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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

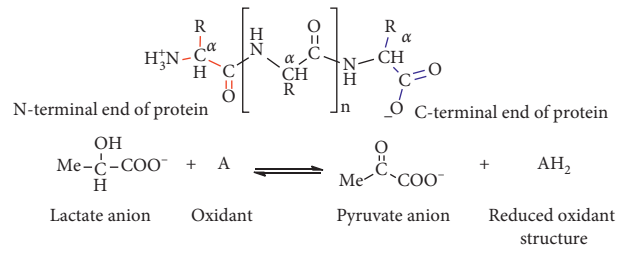


FIGURE 1: The interconversion of lactate and pyruvate using the enzyme lactate dehydrogenase (LDH) and amino acids sequence within one chain.

toward some fungi activities (amylolytic activity) with an objective to obtain new highly bioactive substances.

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, 4H)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-aminonitrile 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^{17}=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 bonds at ν 1235 and 1195 cm^{-1} attributed for C-F and C=S, while that of 5a showed a lack of the C=S group.

The ^{1}H-NMR spectrum of **4a** recorded the resonated singlet signals at δ 1.05, 1.12 for 6H ($^{18}CH_3$-$^{19}CH_3$), triplet signal for β-proton at δ 3.6 for methine H of C_3, singlet signal at δ 5.36 for H of the OH group at C_3, and 13.79 and 10.22 ppm for 2NH of 1,2,4-triazine, in addition to δ at 8.16 (1H, NH bonded) and 7.97–7.16 (aromatic CH). The ^{13}C-NMR spectrum of **4a** gives a signal at δ 70.99 (C_{-3}), 142.53 (C_{-4}), 39.11 (C_{-10}), 42.51 (C_{-13}), 31.99 (C_{-17}), 143.88 (C_{-18}), 19.51 (C_{-19}), 173.43 (C=O), and 201.55 (C=S), in addition to signal s at δ 128–131 (aromatic carbons) and at δ 141.15 ppm (C-F). Finally, a mass spectral study of compound **4a** represented that the molecular ion has the intensity below 5%; however, fragments of the (M^+-1) and (M^+-2) peaks with a base peak at m/z 121 attributed to stable moiety (Figure 2).

The ^{19}FNMR spectrum of compound **4** showed δ at −124 ppm, with a coupling constant at $^{2}J_{F\text{-}H}$ = 8.6 Hz and $^{3}J_{F\text{-}H}$ = 6.3 Hz.

2.2. Biological Evaluation. The four synthesized *a*-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. [28, 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 markably, 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.

