Synthesis of Novel α-Amino Acids Bearing 1,2,4-triazinone and Steroidal Moieties as Enzymetic Affect (Cellobiase Activity) Part I

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Abstract

An imperative class of α-amino acids bearing 1,2,4-triazinone and N,C-disubstituted glycine (5a,b) and/or the related systems (6 and 7) have been synthesized by the condensation of 6-(2'-aminophenyl)-4-phenyl-3-thion-1,2,4-triazin-5-one (1) with the appropriate steroids, Epiandrosteron (2a) and Dehydrosterone (2b), followed by the addition of hydrocyanic acid. Nucleophilic substitution of mercapto group of (5) by 4-fluoroaniline and sulfanilamide give the corresponding systems 6 and 7. Compounds 6, 7, 5, and 4 showed a high enzymatic effect as cellobiase agents against some tested fungi.

Keywords: α-Amino acid, Cellobiase, Triazinone, Steroid, Sulfanilamide

1. Introduction

The α-amino acid is important in the metabolic process. The steroids are considered significant controls in various metabolism related processes, while various steroids with heterocyclic systems show biocidal affects (Janganti, Penthala, Cragle, MacNicol & Crooks, 2004; Guo, Qiu, Yin & Tianjin, 1999).

The 6-(2'-aminophenyl)-3-thiolo-1,2,4-triazin-5-ones derivatives showed biological and medicinal activities (Zhang, Wang & Liu, 2012), on tumors (Abdel-Rahman, 1992 & 2001; Abdel-Rahman, Seada, Fawzy & El-Baz, 1994; Abdel-Mpnem & Abdel-Rahman, 2006) and HIV (Abdel-Rahman, 1991; Abdel-Rahman, Morsy, Hnafy & Amene, 1999; El-Gendy, Morsy, Allimony, Abdel-Monem & Abdel-Rahman, 2001). In addition, it can have an amylolytic effect (Abdel-Rahman & Abdel-Malik, 1999) and effect antimicrobial agents (Ebraheem et al., 2008; Abdel-Rahman & Ali, 2013). Further modification via a redistribution of electron density over the active centers generate new kinds of α-amino acids substituted with 1,2,4-triazine and glycine derivatives in view of their enzymatic affects against Aspergillus nidulans and Apergillus niger fungi.

2. Result and Discussion

2.1 Chemistry

The key compound 6-(2’aminophenyl)3-thiolo-1,2,4-triazin-5(2H)-one (1) was synthesis by heating the isatin sodium salt with 4-phenylthiosemicarbazide at reflux for 2 hour, Scheme 1.

Scheme 1. Synthesis of triazinone 1

The $^{13}$CNMR of compound 1 showed an interesting resonated signals at δ185 and 166 ppm attributed C=S and C=O respectively in addition to the aromatic carbons at δ 130-127 ppm.

The corresponding target of amino-1,2,4-triazineone 1 with steroids such as epiandrosteron and dehydrosterone (2a, 2b) in THF yield the responding target amino-derivatives 3a and 3b, respectively (Scheme 2).

The reactivity of exo and endo C=N groups in the 1,2,4-triazines was studied (Üngören, Dilekoglu & Koca, 1999).
Thus, the addition of HCN to the highly reactive exo C=N of compound 3 gives [3'-thioxo-4'-phenyl-5'-ox-1,2,4-triazin-6-phenylamin-2'-yl]steroids (4a and 4b), Scheme 2.

Scheme 2. Synthesis of amino-derivatives 3a and 3b

The acidic hydrolysis of compounds 4 yields the target N-substituted-C-substituted glycines α-(4'-phenyl-3'-thioxo-5'-ox-2'H-1', 2', 4-triazin-6-phenyl-2yl-α-(steroid-17-yl)glycines (5a and 5b), Scheme 3.

Selective installation of fluorine atom into a therapeutic or diagnostic molecule can enhance a number of pharmacokinetic and physicochemical properties (Delpon, 2008), such as improved metabolic stability and enhanced membrane permeation (Shah & Westwell, 2007; Hagmann, 2008). An increased binding affinity of fluorinated drug candidates to target protein has also been reported (Filler & Saha, 2009).

The isosteric isomer of azacytosine and 6-azauracil, 3-amino-1,2,4-triazin-5-ones is an interesting biological molecules due to its resistance to diamines (Abdel-Rahman & Fawzy, 1992; Makki, Bakhotmah & Abdel-Rahman, 2012). In addition to previous work in cellobiase activates (Makki, Bakhotmah & Abdel-Rahman, 2012; Abdel-Rahman, 1999, 2001; Mohammed, Makki, Abdel-Rahman & Khan, 2014), a simple nucleophilic displacement of the SH group of compounds 5 using 4-fluoroaniline and/or sulfanilamide yield α-[4'-phenyl-3''-(4''-fluorophenyl)-5'-ox-1,2,4-triazin-6-phenyl-2'y]-α-[steroid-17'-yl]glycine (6) and /or α-[4'-phenyl-3' (4'sulfonamoyl phenylamino)glycine (7) respectively. Scheme 3.

