

Synthesis, Characterization, and Antioxidation Evaluation of Novel Spiro-5-(fluoren-9'-yl)-6-azauracil and Their *N,N*-Dialkyl Derivatives

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Abstract

In search for new antioxidant agents derived from 6-azauracil, the spiro-5-(fluoren-9'-yl)-6-azauracil **3** and their *N,N*-disubstituted-6-azauracils **4** - **17** have been synthesized using various methods and reaction conditions. Structure of the new synthesized compounds was deduced from elemental analysis and spectral measurements, for example IR, ¹H/¹³C NMR and mass spectroscopy. The antioxidant evaluation of the new targets showed that the activity increases in the order of **8** > **10** > **6** > **7** > **9** in comparison with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid as standards.

Keywords

N,N-Disubstituted, 6-Azauracil, Fluoren-9-One, 1,2,4-Triazine, Antioxidant

1. Introduction

6-Azauracil (1,2,4-triazine-3,5-(2*H*,4*H*)-dione) **A** is one of the important families of heterocyclic nitrogen systems as biodynamics targets. Recently much effort has been exerted on the synthesis, chemical reactivity, physical properties, and biological evaluation of 6-azauracil **A** and their *N,N*-Disubstituted derivatives. The results have shown that these group of compounds exhibit anticancer [1], antiviral [2] [3], antimalarial [4], herbicidal [5] and antimicrobial [6] [7] [8] [9] [10] behavior, while the *N*⁴-fluoro aromatic substituted uracils **B** possess anticancer [11], anti-depressant hypnotic [12], antiallergic, anxiolytic, antidepressant [13] and anticoccidial properties (Figure 1). JUSTEA patent report "Certain 6-azauracils and derivatives thereof are known in the art. U.S. Pat. Nos. 3,905,971

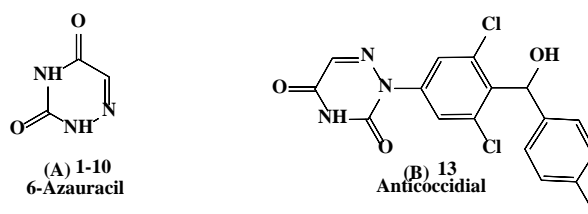


Figure 1. Important medicinal 6-azauracils.

and 3,912,723 disclose certain 2-phenyl-as-triazine-3,5(2*H*,4*H*)diones and certain 2-substituted-phenyl-as-triazine-3,5(2*H*,4*H*)diones and their use as agents for the control of coccidiosis. U.S. Pat. Nos. 3,883,527 and 3,883,528 disclose processes for producing certain 2-aryl-as-triazine-3,5(2*H*,4*H*)-diones, which are useful as coccidiostats”.

Accordingly, the present work focused on investigation for novel *N*-flurinyl-9-spiro-6-azauracils, in view of their antioxidant properties.

2. Chemistry

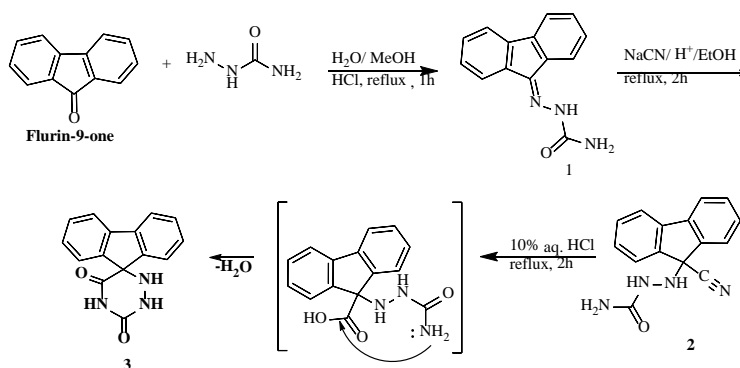
This investigation aims to synthesize novel 6-azauracils derivatives as antioxidant probs. Synthesis strategy starts with the synthesis of spiro 5-(fluoren-9'-yl)-6-azauracil **3**, followed by the formation of *N,N*-disubstituted 6-azauracil **4** - **17**, via a simple alkylation. Thus, condensation of fluoren-9-one with semicarbazide, HCl in reflux with MeOH for 1 h to yield the semicarbazone **1**, which upon reaction of HCN under reflux yields *N*-(carbonitril)-*N*-(flurin-9'-yl)-semicarbazide **2**. Acidic hydrolysis of compound **2** achieved the target spiro 5-(flurin-9'-yl)-hexahydro-6-azauracil **3** (**Scheme 1**). Compound **3** is called the flurinyl spiro-6-azauracile.