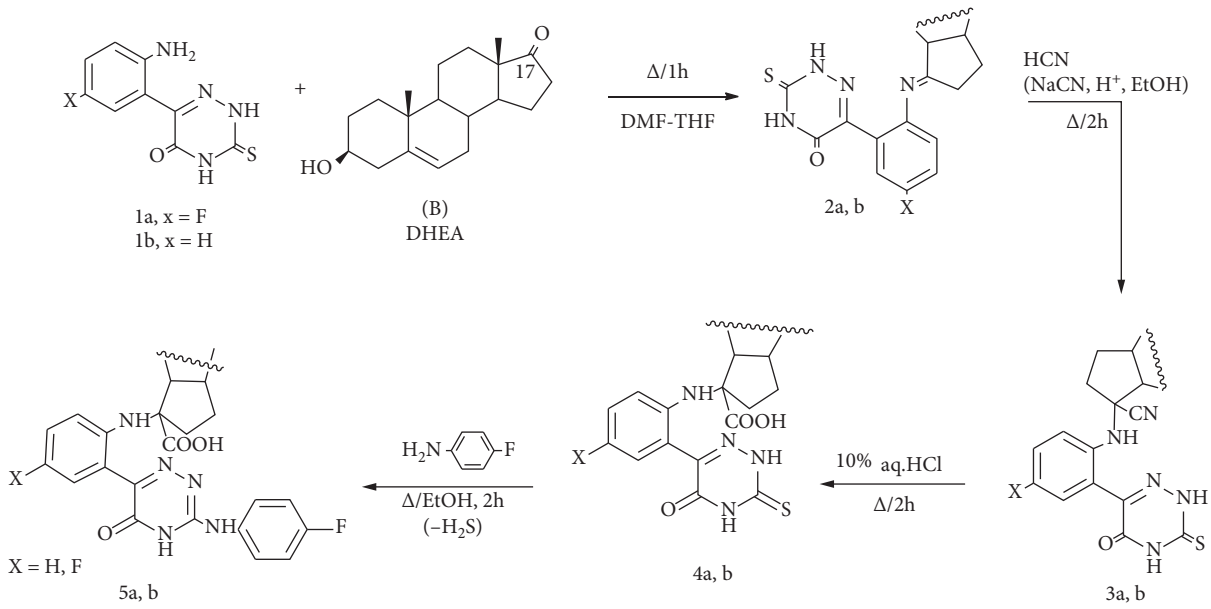
3. Experimental

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, ^{1}HNMR and ^{13}CNMR, were recorded relative to TMS and recorded on a varian-700 spectrometer (DMSO, d_6 ppm).

6-(2-Amino-5-fluorophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one (1a) and 6-(2'-aminophenyl)-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′,4′-triazin-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 $C_{28}H_{33}N_4FSO_2$ (508), C, 66.14; H, 6.49; F, 3.74; N, 11.02; and S, 6.29 %. IR (cm^{-1}) ν = 3550, 3200–3180 (2NH-OH), 3080 (aromatic CH), 2980 (aliphatic CH), 1680 (C=O) 1625 (C=N), 1580 (C=N), 1490, 1440 (defer. CH, CH_2, CH_3), 1250 (C-F), and 1190 (C-S). ^{1}HNMR (δ *ppm*): 13.81, 13.15(each s, 2H, NH, NH-1,2,4–triazine),



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.

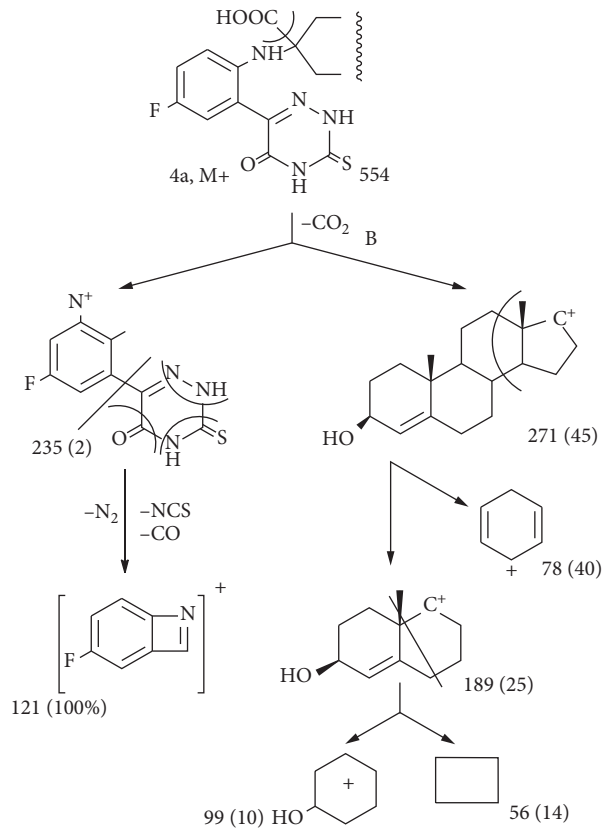


Figure 2: Proposed mass fragmentation pattern of compound **4a**.