Scheme 3. Synthesis of glycine 6 and 7
2.2 Biological evaluation

The effects of the synthesized α-amino acid derivatives on the cellobase activity were studied using the Reese and Mandel procedure (Abdel-Aziz et al., 1996; PH 5, incubated at 40°C for 1 hour). The released reducing sugar was estimated calorimetrically at 540 nm (Ibrahim et al., 1997; Abdel-Rahman, Morsy, Allimon & Abd El-Monem, 1999; Ibrahim et al., 2009), Table 1.

Table 1. Effect on cellobase activity produced by Aspergillus nidulans Aspergillus niger fungi

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aspergillus nidulans</th>
<th>Aspergillus niger</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.66</td>
<td>0.68</td>
</tr>
<tr>
<td>3</td>
<td>0.59</td>
<td>0.58</td>
</tr>
<tr>
<td>4a</td>
<td>0.45</td>
<td>0.46</td>
</tr>
<tr>
<td>4b</td>
<td>0.41</td>
<td>0.43</td>
</tr>
<tr>
<td>5a</td>
<td>0.72</td>
<td>0.75</td>
</tr>
<tr>
<td>5b</td>
<td>0.70</td>
<td>0.72</td>
</tr>
<tr>
<td>6a</td>
<td>0.82</td>
<td>0.86</td>
</tr>
<tr>
<td>6b</td>
<td>0.80</td>
<td>0.81</td>
</tr>
<tr>
<td>7a</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>7b</td>
<td>0.78</td>
<td>0.80</td>
</tr>
</tbody>
</table>

* DMF = 0.73 and 0.72 (μg/mL), blank = 0.97 and 0.80 (μg/mL) (without DMF)

Compounds 6a and 6b both showed a higher amount of activity over the compounds when compared against the other tested fungi. Thus, the introducing of fluorine atoms and/or sulfa-drug moiety resulted in high order of activity in comparison with the corresponding α-amino acids. Additionally, the presence of fluorine substituted α-amino acids bearing 1,2,4-triazine and steroidal moieties led to increases the net-electronegativity, which improve the dielectric constant and enhances the hydrophobic properties. These properties, in all, increase their efficiency as enzymatic parameters.

In conclusion, the relationship between structural parameters, electronic parameter, and antifungal enzymatic activity ensures that the replacement of SH group of 1,2,4-triazine by substituted fluorine enhance the overall enzymatic effects.

3. Experimental

General Procedures

The melting points were determined using a Gallenkamp apparatus and were uncorrected, IR spectrum were recorded with FT-IR Bomem MB 104 using nujol multis and NaCl cells, NMR spectra were obtained on Brukes Avance 400MHz. Chemical shift expressed in δ (ppm) using DMSO-d6. Mass spectrum were measured on GCMS Q1000-Ex at 70eV. Microbiological analyses were performed by the microanalytical center at Ain-Shams University, Egypt.

3.1 6-(2’Aminophenyl)-4-phenyl-3-thioxo-1,2,4-triazin-5-one (1)

A mixture of isatin (0.001 mol, in 5% aqueous NaOH, 50 ml), and 4-phenylthiosemicarbazide (0.001 mol) were refluxed for 2h. The cold reaction mixture was then added to cold HCl. The formed solid is filtered, collected, and crystallized from ethanol to give 1 as orange crystals, yield 80%, m.p. 220-222°C.

IR (cm⁻¹), 3185, 3120 (S, NH₂, NH), 1660 (C=O), 1190 (C=S), 1590 (C=N), 850 (aryl CH); 1HNMR (DMSO-d6) δ (ppm), 11.82, 3.23 (s, NH and NH₂), 7.2-6.8 (m, 9H, aromatic); 13CNMR (DMSO-d6) δ (ppm), 185 (C=S), 166 (C=O), 130-127 (aromatic); M/z, 297 (M+1, 12.11%), 163 (100%); CHNS analysis of the compound 1, C₁₉H₁₂N₂SO (296), calculated: C, 60.8; H, 4.01; N, 18.69; S, 10.81. Found: C, 60.6; H, 4.0; N, 18.67; S, 10.79.