The *N¹,N²*-disubstituted 6-azauracils **3** are used as potential inhibitors [14]. Thus, *N*-Methylation of compound **3** by MeI/1% *aq.* KOH at room temperature gives 1,3-dimethyl-Spiro 5-(fluoren-9'-yl)-6-azauracil **4**, while hydroxymethylation of **3** by refluxing with MeOH/HCHO, yielded 1,3-dihydroxymethyl-spiro-5-(fluoren-9'-yl)-6-azauracil **5**. Similarly, Mannich bases **6** and **7** obtained by the refluxing of compound **3** with secondary amines (such as piperidine and/or morpholine) in MeOH/HCHO (**Scheme 2**).

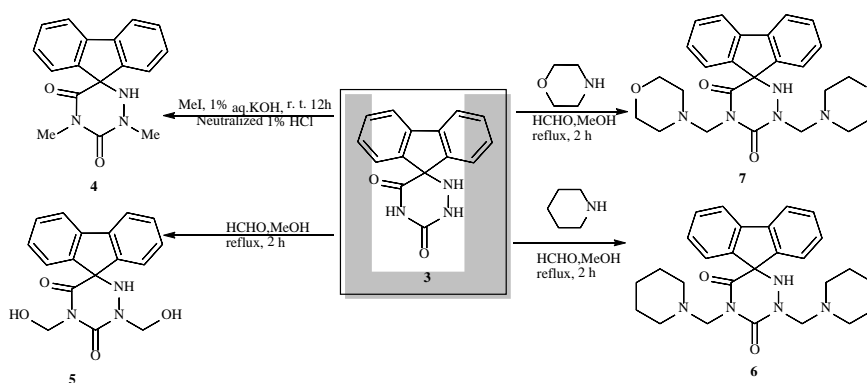
Moreover, reflux compound **3** with primary aromatic amines for example; sulfanilamide 4-amine-3-hydroxy-naphthine sulfonic acid and 4-amino antipyrine drug give the 1,3-di(substituted amino) methyl-spiro-5-(fluoren-9'-yl)-6-azauracils **8** - **10** (**Scheme 3**).

In general, the introduction of acetamide urea and thiourea to heterocyclic moiety almost improves their properties. Thus, reflux compound **3** with acetamide, *N*-phenyl urea and *N*-phenyl thiourea produced 1,3-di(acetyl-spiro-5-(flurin-9'-yl)-6-azauracil **11**, 1,3-Dianilido-spiro-5-(flurin-9'-yl)-6-azauracil **12** and 1,3-dithianilido-spiro-5-(flurin-9'-yl)-6-azauracil **13** respectively (**Scheme 4**).

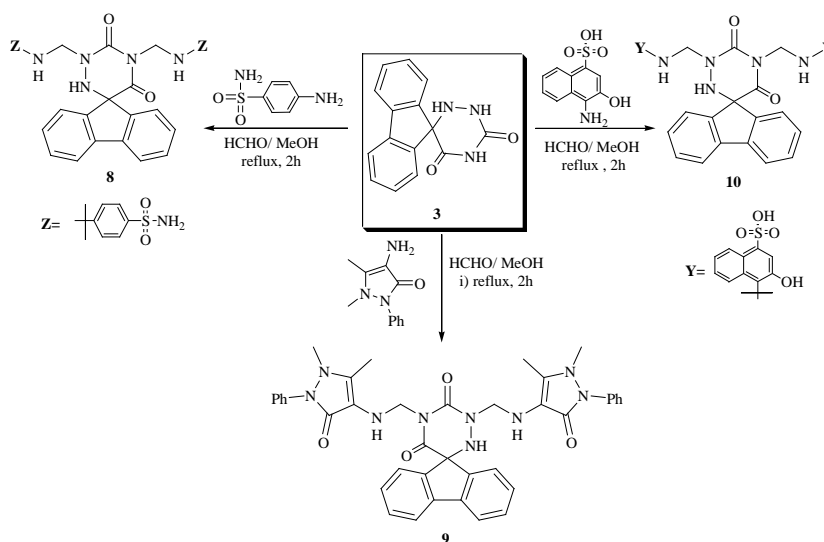
Furthermore, the room temperature reaction stirring of compound **3** with formyl acetate (CH_3COOCHO) in ether for 4 - 6 h, furnished 1,3-diformyl-spiro-5-(flurin-9-yl)-6-azauracil **14** (Scheme 5). Reactivity of compound **4** primarily evaluated by reflux with primary amine for example aniline and hydrazine to give the imino **15** and hydrazone **16** derivatives.



