Research Article

Synthesis of Some Novel Fluorinated/Nonfluorinated α-Amino Acids, Bearing 3-Thioxo-5-oxo-1,2,4-triazin-6-yl and Steroidal Moieties, and Evaluation of Their Amylolytic Effects against Some Fungi, Part-II

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Some new fluorinated/nonfluorinated α-amino acids bearing 3-thioxo-5-oxo-1,2,4-triazin-6-yl and steroidal moieties have been obtained from condensation of the corresponding amino-triazinones with the steroid (Epiandrosterone). This was followed by the addition of HCN and, finally, acidic hydrolysis. The structure of the targets was established from their elemental analysis and spectral data. The amylolytic activity of the new products was evaluated against some fungi.

1. Introduction

α-Amino acids are one of the most important bioactive chemical substances (proteins and nucleoproteins) forming the basic constituents of living cells. Nine proteinogenic amino acids are considered as essential biochemicals for humans: valine, threonine, tryptophan, phenylalanine, leucine, isoleucine, methionine, lysine, and histidine. For instance, 5-fluorocytosine is an analogue of nucleotide, used as a chemotherapeutic antifungal when combined with amphotericin B [1]. Of equal importance, glycine is required for the biosynthesis of the heme group of haemoglobin; also, tryptophan is the precursor of a family of substances important in the biochemistry of the central nervous system (CNS), and tyrosine is the starting material for the biosynthesis of the skin pigment melanin [2].

The enzyme lactate dehydrogenase (LDH) illustrates isozymes very well (Figure 1).

1,2,4-Triazine rings have been reported in the literature as having antifungal properties alongside their antitumor and antiviral activities. [3] Recently, Abdul-Rahman et al. [4–9] reported the synthesis, chemistry, and medicinal and biological activity, especially 6-(2-aminophenyl)3-thioxo-1,2,4-triazin-5-one [10–14]. Among all approved medicinal and pharmaceutical chemicals, nearly 20% have at least one fluorine atom existing which enhances phase II-III clinical trials [15]. Thus, the combination of fluorine with biomolecules such as fluorinated amino acids (FAAs), fluorinated steroids, and fluorinated nucleosides has increased, considerably, of the late years [16].

Incorporation of FAAs is one of the most utilized strategies in peptide and protein science. The effects of the combination of fluorinated α-amino acids into peptides and proteins on the primary and secondary structure have been widely reviewed by Koksch et al [17–19]. Furthermore, the fusion of unnatural/synthesized amino acids into peptides and proteins is generally closely accompanied with antimicrobial [20–22], antiviral [23], and metal chelating properties [24] such as thrombin, trypsin, and factor VIIa inhibitory activity.

Based on all these observations, this present work aims to find new synthetic fluorinated/nonfluorinated α-amino acids bearing both 3-thioxo 5-oxo-1,2,4-triazin-6-yl and steroidal moieties and evaluates their enzymatic effects.
2. Results and Discussion

2.1. Chemistry. The main objective of this work is to synthesize fluorinated/nonfluorinated α-amino acids derived from 1,2,4-triazinone and steroid. Through condensation of either fluorinated or nonfluorinated 1,2,4-triazinones bearing systems, such as 6-((2′-amino-5′-fluorophenyl)-3-thioxo-1,2,4-triazin-5(2H)-one (1a) and/or (1b) a solution in THF with dehydroepiandrosterone (B) (DHEA) in DMF reflux, yielded the 17-imino-derivatives 2a and 2b. Selective addition of HCN to an azomethine (imine) group, the Strecker reaction, is a common way to prepare α-amino acids by simple hydrolysis of the α-amino nitritre addition product. HCN was added to the imino group of 2a or 2b under specific conditions [25, 26] affording the α-amino-acids analogous 3a and 3b, which were hydrolysed to 4a and 4b, respectively (Scheme 1).

Synthesis pathway for the targeted compounds 2–5:

New α-amino acid derivatives 4a and 4b bearing a 6-(fluorinated)/nonfluorinated-aryl-1,2,4-triazin-one and C-(steroid) moieties were isolated by the acidic hydrolysis of 3a and 3b using a diluted HCl. The new fluorinated α-amino acids 5a and 5b were obtained by the reflux of compounds 4a and 4b with 4-fluoroaniline in EtOH (Scheme 1).

The IR spectrum of 4a showed new functional groups at ν 3500, 1710 cm⁻¹, referring to OH and C=O of the carboxylic acid group bearing steroids, with bonds at ν 3500, 3210, and 3090 cm⁻¹ for OH (steroids) and 2NH of 1,2,4-triazine and bearing a 6- thioxo-1-aryl) 17-Imino-(2-(5′-triazin-6′-oxo-3′-yl)-1-aryl) 19FNMR spectrum of compound 4 showed δ at -124 ppm, with a coupling constant at τF-H = 8.6 Hz and τF-H = 6.3 Hz.

