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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

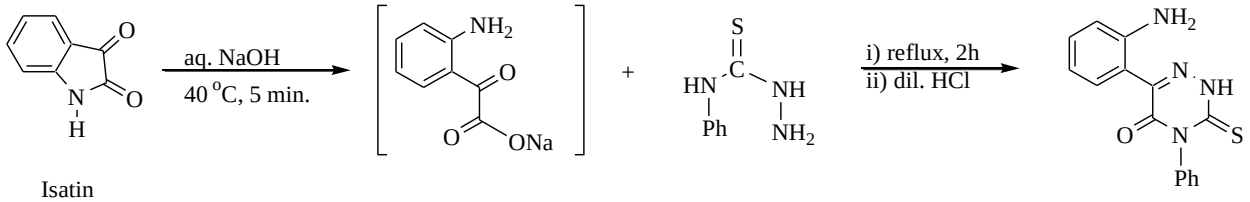
The α-amino acid is important in the metabolic process. The steroids are considered significant controls in various metabolism relatedprocesses, whilevarious steroids with heterocyclic systems show biocidal affects (Janganti, Penthala, Cragle, MacNicol & Crooks, 2004; Guo, Qiu, Yin & Tianjin, 1999).

The 6-(2'-aminophenyl)-3-thioxo-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, 1990) 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-mino acids substituted with 1,2,4-triazine and glycine derivatives in view of their enzymatic affects against *Aspergillus nidlans* and *Apergillus niger* fungi

2. Result and Discussion

2.1 Chemistry

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



Isatin **1**

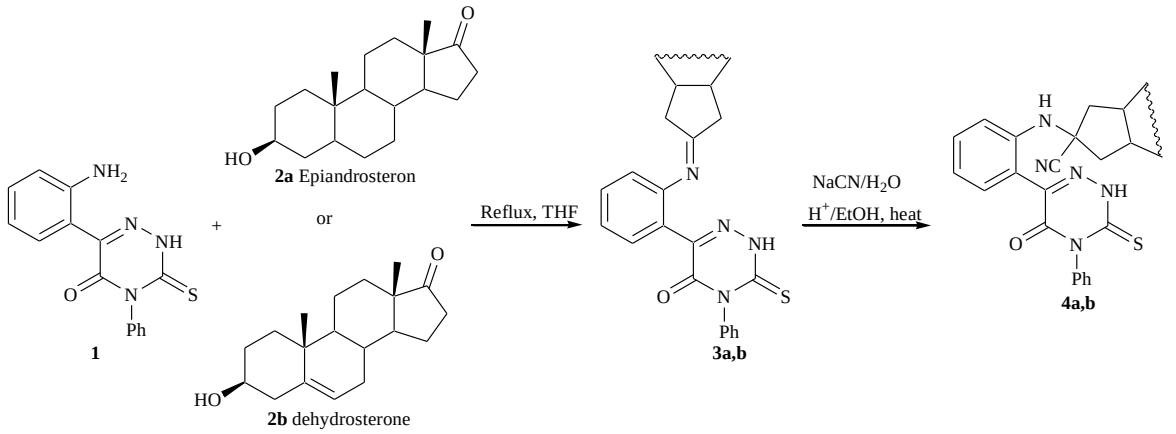
Scheme 1. Synthesis of triazinone 1

The ^{13}CNMR of compound 1 showed an interestingresonated 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, Dilekoğlu & Koca,

2013; Abdel-Raman, 1992). 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'-phenylaimin-2'-yl]steroids (**4a** and **4b**), Scheme 2.

