EVALUATION OF THE SYNTHESIS AND STRUCTURE OF NEW AZETIDIN-2-ONES OF FERULIC ACID

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EVALUATION OF THE SYNTHESIS AND STRUCTURE OF NEW AZETIDIN-2-ONES OF FERULIC ACID (Abstract): Aim: To synthesize some new azetidin-2-ones of ferulic acid and to evaluate them from physicochemical and spectral point of view. Material and methods: The synthesis was carried out in several steps: (i) obtaining the ferulic acid chloride; (ii) obtaining the ferulic acid hydrazide with hydrazine hydrate (98%); (iii) condensation of ferulic acid hydrazide with different benzaldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-flouro-/4-bromo-benzaldehyde) in order to obtain the corresponding hydrazones; (iv) cyclization of ferulic acid hydrazones with chloroacetyl chloride in freshly distilled toluene medium and in the presence of triethylamine, resulting in the corresponding azetidin-2-ones. Results: Six new azetidin-2-ones of ferulic acid were synthesized. They were characterized in terms of their physicochemical properties and their structure was confirmed by IR and ¹H-NMR spectroscopy. Conclusions: Six new azetidin-2-ones of ferulic acid were synthesized, physicochemically characterized and validated spectrally. Keywords: FERULIC ACID, AZETIDIN-2-ONES, SPECTRAL VALIDATION.

In the present days we are witnessing to a growing interest of researchers in the use of antioxidants in reducing oxidative stress. Development of new compounds including in their structure biologically active entities, especially heterocyclic systems is a major concern for researchers. In this context, special attention is directed to azetidin-2-one derivatives.

It is known that the azetidin-2-one ring is the common structure of beta-lactam antibiotics such as penicillins, cephalosporins, carbapenems, monobactams and nocardicine used as chemotherapeutic agents in the treatment of infectious diseases (1). Recent studies assign to this heterocyclic ring many other biological effects: hypoglycemic, antitumor, anti-HIV, anti-inflammatory, analgesic and antioxidant actions (2, 3).

On the other hand, ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a phenolic compound widely distributed in the plant world. Due to its antioxidant effect, ferulic acid may protect DNA and lipids from oxidation by reactive oxygen species and could be useful in the prevention and treatment of several oxidative stress-related disorders including Alzheimer's disease, diabetes mellitus, cancer, hypertension, and atherosclerosis (4, 5).
The aim of this study was to develop new compounds with heterocyclic structure and improved antiinflammatory and antioxidat effects. In order to achieve this goal new azetidin-2-one derivatives of ferulic acid there were synthesized.

**MATERIAL AND METHODS**

Ferulic acid, thionyl chloride, hydrazine hydrate, aromatic aldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde), chloroacetyl chloride, triethylamine, organic solvents (p.a. quality) were purchased from Sigma Aldrich Company and Fluka Company. All reagents and solvents were used without previous purification. For monitoring the reaction, TLC silica gel 60 F254 plates, purchased from Merck Company, were used.

**Synthesis of the azetidin-2-one derivatives of ferulic acid:** Ferulic acid was reacted with thionyl chloride to obtain the ferulic acid chloride that was next treated with hydrazine hydrate 98% to obtain ferulic acid hydrazide. By condensation with different aromatic aldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benzaldehyde) the corresponding hydrazones (4a-f) were obtained. These hydrazones were cyclized with chloroacetyl chloride in freshly distilled toluene medium and in the presence of triethylamine to obtain the corresponding azetidin-2-ones (5a-f) (fig. 1) (6, 7, 8).

**General procedure for azetidin-2-ones of ferulic acid synthesis.** Over 0.025 moles (5 g) of ferulic acid and under continuous stirring 0.15 moles of thionyl chloride were added. The reaction mixture was maintained at 50°C for 4 hours and the excess of thionyl chloride was removed by rotary evaporation. In the second step, over the ferulic acid chloride (0.05 moles) 0.25 moles of hydrazine hydrate 98% were added. The reaction mixture was maintained on ice bath for 2 hours and then at room temperature for another 2 hours, under continuous stirring. Then 15 mL of cold water were added and the precipitate was dried at room temperature. Finally, 0.015 moles (3 g) of ferulic acid hydrazide with a few drops of glacial acetic acid and 0.015 moles of aromatic aldehydes (2-hydroxy-/2-nitro-/4-chloro-/4-fluoro-/4-bromo-benz-
aldehyde) were refluxed for 13-20 hours in absolute ethanol. The reaction was monitored by TLC, using ethyl acetate:methanol:acetone:water (5:2:2:1) as solvent system. In the last step, the 0.0015 moles (0.5 g) of hydrazone with 0.0022 moles of chloroacetyl chloride, in the presence of triethylamine, were refluxed for 15-20 hours in toluene. The reaction was monitored by TLC, using dichloromethane:methanol (9:1) as solvent system. The obtained precipitate was washed with 1M hydrochloric acid, and the organic layer was dried on anhydrous magnesium sulphate and then washed with petroleum ether.