7.98 (1H, dd, $J = 1.7$, 0.5 Hz, H-24), 7.45 (1H, dd, $J = 8.5$, 1.7 Hz, H-26), 7.43 (dd, $J = 8.5$, 0.5 Hz, H-27), 5.35 (s, 1H, OH steroid), 0.94 (3H, s, CH_3 of steroid), 1.21 (3H, s, CH_3 of steroid), 2.44, 2.42, 2.28, 2.22, 2.17, 2.10, 2.09, 2.07, 2.05, 1.85, 1.83, 1.68, 1.64, 1.55, 1.50, 1.47, 1.29, 1.27, 1.07, 1.03, and 1.00 (CH and CH_2 of steroid). ^{13}CNMR (δ *ppm*): 168 (C=O), 160 (C=S), 158.68, 148.09 (C=N), 140.92 (C-F), 140.09 (C-N), 131.21, 128.66, 120.88 (aromatic carbons), 37.2(C1),

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	Amylolytic activity of compounds F^{x}/nonF^{xx} (mg reducing sugar in reaction mixture)	
			4	**5**
A. flavus	113	3.89	3.89/2.80	**4.10**/3.80
A. lumigains	105	6.48	5.18/4.99	5.77/4.59
A. nidulaus	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

[x]The fluorinated α-amino acids. [xx]The nonfluorinated α-amino acids.

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). ^{19}FNMR (δ *ppm*): −124. The coupling constant was $J_{F\text{-}H}$ ($^{2}J_o = 8.6$ Hz, $^{2}J_o = 8.6$ Hz, and $^{3}J_m = 6.3$ Hz).

2b: deep yellow crystals, yield 70%; m.p. 150°C–152°C. Analytical data found C, 67.98; H, 7.11, N: 11.18, and S, 6.33%, calculated for $C_{28}H_{34}N_4SO_2$ (490); C, 68.29; H, 7.31; N, 11.38; and S, 6.50 %. IR (cm^{-1}): 3550 (OH), 3200, 3100 (NH), 1678 (C=O), 1620 (C=N), C 1490, 1448 (deformation CH_2), and 1187 (C=S).

17-Cyano-17-(heteroarylamino)-dehydroepiandrosterone-3β-ol/17-cyano-17-(2-(3-thioxo-5-oxo-1,2,4-triazino(2H,2H)-6-yl)-1-arylamino)-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 and crystallized from ethanol to give **3a** and **3b**, respectively.

3a: deep yellow crystals yield 60% m.p. 213°C–215°C. Analytical data found C, 64.84; H, 6.29; F, 3.35; N, 12.88; and S, 5.89%, calculated for $C_{29}H_{34}N_5FSO_2$ (535); C, 65.04; H, 6.35; F, 3.55; N, 13.08; and S, 5.98%. IR (cm^{-1}) $\nu = 3500$–3300 (b, NH-OH), 2220 (C≡N), 1680 (C=O), 1480, 1445 (deformation. CH_2 and CH_3), 1250 (C-F), and 1198 (C-S). ^{1}HNMR (δ *ppm*): 0.89 (3H, s, CH_3 of steroid)), 1.21 (3H, s, CH_3 of steroid), 10.80, 10.76(each s, NH, NH), 8.0(s, 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 (CH & CH_2 steroid). ^{13}CNMR (δ *ppm*): 172 (C=S), 165 (C-F), 158 (C=O), 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). ^{19}FNMR (δ *ppm*): −124. The coupling constant was $J_{F\text{-}H}$($^{2}J_o = 8.8$ Hz, $^{2}J_o = 8.9$ Hz, and $^{3}J_m = 6.3$ 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%. Calculated for $C_{29}H_{35}N_5SO_2$ (517); C, 67.05; H, 7.12; N, 13.48; and S, 6.16%. IR (cm^{-1}): 3550–3350 (**b**, NH, OH), 2230 (C≡N), 1677 (C=O), 1480, 1440 (deformation CH_2), and 1190 (C=S).

