, the development and application examples of the separation methods may have a crucial role in the progress of molecular chirality level and also to discover the difference in pharmacokinetic, pharmacodynamic, metabolic, and toxic properties between the diastereomers of drug-like compounds and precursors, and other biologically active compounds [9]. To date, many classes of chalcone type compounds have been described as precursors that have generated a range of plant metabolites, revealing interesting biological activities. They have developed by changing aromatic rings in the scaffold, synthetically well-established protocols and using extended π-conjugated systems. Among these ligands, there are only a few examples of chalcones bearing acenaphtene ring, but there is no study about the

separation on chiral columns of diastereomers or the use of chiral additives in the mobile phase. This serious knowledge gap with various biological activities of chalcone rings prompted us to design and synthesis of novel (*E*,*Z*)-2-(4-aminobenzylidene)acenaphthylen-1(2H)-one, **4** and its separation on HPLC system with chiral column since these promising advances, chalcone-based science is expected to be applied in drug discovery [10-12].

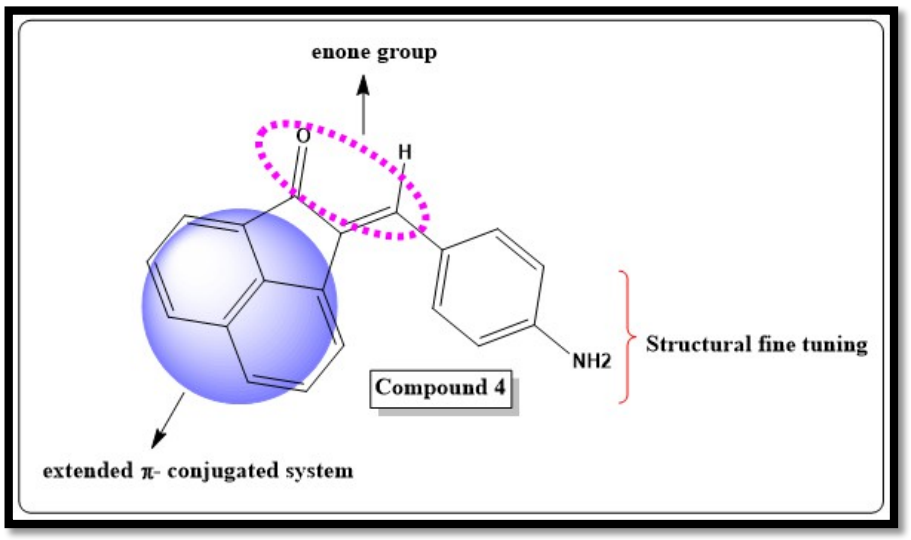

Figure 1: Design of chalcone compound 4

Herein, the author report a facile and versatile procedure for the synthesis of a chalcone derivate that a functionalized (*E*,*Z*)-2-(4-aminobenzylidene)acenaphthylen-1(2H)-one **4** and development of a HPLC method for the seperation of its diastereomers by using chiral column which contains the amylose tris(3,5-dimethylphenylcarbamate) as chiral selector.

2. Materials and Methods

2.1. Sample preparation

The Synthesis of 1-Acenaphtenone: 1-Acenaphtenone was synthesized by using literature methods with slight modification. 1-Naphtylacetylchlorid was obtained quantitative yield by using freshly distilled thionyl chloride and 3h reflux and directly used for the next step [13].

The Synthesis of (*E*,*Z*)-2-(4-nitrobenzylidene)acenaphthylen-1(2H)-one, **3**: Equivalent amounts of 1-acenaphtenone, and p-nitrobenzaldehyde are dissolved 20 mL ethanol. Subsequently, ethanolic solution (1.5 equivalent KOH in 5 mL ethanol) was added slowly to the reaction mixture and kept 24 hours at room temperature under stirring. During the period of reaction the color of mixture changes from yellow to dark red**.** The solution was added to ice water and neutralized

with dilute HCl. The precipitated solid was filtered and recrystallized twice from ethanol afforded compound **3**. (Yield: 80%, yellow solid); m.p. 243–245 °C. lit: 240-242 ^{o}C [14].