2.2. Biological Evaluation. The four synthesized α-amino acids, 4a, 4b, 5a, and 5b, were preliminarily tested toward some fungi such as Aspergillus flavus, Aspergillus fumigains, Aspergillus Niger, Aspergillus nidulaus, and Aspergillus terreus for their amylolytic activity according to the classical methods [27–30]. Using DMF as a solvent, about 0.01 g of each compound was dissolved in the presence of phosphate buffer saline (PBS) at PH 6.5 for 30 minutes. The amylase activity was assayed at the adjusted pH and temperature 38°C, according to the standard method. [29] The activity was estimated (in mg reducing sugar out of reaction mixture), and all the results obtained are reported in Table 1.

From these data, we can conclude that most of the tested compounds exhibited a good to moderate inhibition and/or acceleration activity. In specific, compound 4a, which contains both F and S atoms, showed a stronger effect towards all the tested fungi than other systems, and markedly, fluorinated compounds 4 and 5 showed a complete control on the tested fungi as A. flavus, A. nedulaus, and A. niger. Finally, we report that the active new fluorinated α-amino acids are a promising candidate to be used as enzymatic catalysts in the vital biosynthesis process due to their activity.

3. Experimental

All reagents and solvents were purchased from commercial sources and used without further purification, unless otherwise stated. Melting points were measured using a Stuart SMB3 melting point apparatus and are uncorrected. IR spectra were recorded on a PerkinElmer Lambda 550 S spectrometer (KBr/cm⁻¹). All the chemical shifts, ¹HNMR and ¹³CNMR, were recorded relative to TMS and recorded on a varian-700 spectrometer (DMSO, δ ppm).

6-(2-Amino-5-fluorophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (1a) and 6-(2′-amino-phenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (1b): compounds 1a and 1b were prepared according to the reported method [5, 9].

17-Imino-(2-(5′-oxo-3′-thioxo-1′,2′-thiazin-6′-yl)-1-aryl) dehydroepiandrosterone-3β-ols (2a and 2b): equimolar mixture of 1a and/or 1b and steroid (B), THF (50 ml), and DMF (10 ml) was refluxed for 2 h, cooled, and then, evaporated. The produced solid was crystallized from dioxane to give 2a and/or 2b, respectively.

2a: yellow crystals: yield 80 %, m.p. 212°C–219°C. Analytical data found C, 65.93; H, 6.31; F, 3.59; N, 10.79; and S; 5.99 %, calculated for C₂₈H₃₃N₄FSO₂ (508), C, 66.14; H, 6.49; F, 3.74; N, 11.02; and S, 6.29 %. IR (cm⁻¹) v = 3550, 3200–3180 (2NH-OH), 3080 (aromatic CH), 2980 (aliphatic CH), 1680 (C=O) 1625 (C=N), 1580 (C=N), 1490, 1440 (defer. CH, CH₂, CH₃), 1250 (C-F), and 1190 (C-S). ¹HNMR (δ ppm): 13.81, 13.15(each s, 2H, NH, NH-1,2,4-tria}
Scheme 1: Condensation of fluorinated/nonfluorinated 1,2,4-triazinones as 6-(2′-amino-5′-fluorophenyl)-3-thioxo-1,2,4-triazin-5(2H, 4H) one (1a) and/or (1b) with dehydroepiandrosterone (B) to obtain 4 and 5.

Figure 2: Proposed mass fragmentation pattern of compound 4a.
H-24/26/27), 5.34 (s, 1H, OH of steroid), 3.42, 3.41, 2.58, 2.42 (dd, 3H, CH₃ of steroid), 10.80, 10.76 (each s, NH, NH), 8.0 (s, 1H, OH of acid), 1.67, 1.58, 1.46, 1.49, 1.75, 1.58, and 1.53 Hz (steroid alkene), 3.17, 3.16, 2.85, 2.84, 2.61, 2.59, 2.07, 2.10, 1.98, 1.84, 1.80, 1.78, 1.65, 1.55, 1.48, 1.46, 1.27, 1.25, 1.06, and 1.02 (CH₂ and CH₃ steroid). 13C NMR (δ ppm): 172 (C=O), 165 (C-F), 158 (C=N), 153 (C=N), 140.09 (C=N), 115.5 (C=N), 131.21–118.66 (aromatic carbons), 120.00, 116.50 (alkene of steroid), 37.2, 30.70, 70.99, 42.53, 31.43, 31.42, 51.68, 39.11, 21.81, 39.87, 42.51, 57.53, 30.70, 30.10, 32.99, 42.14, and 19.40 (steroids C). 19F NMR (δ ppm): −124. The coupling constant was JF-H (δJf = 8.8 Hz, δJm = 8.9 Hz, and δJm = 6.3 Hz).