3.2 17-{4-phenyl-3-thioxo-1,2,4-triazin-5-oxo-(phenyl-4-imino)} steroids (3a and 3b)

A mixture of 1 (0.001 mol) and steroid epioandrosterone 2a and Dehydrosterone 2b (0.001 mol) in 50 ml THF was heat to reflux for 2h. The cold solid was filtered and crystallized from ethanol to give 3a (yield 70%; m.p. 212-213°C) and 3b (yield 68%; m.p. 184-185°C).

IR (cm⁻¹) 3a, 3382 (OH), 3010 (aromatic CH), 2985 (aliphatic CH), 1670 (C=O), 1616 (exo C=N), 1185 (C=S), 1480, 1440 (bending aliphatic CH); 1HNMR (DMSO-d6) δ (ppm): 0.85 (s, CH₃, 18-H), 1.85(s, CH₃, 19-H), 2.2 and 2.5 (m, CH₂steroide), 3.69 (s, 17a-H₂), 3.85 (s, 4-H₂), 5.55 (s, OH), 7.8-7.2 (m, 9H, aromatic H), 11.75 (s, NH, 1,2,4-triazine); M/z: 567 (M+1, 5.11%), 163 (100%, C₁₅H₁₀NSO).
3.3 17-carbonitrile- [4-phenyl-3-thioxo-1,2,4-triazin-5-(2H) oxo-6-(2'phenylamino) steroids (4a and 4b)

A solution of NaCN (0.001 mol, 10 ml H2O) was added to compounds 3a or 3b (0.001 mol), followed by the addition of 20 ml of acetic acid/ ethanol mixture (1:1 v/v). The reaction mixture was brought to reflux for 2h. The solid produced after cooling was collected by filtration and crystallized from ethanol to give 4a (yield 66%, m.p. 219-220 °C) or 4b (yield 68%, m.p. 16-218 °C).

IR (cm⁻¹): 3400 (OH), 3180 (exon OH), 3150 (endo NH), 3050 (ar CH), 2880 (aliphatic CH), 1666 (C=O), 1180 (C=S); ¹H NMR (DMSO-d₆) δ (ppm): 11.45, 8.85 (s, exo and endo NH respectively), 0.88 (s, CH₃ for 18-H), 1.86 (s, CH₃ for 19-H), 2.25 and 2.55 (m, CH₂ steroidal); ¹³CNMR (DMSO-d₆) δ (ppm): 12.23 (C₁₁₈Me), 18.70 (C₁₁₉Me), 81.38 (C=OH), 118.75 (CN), 168.35 (C=O), 188.11 (C=S); M/z: 597 (M+1, 6.55%), 165 (100%), CHN analysis: Calculated: C, 69.0; H, 6.7; N, 8.9; S, 5.11%. Found: C, 68.5; H, 6.6; N, 8.6; S, 4.9%. 5a: C₁₇H₁₄N₂SO₃ (596), Calculated: C, 70.5; H, 6.9; N, 11.7; S, 5.4%. Found: C, 70.1; H, 6.4; N, 11.6; S, 5.4%. 4b: C₁₇H₁₈N₂SO₃ (593), Calculated: C, 70.80; H, 6.58; N, 11.79; S, 5.41%. Found: C, 70.78; H, 6.56; N, 11.8; S, 5.39%.

3.4 17-Carboxy-17-[4-phenyl-3-thioxo-1,2,4-triazin-5(2H) oxo-6-(2'phenylamino)] steroids (5a and 5b)

Compounds 4a and 4b (0.001 mol) in HCl (5%, 20 ml) was reflux for 1h, the formed solid was filtered and crystallized from ethanol to give 5a (yield 65%, m.p. 148-150 °C) or 5b (yield 60%, m.p. 222-224 °C).

IR (cm⁻¹): 3400-3350 (br, OH, NH), 3180-3130 (2NH), 3030 (ar CH), 2890 (aliphatic CH), 1685 and 1665 (2C=O), 1620 (C=N), 1480 and 1445 (bending CH₂ steroidal), 1180 (C=S); ¹H NMR (DMSO-d₆) δ (ppm): 0.86 (s, C₁₈Me), 1.88 (s, C₁₉Me), 2.25 and 2.55 (m, CH₂ steroidal); ¹³CNMR (DMSO-d₆) δ (ppm): 8.65 and 11.85 (s, 2NH), 10.55 (s, OH); ¹³CNMR (DMSO-d₆) δ (ppm): 12.12 (C₁₁₈), 18.55 (C₁₁₉), 81.33 (C₁₂), 168.15 (C=O), 180.11 (COOH), 188.0 (C=S); M/z: 627 (M+1, 13.0%), 165 (100%).