The Synthesis of (*E*,*Z*)-2-(4-aminobenzylidene)acenaphthylen-1(2H)-one, **4**: To a solution of compound **3** (1mmol, 301 mg) in 15 mL ethanol was added to $SnCl_2 \cdot 2H_2O$ (4.6 mmol, 1.0 g). The mixture was heated at 90 °C for 4 h by monitoring thin layer chromatography (TLC). After cooling to the room temperature, the reaction mixture was poured over ice to precipitate and treated with a 25% aqueous NaOH solution to adjust pH to 9. The obtained residue was extracted with 2 × 50 mL ethyl acetate, dried over Na_2SO_4, and evaporated to dryness. The solid was purified by column chromotography using ethyl acetate/n-hexane (2:1) as eluent to afford compound **4**. (Yield: % 60 dark orange solid); m.p. 327–330 °C. IR (KBr) ν_{max}: 3427, 3206, 3041, 2926, 2845, 1687,1517, 820, 770 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.25 (d, *J* = 7.0 Hz, 1H), 8.11 (d, *J* = 7.1 Hz, 1H), 8.01 (dd, *J* = 7.0, 0.8 Hz, 1H), 7.97 (d, *J* = 8.3 Hz, 1H), 7.90-7,80 (m, 2H), 7.73-7,60 (m, 3H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.05 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186, 2, 148.2, 137.1, 134.9, 134.4, 133.9, 132.1, 131.4, 130.4, 129.9, 129.6, 129.2, 127.9, 126.9, 125.7, 123.2, 115.6, TOF- MS (m/z), calcd for $C_{19}H_{14}NO$ $[M-H]^{+}$, 272,1075; found, 272,1046.

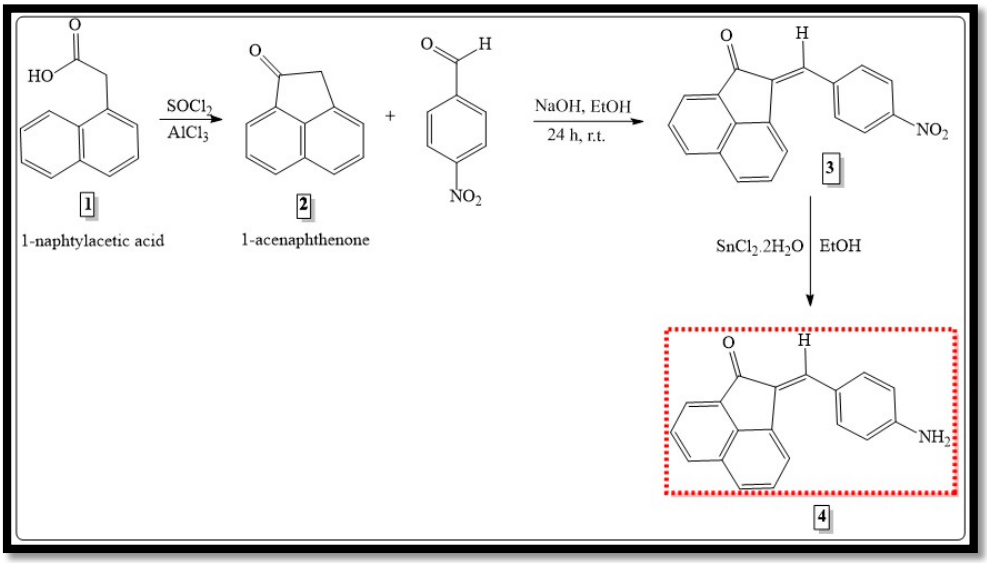

Figure 2: Synthetic routes to obtain compound 4

2.2. Materials and methods

All chemicals were purchased from commercial sources, and all of them are analytical grade. The progress of the reaction was monitored by TLC which was performed by using Merck

silica gel (60 F254) plates (0.25 mm) and visualized under ultraviolet light (UV). The melting points were measured by using the Electrothermal IA9200 apparatus. FT-IR (ATR) spectra were recorded on Perkin-Elmer Spectrum 100 FT-IR spectrophotometer. NMR spectra were measured in DMSO-$_{d6}$ solvent with TMS as an internal standard. Chemical shifts are expressed in δ units (ppm on a Bruker Avance III 400 MHz (^{1}H: 400 MHz, ^{13}C: 100 MHz) NaNoBay FT-NMR spectrometer at the Mersin University Advanced Technology Education, Research and Application Center (MEITAM). Coupling constants (*J*) are given in hertz (Hz). The Mass spectrum was recorded by Agilent 6530 Accurate-Mass (Q-TOF LC/MS) in m/z (rel. %).