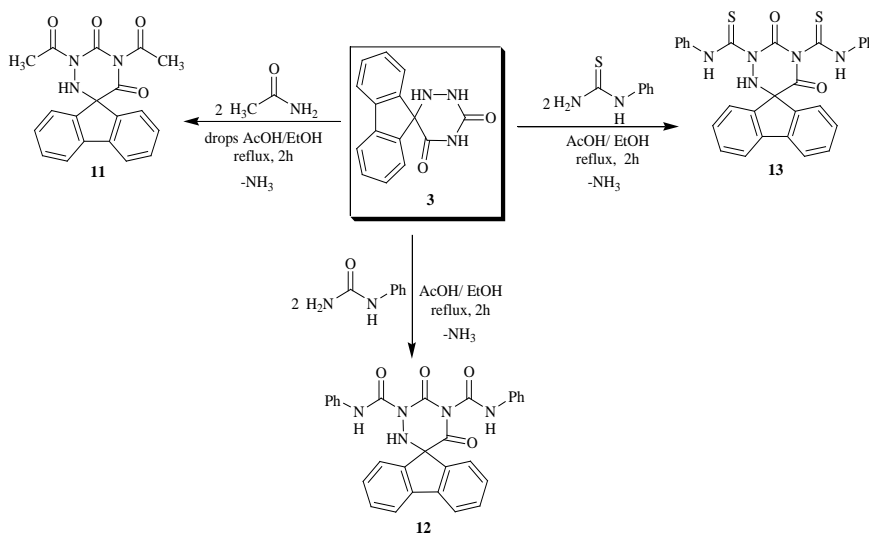
Scheme 1. Synthesis of fluriny spiro-6-azauracile **3**.



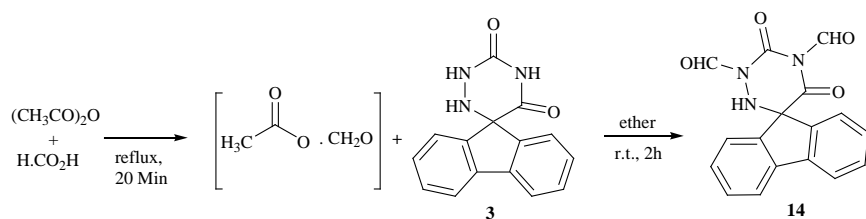
Scheme 2. Formation of compounds **4 - 7**.



Scheme 3. Formation of compounds **8 - 10** from **3**.



Scheme 4. Formation of compound 11 - 13 from 3.



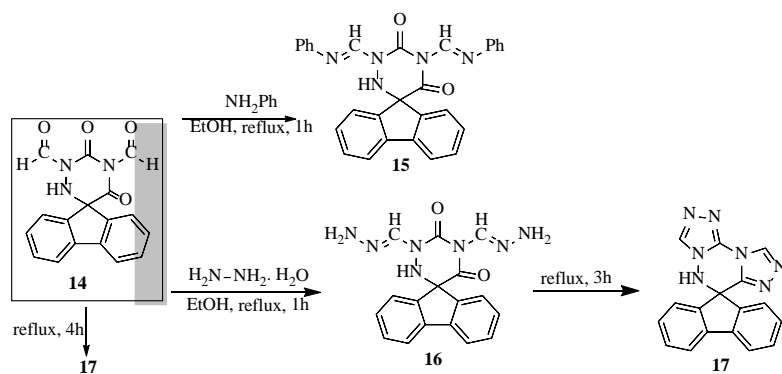
Scheme 5. Formation of compound 14 from 3 and formyl acetate.