CHN Analysis for 5a: C₁₇H₁₄N₂SO₃ (614), Calculated: C, 69.0; H, 6.7; N, 8.9; S, 5.11%. Found: C, 68.5; H, 6.6; N, 8.6; S, 4.9%. 5b: C₁₇H₁₈N₂SO₃ (612), Calculated: C, 69.20; H, 6.41; N, 8.97; S, 5.10%. Found: C, 69.18; H, 6.39; N, 8.95; S, 4.09%.

3.5 17-Carboxy-17-[3-(4'fluorophenylamino)-4-phenyl-5-oxo-1,2,4-triazine-6-(2'-phenylamino)] steroids (6a and 6b)

An equimolar of 4-fluoroaniline and 5a or 5b in 100 ml ethanol was refluxed for 4h. The cold reaction mixture was then poured onto ice. The solid formed is collected by filtration and crystallized from ethanol to give 6a (yield 78%, m.p. 185-187 °C) or 6b (yield 75%, m.p. 130-132 °C).

IR (cm⁻¹): 3400-3380 (br, OH and NH), 3200-3150 (br, 2NH), 1680 and 1660 (2C=O), 1610 (C=N), 1484, 1444 (bending CH₂), 1250 (C-F), 905, 854 (aryl CH), 675 (C-F); ¹H NMR (DMSO-d₆) δ (ppm): 0.88, 1.88 (s, C₁₈Me and C₁₉Me), 2.20 and 2.50 (m, CH₂ steroidal), 3.85 (s, 1H,C₁₃), 6.41-6.60, 6.82-7.23 and 7.41-7.80 (m, 13H, aromatic), 8.55 and 11.85 (s, 2NH), 10.55 (s, OH); ¹³CNMR (DMSO-d₆) δ (ppm): 12.6 (C₁₁₈Me), 17.0 (C=CH₂), 18.7 (C₁₁₉Me), 44.0 (C=CH), 81.66 (C=OH), 142.11 (C-F) 138.0 (C=N), 162.0 (COOH), 168.0 (C=O);

CHN Analysis for 6a: C₁₇H₁₄N₂F₄O₄ (692), Calculated: C, 71.0; H, 6.6; N, 10.1; F, 2.7%. Found: C, 71.0; H, 6.3; N, 9.7; F, 2.4%; 6b: C₁₇H₁₄N₂F₄O₄ (690), Calculated: C, 71.03; H, 6.24; N, 10.10; F, 2.68%. Found: C, 71.01; H, 6.21; N, 9.08; F, 2.65%.

3.6 17-Carboxy-17'-[3'-4' -aminosulfanomethylphenylamino]-4-phenyl-5-oxo-1,2,4-triazine-6-(2'-phenylamino) steroids (7a and 7b)

A mixture of 5a or 5b (0.011 mol) and sulfanilamide (0.001 mol) in absolute ethanol (50 ml) was refluxed for 4h. The cold solid formed was filtered and crystallized from ethanol to give 7a (yield 66%, m.p. 140-142 °C) or 7b (yield 68%, m.p. 199-200 °C).

IR (cm⁻¹): 3450-3340 (br, OH and NH), 3200-3180 (br, 2NH), 1686 and 1660 (2C=O), 1615 (C=N), 1480, 1440 (bending CH₂), 1350 (SO₂-NH-R), 910, 850 (aryl CH); ¹H NMR (DMSO-d₆) δ (ppm): 0.81, 1.87 (s, C₁₈Me and C₁₉Me), 2.25 and 2.55 (each m, CH₂ steroidal), 3.78 (s, 1H,C₁₃), 6.41-6.60, 6.85-7.15 and 7.31-7.70 (each m, 12H, aromatic), 8.51 and 8.66 (each s, 2CH), 10.55 (s, OH), 11.80 and 11.40 (2NH); CHN Analysis for 7a: C₁₇H₁₄N₂SO₆ (753), Calculated: C, 65.3; H, 6.4; N, 11.2; S, 4.24%. Found: C, 65.01; H, 6.1; N, 11.0; S, 4.1%; 7b: C₁₇H₁₄N₂SO₆ (751), Calculated: C, 65.51; H, 6.01; N, 11.02; S, 4.30%. Found: C, 64.9; H, 5.89; N, 11.03; S, 4.29%.
4. Conclusion
This study showed that the presence of fluorine atoms and/or sulfu-drug moiety, combined with \(\alpha\)-aminoacids, increases the cellobiase activity, while the carbonitrile derivatives decrease the tested bioactivity over the synthesized amino acid.

In addition, the incorporation of 5-ox-1,2,4-triazin-3-thione and a type of steroids to amino acid (glycine) initiates the potency of the novel synthesis systems, leading to the inhibition. It also accelerated its enzymatic affects.

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