The structure of the studied analyte is shown in Fig. 1. HPLC-grade n-hexane and isopropanol as well as chemical-grade formic acid, were supplied by Karl Roth (Karlsruhe, Germany). Chiral column included Amylose tris(3,5-dimethylphenylcarbamate) as CSP was provided by Phenomenex Inc. (Torrance, CA, USA). and the dimensions of the analytical column was of 250×4.6 mm dimensions packed with silica particles of 5µm nominal particle size. An Agilent 1100 HPLC instrument (Agilent Technologies, Palo Alto, CA, USA) including the Chemstation software (version B.03.02-SR2) was used for instrument control, data acquisition, and data processing. HPLC separations were performed at 45 ^{o}C at 1.00 mL/min mobile phase flow rate and 10 µL injection volume if not mentioned otherwise. The mobile phases contained n-hexane : isopropanol, 60:40 (v/v). UV detection was performed at 225 nm.

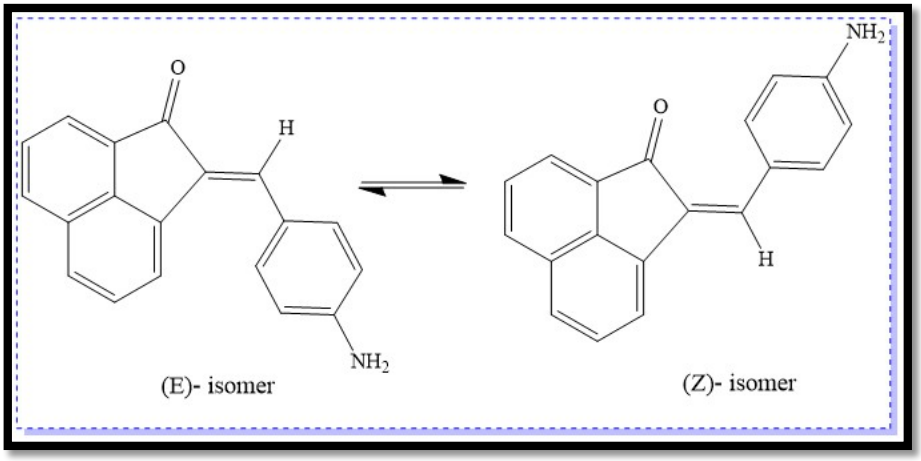

Figure 3: Diastereomeric forms of compound 4

3. Results and Discussion

3.1. Ligand design and synthesis

Synthetic access to the novel acenaphtenopyrimidine derivative compound 4 is outlined in Fig. 2. Compound **4** was synthesized from commercially available starting materials 1-naphtylacetic acid (1) and thionyl chloride via ring closure by intramolecular Friedel-Crafts reaction following reported procedures with slight modifications [13]. Preparation of novel intermediate compound **3** was carried out via claisen schmidt condensation using 1-acenaphthenone, **2** and p-nitrobenzaldehyde under the alkaline condition to obtain 80% yield. (*E*,*Z*)-2-(4-nitrobenzylidene)acenaphthylen-1(2H)-one **3** was readily reduced by $SnCl_2$, 2 H_2O in ethanol to afford compound **4** in 60 % yield. All compounds were identified and fully characterized by FT-IR, NMR, and mass spectroscopy.