Fusion heating of compound **16**, furnished the thermal cyclisation compound 1,2,4-triazolo [1,2,4]triazino-1,2,4-triazole **17**. Likewise, Compound **17** obtained from cyclocondensation reaction of **14** with hydrazine hydrate (**Scheme 6**).

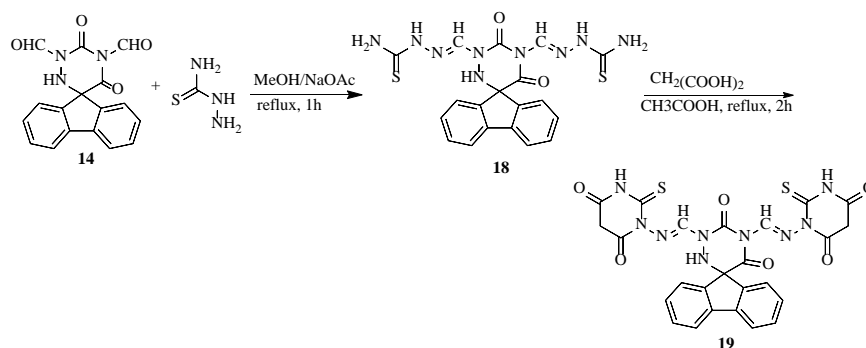
Moreover, condensation of compound **14** with thiosemicarbazide in acetic acid afforded the thiosemicarbazone **18** which upon ring closure reactions with malonic acid in refluxing AcOH, yielded the thiobarbituric acid derivatives **19** (**Scheme 7**).

3. Results and Discussion

6-Azaauracil derivatives showed significant biological effects in comparison with uracil moiety, 6-azauracil enhanced the electronegativities over the structures center. This improves distribution, dielectric constant and hydrophobic properties within the body [1] [2]. Correspondingly, 6-azauracil used as amphipathic prodrugs of 1,2-diol drugs via the regioselective enzymatic protocol [3]. Thus, this investigation tends to synthesis a novel 6-azauracil followed by obtaining their 1,3-disubstituted spiro-(5-fluoren-9'-yl)-6-azauracil **4** - **19** (**Schemes 1-7**). Structures of the new targets are deduced from their elemental analysis and spectral measurement.



Scheme 6. Formation of compound 15 - 17 from 14.



Scheme 7. Formation of 19, 18 from 14.

IR spectrum of compound **3** showed ν^{-1} at 3200 - 3100 cm^{-1} for 3 NH of 1,3- and 6-position with ν^{-1} at 1700 - 1680 cm^{-1} for two C=O. While, all IR spectra of compounds **4** - **19** recorded the lack of N¹ and N³ of 6-azauracil which confirm that substitution reactions take place on NH, with appearances of two C=O at ν^{-1} 1710 - 1680 cm^{-1} for 2- and 4-positions. IR spectrum of **17** showed a lack of both ¹NH and ³NH and C=O of 6-azauracil, while that of **18** recorded ν^{-1} 3200 - 3100, 1200 attribute to both C=S and -CH₂- groups respectively which confirm that full fused heteropoly cyclic system **17**.

The ¹H NMR spectrum, showed broad peak at 14 - 11 ppm attribute to presence of the unreactive NH at 6-position of 6-azauracil.

Only, compounds **4** - **11** showed at ~4 ppm attribute to CH₂-N for N¹ and N³, while compounds **8**, **9**, **10**, **12**, **13** and **15** recorded the additional, new aromatic protons at 8 - 6 ppm, while that of **5** showed 5.5 ppm for OH protons.

In addition, the oriental aromatic protons of flurin-9'-yl moiety shows aromatic multiple at 6 - 8 ppm for compounds **3** - **18**. Compound **14** exhibit peak at 9.5 ppm for the aldehydic proton, which lacks in the structure of **15** - **18**. ¹³C NMR spectral study of the new synthesized compound **4** - **11** showed at 30-15 ppm attribute to presence of aliphatic carbon. Only the compound **13**, **18** and **19** recorded at 180 - 170 ppm for C = S carbons. All the compounds, showed at 130 - 120 ppm for the aromatic carbon with at 170 - 160 ppm for C = O carbons which disappear in the structure of **17**. Only compound **19** recorded signals at

40 ppm attribute a cyclic CH_2 of thiobarbituric acids.