Physicochemical characterization: The azetidin-2-one derivatives of ferulic acid were physicochemically characterized: melting point, output, molecular formula, relative mass, solubility in water and other organic solvents. The general synthesis was optimized in order to gain great output and advanced purity for the obtained derivatives. The reaction was monitored by TLC on 60 F254 silica gel plates, and spots were visualized by UV light at 254 nm. The melting point was determined by using Buchi M 565 (Buchi, Switzerland).

Spectral characterization: The IR spectra of the synthesized derivatives were recorded using a Fourier transform spectrometer, ABB-MB 3000 FT-IR MIRacle™ Single Bounce ATR-crystal ZnSe, and the studied spectral range was between 4000–5000 cm⁻¹, at a resolution of 4 cm⁻¹, 16 scans being carried out for each sample. The spectra were interpreted using the Horizon MB™ FT–IR software (9, 10). For ¹H–RMN, the samples were dissolved in hexadeuterio-dimethyl- sulfoxide (DMSO-d₆) and the Bruker 400 MHz NMR spectrometer (Germany) was used. The spectral signals were automatically integrated and the chemical shift values were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The spectra were interpreted using MestReNova software (11).

RESULTS AND DISCUSSION

The azetidin-2-one derivatives of ferulic acid are crystalline powders, colored in different shades of brown, very slightly soluble in dimethylsulfoxide (DMSO) and dimethylformamide (DMF), partially soluble in absolute ethanol, methanol, chloroform, acetone, dioxane and insoluble in distilled water, benzene and ethyl ether. The physicochemical and spectral characteristics are listed (tab. I, II).

<table>
<thead>
<tr>
<th>Comp.</th>
<th>R</th>
<th>Physicochemical characteristics</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Molecular formula</td>
<td>yield (%)</td>
<td>melting point (°C)</td>
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</tr>
<tr>
<td>5a</td>
<td>-H</td>
<td>C₁₉H₁₇ClN₂O₄</td>
<td>372.80</td>
<td>24.00</td>
<td>72</td>
</tr>
<tr>
<td>5b</td>
<td>-OH(2)</td>
<td>C₁₉H₁₇ClN₂O₅</td>
<td>388.80</td>
<td>25.93</td>
<td>107-109</td>
</tr>
<tr>
<td>5c</td>
<td>- NO₂(2)</td>
<td>C₁₉H₁₆ClN₃O₆</td>
<td>417.80</td>
<td>17.46</td>
<td>123</td>
</tr>
<tr>
<td>5d</td>
<td>-Cl(4)</td>
<td>C₁₉H₁₆Cl₂N₂O₄</td>
<td>407.25</td>
<td>39.90</td>
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<tr>
<td>5e</td>
<td>-F(4)</td>
<td>C₁₉H₁₆ClF₂N₂O₄</td>
<td>390.79</td>
<td>39.10</td>
<td>150</td>
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<tr>
<td>5f</td>
<td>-Br(4)</td>
<td>C₁₉H₁₆BrClN₂O₄</td>
<td>450.00</td>
<td>31.12</td>
<td>89</td>
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</table>
Evaluation of the synthesis and structure of new azetidin-2-ones of ferulic acid

Spectral characteristics of azetidin-2-one derivatives of ferulic acid (5a-f)