17-(6-(2–Arylamino)-3-thioxo–5–oxo-1,2,4-triazino-6-yl)-17-carboxy-17-heteroaryl amino or (2-(3-thioxo-5-oxo-1,2,4-triazin(2H, 4H)-6-yl-4-fluoro-1-amino)-dehydroepiandrosterone-3-β-ols (4a and 4b): compounds **3a** and/or **3b** (0.5 gm) diluted HCl (10%, 50 ml) were in reflux for 2 h, cooled, and poured onto ice. The solid, thus, obtained was filtered off and crystallized from ethanol to give **4a** and/or **4b**, 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 $C_{29}H_{35}N_4FSO_4$ (554); C, 62.81; H, 6.31; F, 3.42; N, 10.10; and S, 5.77%. IR (cm^{-1}) $\nu = 3500$, 3300 and 3210 (OH, NH, NH), 1710, 1680 (2C=O), 1235 (C-F), and 1195 (C-S). ^{1}HNMR((δ *ppm*): 0.89 (3H, s, CH_3), 1.21 (3H, s, CH_3), 13.79, 10.22(each s, 2H, NH, NH), 8.16(s, 1 H, NH bounded), 1.39–1.85 (12H, 1.63 (m), steroids), 5.19 (1H, dd, $J = 7.7$, 7.1 Hz steroid alkene), 4.28 (dddd, $J = 7.6$, 3.2, 2.6, 2.3 Hz, H3), 7.07 (1H, dd, $J = 8.8$, 1.6 Hz, H24/26/27), 7.32 (1H, dd, $J = 8.8$, 0.5 Hz, H24/26/27), 7.39 (1H, dd, $J = 1.6$, 0.5 Hz, H24/26/27), 5.36 (s, 1H, OH of steroid), 6.5 (s, 1H, OH of acid), 1.67, 1.58, 1.46, 1.49, 1.75, 1.58, and 1.53 Hz (CH and CH_2 of steroid). ^{13}CNMR (δ *ppm*): 172 (C=S), 165 (C-F), 158 (C=O), 151.55 (C=O), 131.21–120.88 (aromatic carbons), 37.20 (C_1), 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), 30.70 (C16), 32.99 (C17), 42.14 (C18), 19.42 (C19), M/Z (Int.%) 554 (0.0), 275 (5), 235 (5), 197 (100), 121 (100), 189 (25), 99 (10), 78 (40), and 56 (14). ^{19}FNMR (δ *ppm*): −124. The coupling constant was $J_{F\text{-}H}$($^{2}J_o = 8.8$ Hz, $^{2}J_o = 8.9$ Hz, and $^{3}J_m = 6.3$ Hz).

t**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 $C_{29}H_{36}N_4SO_4$ (536); C, 64.68; H, 7.06; N, 10.40; and S, 5.94%. IR (cm^{-1}): $\nu = 3500$ (OH), 3320, 3180 (NH), 1690, 1670 (C=O), 1480, 1440 (deformation CH_2), and 1190 (C=S).

17-(6-(2'-Arylamino)-3(4'-fluoro-phenylamino)-1,2,4-triazin-5(4H)ones)-17-carboxy-dehydroeniandrosterone-3-

β-ols(5a and 5b): equimolar of **4a** and/or **4b** and 4-fluoroailine with ethanol(100 ml) was refluxed for 4 h, cooled, and then, poured onto 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 $C_{35}H_{41}N_5F_2O_4$(633); C, 66.56; H, 6.18; F, 6.02; N, and 11.09%. IR(cm^{-1}): ν = 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 = 8.8, 1.6 Hz), 7.01 (ddd, 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, CH3 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=O), 160.0 (C=O), 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), 30.70 (C15), 30.72 (C16), 32.98 (C17), 42.11 (C18), and 19.40 (C19). ^{19}FNMR((δ *ppm*): −124. The coupling constants are $J_{F\text{-}H}$(^2J_o = 8.8 Hz, ^2J_o = 8.9 Hz, and ^3J_m = 6.3 Hz) and $J_{F\text{-}H}$(^2J_o = 8.9 Hz and ^3J_m = 5.6 Hz).

5b: orange crystals, yield 70%; m.p. 245°C–247°C. Analytical data found C, 67.99; H, 6.66; F, 2.91; and N, 11.19%, calculated for $C_{35}H_{42}FN_5O_4$ (615); C, 68.29; H, 6.82; F, 3.08; and N, 11.38%. 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 steroidal 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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