3b: orange crystals yield 70%; m.p. >290°C. Analytical data found C, 66.95; H, 6.85; N, 13.25; and S, 5.90%.

30.70 (C2), 70.99 (C3), 42.53 (C4), 141.37 (C5), 122.55 (C6), 31.43 (C7), 31.42 (C8), 51.68 (C9), 39.11 (C10), 21.81 (C11), 39.87 (C12), 42.51 (C13), 57.53 (C14), 30.70 (C15), 30.10 (C16), 32.99 (C17), 42.14 (C18), and 19.40 (C19). 19F NMR (δ ppm): −124. The coupling constant was JF-H (δJf = 8.8 Hz, δJm = 8.9 Hz, and δJm = 6.3 Hz).

Table 1: The effects of fluorinated/nonfluorinated α-amino acids on the activity of the crude amylase.

| Test organism/final ph N-4 | Mycelia dry weight (mg 50 ml) culture med | Control | 4 | 5 |
|---------------------------|------------------------------------------|---------|---|---|
| A. flavus                 | 113                                      | 3.89    | 3.89/2.80 | 4.10/3.80 |
| A. tumiginis              | 105                                      | 6.48    | 5.18/4.99 | 5.77/4.59 |
| A. nidulatus              | 145                                      | 5.18    | 6.49/4.88 | 5.13/4.33 |
| A. niger                  | 123                                      | 3.37    | 3.89/3.38 | 3.37/2.99 |
| A. terreus                | 102                                      | 5.18    | 4.14/4.00 | 3.99/3.78 |

*The fluorinated α-amino acids. **The nonfluorinated α-amino acids.

**17-Cyano-17-(heteroarylaminio)-dehydroepiandrosterone-3β-ol/17-cyano-17-(3-thioxo-5-oxo-1,2,4-triazino(2H,2H)-6-yl)-1-epiandrosterone-3-β-ols (3a and 3b):** A mixture of 2a and/or 2b (0.001 mol) and NaCN (0.001 mol, in 10 ml) with acetic acid-ethanol (1:1, 50 ml) was refluxed for 2 h, cooled, and was then poured onto ice. The solid was filtered off and crystallized from ethanol to give 3a and 3b, respectively.

4a: orange-yellow crystals, yield 65%; m.p. 225–227°C. Analytical data found C, 62.66; H, 6.05; F, 3.21; N, 10.00; and S, 5.55%, calculated for C₂₂H₂₁N₅F₂O₃ (554); C, 62.81; H, 6.31; F, 3.42; N, 10.10; and S, 5.77%. IR (cm⁻¹): 3500, 3350, and 3210 (OH, NH, NH), 1710, 1680 (C=O), 1268 (C−F), and 1195 (C−S). 1H NMR (δ ppm): 0.89 (3H, s, CH₃ of steroid), 1.21 (3H, s, CH₃ of steroid), 10.80, 10.76 (each s, NH, NH), 8.0 (1H, OH of 1,2,4-triazine), 5.19 (1H, dd, J = 7.7, 7.1 Hz, =C−H steroid alkene), 7.08 (1H, dd, J = 8.7, 1.6 Hz, H-24/26/27), 7.39 (1H, dd, J = 1.6, 0.5 Hz, H-24/26/27), 7.56 (1H, dd, J = 8.7, 0.5 Hz, H-24/26/27), 5.34 (s, 1H, OH of steroid), 3.42, 3.41, 2.58, 2.42, 2.26, 2.25, 2.09, 2.07, 1.98, 1.84, 1.80, 1.78, 1.65, 1.55, 1.48, 1.46, 1.27, 1.25, 1.06, and 1.02 (CH₂ and CH₃ steroid). 13CNMR (δ ppm): 172 (C=O), 165 (C-F), 158 (C=N), 153 (C=N), 140.09 (C=N), 115.5 (C=N), 131.21–118.66 (aromatic carbons), 120.00, 116.50 (alkene of steroid), 37.2, 30.70, 70.99, 42.53, 31.43, 31.42, 51.68, 39.11, 21.81, 39.87, 42.51, 57.53, 30.70, 30.10, 32.99, 42.14, and 19.40 (steroids C). 19F NMR (δ ppm): −124. The coupling constant was JF-H (δJf = 8.8 Hz, δJm = 8.9 Hz, and δJm = 6.3 Hz).