<table>
<thead>
<tr>
<th>Comp.</th>
<th>FT-IR characteristic band (cm⁻¹)</th>
<th>¹H-RMN (400 MHz, DMSO-d₆, δ ppm)</th>
</tr>
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<tbody>
<tr>
<td>5a</td>
<td>3190 (NH), 2966, 1587, 839 (aromatic ring), 2839, 2773, 1447, 1402 (CH aliphatic), 1724 (C=O cyclic), 1651 (CO-NH), 1587 (C=N), 1394 (CH-N), 1255 (C-N), 723 (CH-Cl)</td>
<td>3.74 (s, 3H, OCH₃), 5.04 (d, 1H, CH-Cl), 5.34 (d, 1H, Ar-CH-N), 6.79-6.82 (m, 1H, Ar-H), 6.98-7.12 (m, 2H, Ar-H), 7.39-7.43 (m, 1H, Ar-H), 7.48-7.52 (m, 1H, Ar-H), 7.54-7.60 (m, 3H, Ar-H), 7.65-7.68 (m, 2H, Ar-H)</td>
</tr>
<tr>
<td>5b</td>
<td>3065 (NH, OH), 2974, 1627, 1524, 893 (aromatic ring), 2845, 2754, 1487, 1387 (CH aliphatic), 1732 (C=O cyclic), 1572 (CO-NH), 1524 (C=N), 1380 (CH-N), 1269 (C=N), 1196 (C-O), 690 (CH-Cl)</td>
<td>3.81 (s, 3H, OCH₃), 5.02 (d, 1H, CH-Cl), 5.32 (d, 1H, Ar-CH-N), 6.55-6.59 (m, 1H, Ar-H), 6.80-6.87 (m, 1H, Ar-H), 7.16-7.29 (m, 2H, Ar-H), 7.33-7.35 (m, 1H, Ar-H), 7.60-7.65 (m, 1H, Ar-H), 7.72-7.79 (m, 1H, Ar-H), 7.94 (s, 1H, Ar-H)</td>
</tr>
<tr>
<td>5c</td>
<td>3250 (NH), 2943, 1724, 1510, 851 (aromatic ring), 2895, 2856, 1471, 1419, (CH aliphatic), 1728 (C=O cyclic), 1628 (CO-NH), 1607 (C=N), 1387 (CH-N), 1232 (C-N), 1570, 1342 (NO₂), 709 (CH-Cl)</td>
<td>3.83 (s, 3H, OCH₃), 5.32 (d, 1H, CH-Cl), 5.54 (d, 1H, Ar-CH-N), 6.69-6.75 (m, 1H, Ar-H), 6.88-6.93 (m, 1H, Ar-H), 6.99 (dd, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.35-7.43 (m, 1H, Ar-H), 7.59-7.64 (m, 1H, Ar-H), 7.91-7.98 (m, 2H, Ar-H), 8.09 (dd, 1H, Ar-H)</td>
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<td>5d</td>
<td>3254 (NH), 2945, 1724, 1510, 862 (aromatic ring), 2901, 2847, 1470, 1402 (CH aliphatic), 1734 (C=O cyclic), 1595 (CO-NH), 1510 (C=N), 1396 (CH-N), 1230 (C-N), 814 (C-Cl), 703 (CH-Cl)</td>
<td>3.82 (s, 3H, OCH₃), 4.92 (d, 1H, CH-Cl), 5.24 (d, 1H, Ar-CH-N), 6.80-6.85 (m, 2H, Ar-H), 6.95-7.04 (m, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.37 (dd, 1H, Ar-H), 7.52 (dd, 2H, Ar-H), 7.77 (dd, 2H, Ar-H)</td>
</tr>
<tr>
<td>5e</td>
<td>3252 (NH), 2945, 1680, 1506, 825 (aromatic ring), 2847, 1470, 1419 (CH aliphatic), 1730 (C=O cyclic), 1634 (CO-NH), 1601 (C=N), 1384 (CH-N), 1227 (C-N), 1095 (C-F), 714 (CH-Cl)</td>
<td>3.83 (s, 3H, OCH₃), 4.94 (d, 1H, CH-Cl), 5.26 (d, 1H, Ar-CH-N), 6.79-6.89 (m, 2H, Ar-H), 6.99-7.03 (m, 1H, Ar-H), 7.11 (s, 1H, Ar-H), 7.29-7.37 (m, 3H, Ar-H), 7.81 (td, 2H, Ar-H)</td>
</tr>
<tr>
<td>5f</td>
<td>3252 (NH), 2943, 1724, 1510, 836 (aromatic ring), 2893, 2837, 1472, 1419 (CH aliphatic), 1726 (C=O cyclic), 1583 (CO-NH), 1562 (C=N), 1386 (CH-N), 1232 (C-N), 858 (C-Br), 724 (CH-Cl)</td>
<td>3.85 (s, 3H, OCH₃), 4.95 (d, 1H, CH-Cl), 5.28 (d, 1H, Ar-CH-N), 6.77-6.85 (m, 2H, Ar-H), 6.93-6.99 (m, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.30-7.37 (m, 1H, Ar-H), 7.58-7.63 (m, 2H, Ar-H), 7.72-7.75 (m, 2H, Ar-H)</td>
</tr>
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</table>

The azetidin-2-one derivatives of ferulic acid were characterized by IR absorption. The FT-IR spectra revealed the presence of C-O stretching band cyclic at 1724–1734 cm⁻¹. The IR spectra also showed the characteristic absorption band of CH-N at 1380–1396 cm⁻¹ and the characteristic absorption band of CH-Cl in the region 690–724 cm⁻¹. These data confirm the structure of derivatives and the successful azetidine ring formation.

The structure of derivatives is also supported by NMR spectra. The apparent resonance multiplicity is described as s (singlet), d (doublet), m (multiplet), dd (double doublet), and td (triple doublet) signal.

The signals of CH-Cl protons resonate as a doublet in a range of δ 4.92–5.04 ppm.
and the protons linked by the N from azetidine ring (Ar-CH-N) exhibited a doublet at δ 5.24–5.54 ppm.

**CONCLUSIONS**

We synthesized six new azetidin-2-one derivatives of ferulic acid. The optimal reaction conditions for obtaining high yield and high purity compounds were established. The new derivatives were characterized by their physical constants (melting point, yield, molecular formula, molecular weight, solubility in different organic solvents). The chemical structure was confirmed proved by FT-IR and 1H-RMN spectroscopy. All the obtained data encourage us to further investigate *in vitro* and *in vivo* these new azetidin-2-one derivatives of ferulic acid.

**ACKNOWLEDGMENTS**


**REFERENCES**