Mass fragmentation pattern shows the molecular ion peaks and base peak. This observation explains the degree of stabilities for the study systems, non-aromatic, thus, the base peak of the compound 3, 6 and 7 is m/z 152 for C_{12}H_9 while that of compounds of 12, 13 and 17 is 56.63 attribute to a type of cyclic (Table 1). While Figure 2 and Figure 3 showed two examples for suggested mass cyclic fragmentation of compound 3 and 17.

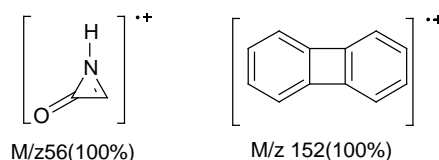


Table 1. Mass fragmentation study of new compounds 3 - 17 (M/Z/Int. %).

| Compound No. | Fragmentations |
|--------------|--|
| 3 | 41 (52), 56 (10), 57 (50), 70 (47), 71 (49), 85 (5), 104 (10), 113 (8), 148 (50), 151 (5), 152 (100), 167 (45), 178 (1), 208 (10; M-57). |
| 6 | 41 (2), 56 (5), 81 (5), 152 (100), 163 (5), 208 (1), 281 (1), 374 (10), 379 (49), 394 (35), 410 (15), 428 (65; M-42). |
| 7 | 41 (12), 44 (10), 71 (1), 83 (10), 133 (12), 289 (25), 291 (75), 346 (70), 401 (30), 410 (15), 428 (65), 178 (1), 208 (10; M-36). |
| 9 | 41 (5), 56 (88), 152 (100), 163 (89), 187 (75), 322 (18; M-374). |
| 11 | 41 (10), 43 (15), 57 (100), 85 (60), 70 (47), 152 (25), 163 (38), 185 (10), 203 (5). |
| 13 | 56 (100), 70 (60), 83 (40), 97 (10), 112 (51), 179 (2), 187 (1), 187 (5), 203 (10), 319 (2; M-185). |
| 16 | 57 (100), 75 (60), 83 (39), 152 (5), 165 (3), 187 (1), 203 (10), 238 (3), 278 (2), 349 (1; M-187). |
| 17 | 56 (100), 67 (59), 83 (36), 98 (10), 152 (1), 165 (1), 203 (5), 278 (1; M-35). |

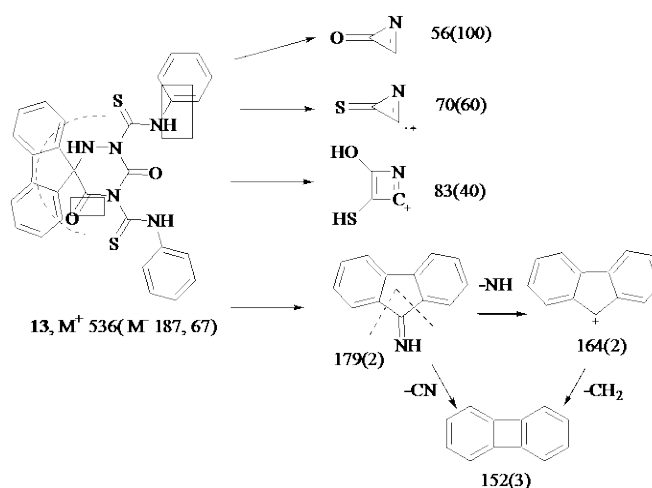


Figure 2. Mass fragmentation pattern of 3.

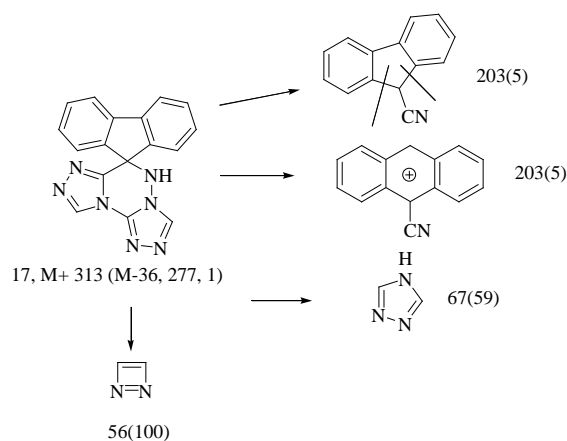


Figure 3. Mass fragmentation pattern of compound 17.