3.2. Method development for the separation of diastereomers

Several types of chiral selectors were used in the present study. The nature and the backbone of these chiral selectors have significantly affected the separation behaviour of the diastereomers. There was no separation obtained by using Lux Cellulose 2, Lux Cellulose 3, Lux i-Cellulose 5 and partially separation observed by using Lux Cellulose 4, Lux Amylose 1. Furthermore, the successful separation between the diastereomers achieved via Lux Amylose 2, Lux Cellulose 1 and immobilised version of Lux i- Amylose 1. In the chromatographic analysis, the resolution between the target analytes has priority before the detection and quantification. On the other hand, while solvent consumption is another problem, the analysis time should be reduced as much as possible. Because of this reason, Lux i-Amylose 1 column was selected for the further steps due to the elution time of the target analytes. After selecting the optimum stationary phase, mobile phase trials were realized to obtain better peak shapes as well as the shortest analysis time. As a mobile phase, composition alcohol-hydrocarbon mixtures (n-hexane : isopropanol) with varied percentages were tested. The amount of isopropanol changed from 20% to 40% and due to the increased resolution between the diastereomers with the decreased analysis time 40% was selected as an optimum condition. After optimizing the major parameters, minor ones such as the temperature of the column and the flow rate were arranged. The flow rate was changed from 1 mL/min to 2 mL/min. As expected, the analysis time decreased significantly, but due to the high column backpressure, flow rate kept constant as 1 mL/min. Lastly, the effect of temperature on the separation was evaluated from 25-50 ^{o}C. It was observed that when using higher temperatures the elution times of the analytes decreased with the increasing of the resolution. Because of this reason, 45 ^{o}C was selected for the further steps while there is no significant change between 45 and 50 ^{o}C. After all of these optimization studies, the resultant chromatogram with the system suitability test parameter results were demonstrated in Fig. 4. and Table 1. A system suitability

test is to prove that the method can provide results of acceptable precision and accuracy. In general, the performed method suitability is required at least two of these criteria.

Table 1: System suitability test parameters for the analytes

Parameters	Isomer I	Isomer II
Retention time (min)	8.797	11.713
Retention factor (k')	2.258	3.338
Theoretical plates numbers (N)	10320	10020
Separation factor (α)	-	1.478
Resolution (Rs)	-	7.149
Symmetry	1.066	1.052
Tailing factor (Tf)	1.101	1.091

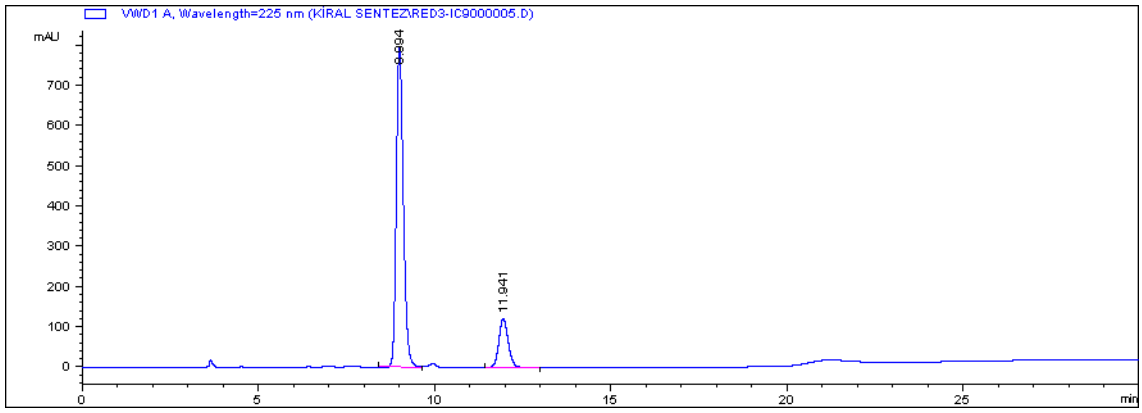

Figure 4: Chromatogram related with optimized conditions of Compound 4 isomers

3.3. Method validation

International Council on Harmonization (ICH) guidelines were used for the analytical method development and validation [15]. The validation of the methods were quantified for the isomers of Compound **4** with the following parameters: the limit of detection (LOD), limit of quantification (LOQ), accuracy, range, precision, specificity, linearity, etc. As for the raw compound **4,** stock solutions were prepared by weighing 10 mg solid and dissolving 10 mL in methanol. Standard solutions for HPLC were prepared by differentiating the concentration in the range between 1-250 µg/mL. Calibration curve was created by plotting the peak area of the isomers of compound **4** to the concentration (Table 2). Biological samples were prepared similar to the raw materials with an additional step of protein precipitation by using methanol. After the precipitation step, the working standards of synthetic urine samples were applied to the system

by dilute and shoot methodology. All results were demonstrated in Table 2, and the chromatogram related to the biological samples shown in Fig. 5.