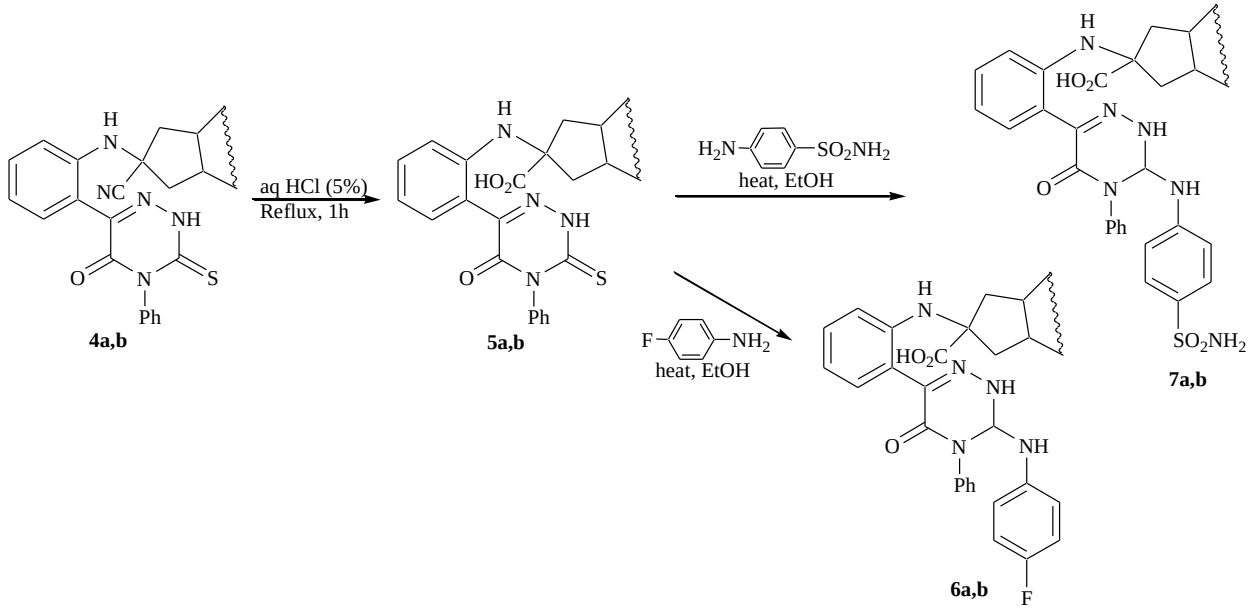


Scheme 2. Synthesis of amino-derivatives 3a and 3b

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 diaminese (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'yl]-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 Biologicalevaluation

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^{o}C for 1hour). 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 cellobiase activity produced by *Aspergillus nidulans Aspergillns niger* fungi

Compound	Amount of glucose (μg/mL)*	
	Aspergillus nidulans	*Aspergillns niger*
1	0.66	0.68
3	0.59	0.58
4a	0.45	0.46
4b	0.41	0.43
5a	0.72	0.75
5b	0.70	0.72
6a	0.82	0.86
6b	0.80	0.81
7a	0.76	0.78
7b	0.78	0.80

* 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-trizine 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 enzymetic parameters.

In conclusion, the relationship between structural parameters, electronic parameter, and antifungalenzymatic activity ensures that the replacement of SH group of 1,2,4-triazine by substituted fluorine enhance the overall enzymetic 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 mults and NaCl cells, NMR spectrums were obtained on Brukes Avance 400MHz. chemical shift expressed in δ (ppm) using DMSO-d_6, 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-phenylthiosemicarbzide (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^{o}C.

IR (cm^{-1}), 3185, 3120 (S, NH_2, NH), 1660 (C=O), 1190 (C=S), 1590 (C=N), 850 (aryl CH); ^{1} HNMR (DMSO-d_6)δ (ppm), 11.82, 3.23 (s, NH and NH_2), 7.2-6.8 (m, 9H, aromatic); ^{13}CNMR (DMSO-d_6) 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**, $C_{15}H_{12}N_4SO$ (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-6-(phenyl-4-imino)] steroids (3a and 3b)

A mixture of **1** (0.001 mol) and steroid epiandrosterone **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 ^{o}C) and **3b** (yield 68%; m.p. 184-185 ^{o}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); ^{1}HNMR (DMSO-d_6) δ (ppm): 0.85 (s, CH_3, 18-H), 1.85(s, CH_3, 19-H), 2.2 and 2.5 (m , CH_2steroide), 3.69 (s, 17a-H_2), 3.85 (s, 4-H_2), 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%, C_8H_5 NSO).

CHNS Analysis for **3a**: $C_{36}H_{40}N_4SO_2$ (566), Calculated: C, 72.1; H, 7.1; N, 9.9; S, 5.7%. Found: C, 72.4; H, 6.97; N, 9.7; S, 5.6%. **3b:**$C_{34}H_{38}N_4SO_2$ (564), Calculated: C, 70.28; H, 6.62; N, 9.71; S, 5.54 %. Found: C, 70.30; H, 6.64; N, 9.74; S, 5.49%.

3.3 17-carbonitrile-17-[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 H_2O) 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 ^oC) or **4b** (yield 68%, m.p. 16-218 ^oC).