4. Experimental

The commercial chemicals and solvents used in the synthesis were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), Fisher Scientific Inc. (Springfield, NJ), or Lancaster (Windham, NH) and were used without further purification. Analytical grade reagents were purchased from standard commercial sources. Melting points determined with an electrothermal Bibby Stuart Scientific melting point sample (UK). A Perkin Elmer Model RXI-FT-IR system 55529 was used for recording the IR spectra of the prepared compounds. A Bruker advance DPX 400 MHz model uses TMS as internal standard was used for recording the ^1H and ^{13}C NMR spectra of the compounds on deuterated DMSO- D_6 . A GC-MS-GP 1000 Ex model was used for recording the mass spectra of the compounds. Electronic spectra were recorded in ethanol on Shimadzu UV and visible 310 IPC Spectrophotometer. Elemental analysis was performed in micro analytical center of Cairo University, Cairo, Egypt.

1) Semicarbazone-*N*-(fluorene-9-yl) (1)

Fluorene-9-one (0.01 mol) and semicarbazide. HCl (0.01 mol, in 10 ml H_2O) in MeOH (20 ml) refluxed for 1 h, cooled then poured onto ice. The solid obtained filtered and crystallized from MeOH to give **1**. Yield 80%. m.p. 210°C - 212°C . IR (vcm^{-1}): 3362, 3200, 3127 (NH-NH₂), 1666 (CONH), 1626 (NH₂), 1571 (C=N), 1317 (NCN), 949, 916, 877 (aromatic ring). ^1H NMR (DMSO- d_6) (δ): 10.71 (s, NH), 8.51 (d, 4H, aromatic), 7.1 - 7.6 (m, 2H, aromatic), 7.19 - 7.15, 7.07 - 6.76 (m, 2H, aromatic), 3.35 (s, 2H, NH₂). ^{13}C NMR (DMSO- d_6) (δ): 162 (C=O), 140 (C=N), 120 - 116 (aromatic carbons), 109 (C-C). Anal.%Calcd for $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}$ (237.26): C, 70.87; H, 4.67; N, 17.71. Found: C, 70.66; H, 4.51; N, 17.49.

2) Fluorene-9-(*N*-semicarbazide)-9-carbonitrile (2)

A mixture of **1** (0.01 mol) and NaCN (0.01 mol, in 10 ml H_2O) in EtOH/AcOH (1:1, 50 ml) refluxed 2 h, cooled then poured onto ice. The solid product filtered off and crystallized from EtOH to give **2**, yield 70%, m.p. 230°C - 232°C . IR (vcm^{-1}): 3203, 3128 (NH, NH), 2220 (CN), 1669 (C=O), 1573 (C=N), 1356 (NCN), 1170 (N-N), 946, 916, 870 (aromatic rings). ^1H NMR (DMSO- d_6) (δ):

8.31 (s, NH), 8.61 - 8.83 (m, 2H, aromatic), 7.1 - 7.6 (m, 1H, aromatic), 7.21 - 7.43, 7.07 - 6.76 (m, 2H, aromatic), 6.35 (s, 2H, NH₂). ¹³CNMR (DMSO-d₆) (δ): 163.7 (C=O), 133.8 (C=N), 120.12 - 115.66 (aromatic carbons). Anal.%Calcd for C₁₅H₁₂N₄O (264.29): C, 68.17; H, 4.58; N, 21.20. Found: C, 68.11; H, 4.39; N, 20.09.