4b: orange crystals, yield 78%; m.p. 270–272°C. Analysis data found C, 64.53; H, 6.88; N, 10.25; S, 5.7% calculated for C₂₂H₂₁N₅F₂O₃ (536); C, 64.68; H, 7.06; N, 10.40; and S, 5.94%. IR (cm⁻¹): ν = 3500 (OH), 3320, 3180 (NH), 1690, 1670 (C=O), 1480, 1440 (deformation CH₂), and 1190 (C=S).
$\beta$-ols (5a and 5b): equimolar of 4a and/or 4b and 4-fluoroalilne with ethanol (100 ml) was refluxed for 4 h, cooled, and then, poured on ice. The solid produced filtered off and crystallized from ethanol to give 5a and 5b, respectively.

5a: orange-yellow crystals, yield 65%; m.p. 220–221°C. Analytical data found C, 66.3; H, 5.89; F, 5.89; and N, 10.98%, calculated for C$_{15}$H$_{14}$F$_{2}$O$_{4}$ (633); C, 66.56; H, 6.18; F, 6.02; and 11.09%. IR (cm$^{-1}$): 3480, 3300, 3210, 3190 (3NH, OH), 2980, 2880 (aliphatic CH), 1610 (C=N), 1250 (C-F), and 650 (C-F). $^1$HNMR (δ ppm): 13.73, 12.96, 10.23 (each s, 3NH), 7.96, 7.95, 7.35, 7.17, 7.16 6.87 (2H, ddd, J = 8.5, 1.6, 0.5 Hz), 6.94–7.04 (3H, 6.97 (dd, J = 8.5, 1.9, 0.5 Hz), 7.27–7.34 (2H, 7.33 (dd, J = 1.6, 0.5 Hz), 7.30 (dd, J = 8.8, 0.5 Hz) (aromatic CH), 5.36 (s, 1H, OH of steroid), 6.20 (s, 1H, OH of acid), 4.19 (1H, dd, J = 7.7, 7.1 Hz, alkene steroid), 0.89 (3H, s, CH$_3$ steroid), 1.21 (3H, s, CH$_3$ steroid), 1.39–1.85 (12H, 1.63 (dddd, J = 13.1, 7.6, 2.9, 2.0 Hz), 3.49, 2.56–2.68, 2.59, 2.30, 2.11, 2.09, 2.08, and 1.94–1.88 (CH & CH$_2$ of steroid). $^{13}$CNMR (δ ppm): 170.43 (C-F), 165.5 (C-F), 162.1 (C-F), 141.53 (C=N), 140.15 (C-N), 130–123 (aromatic carbons), 119, 116 (C5-C6, -1,2,4-triazine), 37.11 (C1), 30.70 (C2), 71.01 (C3), 42.6 (C4), 141.3 (C5), 122.6 (C6), 32.0 (C7), 31.5 (C8), 51.7 (C9), 39.0 (C10), 21.9 (C11), 21.9 (C11), 39.9 (C12), 45.6 (C13), 57.63 (C14), 37.11 (C1), 30.70 (C15), 30.72 (C16), 39.9 (C12), 42.11 (C18), and 19.40 (C19). $^{13}$FNMRE (δ ppm): -124. The coupling constants are $J_{F-H}$ ($J_{F-o}$ = 8.8 Hz, $J_{F-o} = 8.9$ Hz, and $J_{F-o}$ = 6.3 Hz) and $J_{F-H}$ ($J_{F-o} = 8.9$ Hz and $J_{F-o} = 5.6$ Hz).

5b: orange crystals, yield 70%; m.p. 245°C–247°C. Analytical data found C, 66.3; H, 5.89; F, 5.89; and N, 11.19%, calculated for C$_{15}$H$_{14}$F$_{2}$O$_{4}$ (633); C, 66.56; H, 6.18; F, 6.02; and 11.09%. IR (cm$^{-1}$): 3500 (OH), 3300, 3200, 3180 (NH), 2980, 2880 (aliphatic CH), 1610 (C=N), 1230 (C-F), and 680 (C-F).

4. Conclusions
In a search for new α-amino acids, the present work reports a simple route to synthesize fluorinated and nonfluorinated α-amino acids derived from the corresponding 1,2,4-triazinone bearing an amino group and a steroid component. The new fluorinated synthetic skeletons exhibit an amylolytic activity greater than nonfluorinated systems against some fungi.

Data Availability
The data used to support the study can be made available upon request to the corresponding author.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Supplementary Materials
Figure I: a graphical abstract of all newly synthesized compounds 4a, 4b, 5a, and 5b. (Supplementary Materials)
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