IR (cm^{-1}) **4a**: 3400 (OH), 3180 (exo NH), 3150 (endo NH), 3050 (ar CH), 2880 (aliphatic CH), 2215 (CN), 1666 (C=O), 1180 (C=S); ^{1}HNMR (DMSO-d_6) δ (ppm): 11.45, 8.85 (s, exo and endo NH respectively), 0.88 (s, CH_3 for 18-H), 1.86 (s,CH_3 for 19-H), 2.25 and 2.55 (m, CH_2steroid); ^{13}CNMR (DMSO-d_6) δ (ppm):12.23 (C_{18}, Me), 18.70 (C_{19}, Me), 81.38 (C-OH), 118.75 (CN), 168.35 (C=O), 188.11 (C=S); M/z: 597 (M+1, 6.55%), 165 (100%, C8H7NSO);CHNS Analysis for **4a**: $C_{37}H_{41}N_5SO_2$ (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**: $C_{37}H_{39}N_5SO_2$ (593), Calculated: C, 70.80; H, 6.58; N, 11.79; S, 5.41%. Found: C, 70.78; H, 6.56; N, 11.78; 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 ^oC**)** or **5b** (yield 60%, m.p. 222-224 ^oC).

IR (cm^{-1})**:** 3400-3350 (br, OH, NH), 3180-3130 (2NH), 3030 (ar CH), 2890 (aliphatic CH), 1685and 1665 (2 C=O), 1620 (C=N), 1480 and 1445 (bending CH_2 steroid), 1180 (C=S);); ^{1}HNMR (DMSO-d_6) δ (ppm): 0.86 (s, C18, Me), 1.88 (s, C19, Me), 2.25 and 2.55 (m, CH_2steroid), 3.70 (s, 1H), 7.75 and 7.18 (m, 9H, aromatic), 8.55 and 11.85 (s, 2NH), 10.55 (s, OH);^{13}CNMR (DMSO-d_6) δ (ppm): 12.12 (C_{18}), 18.55 (C_{19}), 81.33 (C_3), 168.15 (C=O), 180.11 (COOH), 188.0 (C=S); M/z: 627 (M+1, 13.0%), 165 (100%).

CHNS Analysis for **5a**: $C_{37}H_{42}N_4SO_4$ (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**: $C_{37}H_{40}N_4SO_4$ (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-phenl-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 ^oC) or **6b** (yield 75%, m.p. 130-132 ^oC).

IR (cm^{-1}): 3400-3380 (br, OH and NH), 3200-3150 (br, 2NH), 1680 and 1660 (2 C=O), 1610 (C=N), 1484, 1444 (bending CH_2), 1250 (C-F), 905, 854 (aryl CH), 675 (C-F);^{1}HNMR (DMSO-d_6) δ (ppm):): 0.88, 1.88 (s, C18, Me andC19, Me), 2.20 and 2.50 (m, CH_2 steroid), 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);^{13}CNMR (DMSO-d_6) δ (ppm): 12.6 (C_{18}-Me), 17.0 (C-CH_2), 18.7 (C_{19}-Me), 44.0 (C-CH), 81.66 (C-OH),142.11(C-F) 138.0 (C=N), 162.0 (COOH), 168.0 (C=O);

CHNS Analysis for **6a**: $C_{41}H_{46}N_5FO_4$ (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**: $C_{41}H_{44}N_5FO_4$ (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''-aminosulfanomylphenylamino)-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 (0.001mol) 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 ^oC) or **7b** (yield 68%, m.p. 199-200 ^oC)

IR (cm^{-1}): 3450-3340 (br, OH and NH), 3200-3180 (br, 2NH), 1686 and 1660 (2 C=O), 1615 (C=N), 1480, 1440 (bending CH_2), 1350 (SO_2-NH-R), 910, 850 (aryl CH);^{1}HNMR (DMSO-d_6) δ (ppm):): 0.81, 1.87 (s, C18, Me andC19, Me), 2.25 and 2.55 (each m, CH_2 steroid), 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 (s, 2NH); CHNS Analysis for **7a**: $C_{41}H_{48}N_6SO_6$ (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**: $C_{41}H_{46}N_6SO_6$ (751), Calculated: C, 65.51; H, 6.01; N, 11.02; S, 4.30%. Found: C, 6.49; H, 5.89; N, 11.03; S, 4.29%.

4. Conclusion

This study showed that the presence of fluorine atoms and /or sulfa-drug moiety, combined with α-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) initiatesthe potency of the novel synthesis systems, leading to the inhibition.It also accelerated its enzymatic affects.

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