Table 2: The obtained results from HPLC method

	Raw Material		Biological Sample	
	Isomer I	Isomer II	Isomer I	Isomer II
Linearity range (µg/mL)	1-100	1-100	5-100	5-100
Slope	66.887	26.6	66.138	26.292
Determination coefficient (R^2)	0.999	0.9991	0.9993	0.9968
Intercept	71.658	49.991	80.941	68.102
Limit of detection (µg/mL)	0.06	0.2	0.56	1.25
Limit of quantificatioin (µg/mL)	0.19	0.59	1.67	3.75
Within-day precision (RSD%)	<%5	<%5	<%5	<%5
Added Amount	-	-	10	10
Found Amount	-	-	10.56	10.54
Recovery (%)	-	-	105.65	105.46
Bias	-	-	-5.65	5.46

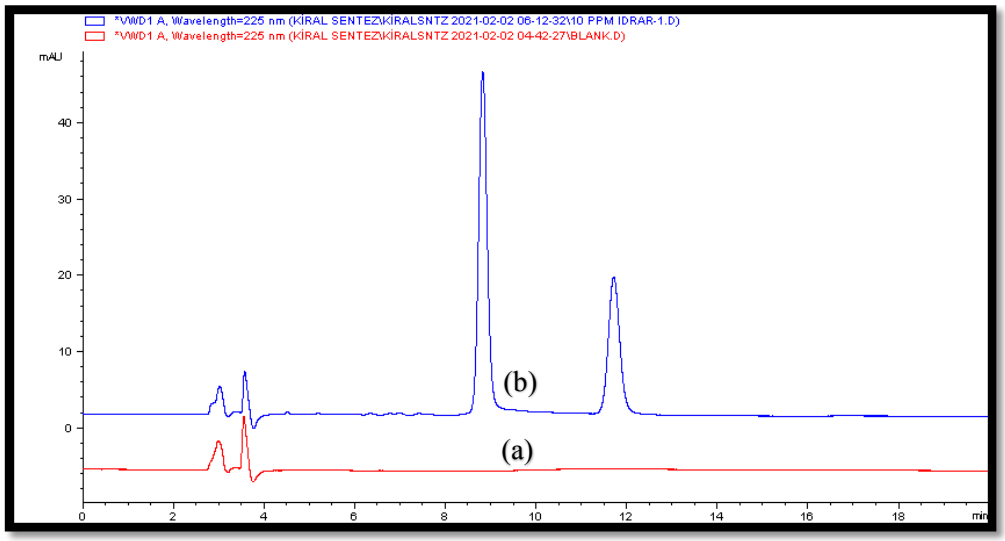

Figure 5: The HPLC chromatogram of the synthetic urine blank (a) and spiked with Compound **4** (b) (10 µg/mL)

4. Conclusion

A novel chalcone derivate was prepared by using a facile and versatile procedure for the synthesis of a functionalized (*E*,*Z*)-2-(4-aminobenzylidene)acenaphthylen-1(2H)-one, **4**, separated its diastereomers by using a chiral column that included amylose tris(3,5-dimethylphenylcarbamate) as chiral selector. The developed HPLC method has come to the

forefront since it presents a selective, sensitive, and accurate determination of chalcone diastereomers to contribute to the progress of chalcone-based drug discovery [16-20]. The optimized method was fully validated as per the ICH guidelines. High percentage recovery claims that the presented method was not affected by the impurities in the biological media. RSD% values for the repeatability studies suggest that the optimized method was highly precise and reproducible.

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