3) Spiro-(5-fluoren-9'-yl)-6-azauracil (3)

Compound **2** (1 gm, 0.01 mol) and aq. HCl (10%, 10 ml) heated under reflux 2 h, cooled, the resultant solid, filtered off and crystallized from MeOH to give **3**; Yield 66%; m.p. 250°C - 252°C, IR (vcm⁻¹): 3459 (OH), 1668 (C=O), 1617 (C=C), 1596 (C=N), 1316 (NCN), 948, 876 (aromatic rings). ¹HNMR (DMSO-d₆) (δ): 11.5 - 12.11 (m, 3NH), 8.55 (m, 4H, aromatic), 7.12 (d, 4H, aromatic). ¹³CNMR (DMSO-d₆) (δ): 169.6 (C=O), 141.12 (C=O), 130.99 (C=N), 120.76 - 117.11 (aromatic carbons), 89.7 (C-C-N). Anal.%Calcd for C₁₅H₁₁N₃O₂ (237.26): C, 67.92; H, 4.18; N, 15.84. Found: C, 67.79; H, 4.70; N, 15.80.

4) 1,3-Dimethyl-spiro-5-(fluoren-9'-yl)-6-azauracil (4)

A mixture of **3** (0.01 mol), MeI (0.01 mol) in aq. KOH (1%, 50 ml) stirred for 12 h at room temperature. The reaction neutralized by dil. HCl treated with MeOH. The yielded solid filtered off and crystallized from dioxan to give **4**. Yield 60% m.p. 150°C - 152°C IR (vcm⁻¹): 3380 (OH), 3320 (NH), 3080 (aromatic CH), 2950, 2870 (aliphatic CH), 1700, 1680 (2C=O), 1490, 1440 (deformation CH₂), 1310 (NCN), 940, 910 (aromatic rings). ¹HNMR (DMSO-d₆) (δ): 7.90 (m, 4H, aromatic), 7.28 (m, 4H, aromatic protons), 3.51 (s, 3H, CH₃), 3.2 (s, 3H, CH₃). ¹³CNMR (DMSO-d₆) (δ): 162.2, 159.4 (2C=O), 130.81 - 121.72 (aromatic carbons), 112.89 - 110.70 (C-C). Anal.%Calcd for C₁₇H₁₅N₃O₂ (293.33): C, 69.61; H, 5.15; N, 14.33. Found: C, 69.51; H, 5.05; N, 14.12.

5) 1,3-Dihydroxymethyl-spiro-5-(fluoren-9'-yl)-6-azauracil (5)

A mixture of **3** (0.01 mol), HCHO (0.02 mol) in MeOH (50 ml) refluxed 2 h then poured onto ice. The solid produced filtered off and Crystallized from MeOH to give **5**. Yield 60%, m.p. 160°C - 162°C. IR (vcm⁻¹): 3380 (NH₂), 3050 (aromatic CH), 2980, 2890 (aliphatic CH), 1710, 1680 (2C=O), 1560 (C=N), 1490, 1440 (deformation CH₃), 1310 (NCN), 960, 910 (aromatic rings). ¹HNMR (DMSO-d₆) (δ): 8.87 (m, 4H, aromatic), 7.93 (m, 4H, aromatic protons), 5.9 (s, 1H, OH), 5.32 (s, 2H, CH₂), 5.22 (s, 2H, CH₂). ¹³CNMR (DMSO-d₆) (δ): 142.2, 155.4 (2C=O), 141.81 - 126.72 (aromatic carbons), 77.9 (CH₂), 68.6 (CH₂). Anal.%Calcd for C₁₇H₁₅N₃O₄ (325.32): C, 62.76; H, 4.65; N, 12.92. Found: C, 62.55; H, 4.39; N, 12.80.

6) Mannich Bases 6 & 7

A mixture of **3** (0.01 mol) and sec. amines as piperidine and/or morpholine (0.02 mol), HCHO (0.02 mol) and MeOH (50 ml) refluxed 2 h, cooled then poured onto ice. The solid obtained filtered off and crystallized from MeOH to give **6** and/or **7** respectively.

6, yield 70%, m.p. 195°C - 196°C.

7, yield 72%, m.p. 200°C - 201°C.

6, Anal.%Calcd for C₂₇H₃₃N₅O₂ (459.59): C, 70.56; H, 7.24; N, 15.24. Found: C,

70.44; H, 7.12; N, 15.10.

7, Anal.%Calcd for $C_{25}H_{29}N_5O_2$ (459.59): C, 64.78; H, 6.31; N, 15.11; O, 13.81 Found: C, 64.58; H, 6.21; N, 15.31; O, 13.41.

6, IR (vcm^{-1}): 3059 (NH), 2933 (CH_2), 1712, 1665 (2C=O), 1610 (C=C), 1598 (C=N), 1471, 1448 (deformation CH_2), 1179 (NCN), 949, 917 (aromatic rings).

7, IR (vcm^{-1}): 3310 (NH), 3050 (aromatic CH) 2980 (aliphatic CH), 1680, 1644 (2C=O), 1578 (C=N), 1478, 1449 (deformation CH_2), 1303 (NCN), 1681 (C-O-C), 950, 915 (aromatic rings).

6, $^1\text{HNMR}$ (DMSO- d_6) (δ): 7.5 - 8.0 (m, 4H, aromatic protons), 8.1 - 8.4 (m, 4H, aromatic protons), 1.30 (pent, 4H, 2CH_2), 1.45 - 1.50 (m, 2H, CH_2), 2.4 (t, 8H, 4CH_2), 5.1 (s, 1H, $\text{CH}_2\text{-N}$), 5.1 (s, 1H, NH).

7, $^1\text{HNMR}$ (DMSO- d_6) (δ): 7.30 - 7.90 (m, 4H, aromatic protons), 8.0 - 8.2 (m, 4H, aromatic protons), 1.30 (pent, 4H, 2CH_2), 1.45 - 1.50 (m, 4H, 2CH_2), 5.1 (s, 1H, $\text{CH}_2\text{-N}$), 5.1 (s, 1H, NH). $^{13}\text{CNMR}$ (DMSO- d_6) (δ): 148.2, 156.4 (2C=O), 140.66 - 125.12 (aromatic carbons), 84.9(CH_2), 77.9, 65.1 ($2\text{CH}_2\text{-N}$), 66.4, 52.8 (4 CH_2).

7) 1,3-Di(4'-tolyl)sulfonamido

methyl-spiro-(5-fluoren-9-yl)-6-azauracile (8)

A mixture of **3** (0.01 mol) and sulfanilamide drug (0.02 mol) with MeOH (50 ml), HCHO (0.02 mol) refluxed 2 h, cooled then poured onto ice. The solid yielded filtered off and crystallized from EtOH to give **8**. Yield 66% m.p. 180°C - 181°C. IR (vcm^{-1}): 3461, 3368, 3212 (NH, NHCH_2 , NH_2 , SO_2), 1702, 1669 (2C=O), 1619 (C=C), 1595 (C=N), 1485, 1459 (deformation CH_2), 1304 (SO_2NH), 1177 (C-N), 946, 916, 824 (aromatic rings). $^1\text{HNMR}$ (DMSO- d_6) (δ): 7.90 - 7.85 (m, 4H aromatic protons), 7.71 - 7.52 (m, 4H, aromatic protons), 7.54 - 7.12 (m, 8H, phenyl protons), 6.89 (s, 4H, 2NH_2), 6.3 (s, 1H, NH), 6.2(s, 1H, NH), 5.55 (s, 1H, NH), 4.9, 4.52 (s, 4H, 2CH_2). $^{13}\text{CNMR}$ (DMSO- d_6) (δ): 162.2, 156.4 (2C=O), 140.66 - 125.12 (aromatic carbons), 84.9 (CH_2), 77.9, 65.1 ($2\text{CH}_2\text{-N}$), 66.4, 52.8 (4 CH_2). Anal.%Calcd for $C_{29}H_{27}N_7O_6S_2$ (633.70): C, 56.34; H, 3.68; N, 12.17; O, 16.68; S, 11.14. Found: C, 56.24; H, 3.78; N, 12.57; O, 16.68; S, 11.69.

8) 1,3-Di(1'-phenyl-2',3'-dimethyl-5'-exo-pyrazol-4-yl)

aminomethyl-spiro-(5'-fluoren-9-yl)-6-azauracil (9)

A mixture of **3** (0.01 mol) and 4-aminoantipyrine (0.02 mol), HCHO, (0.02 mol), MeOH (50 ml) refluxed 2 h, cooled, the solid obtained filtered off and crystallized from EtOH to give **9**. Yield 60%; m.p. 205°C - 207°C. IR (vcm^{-1}): 3400 (NH), 3302, 3131 (NH, NH), 3050 (aromatic CH), 1702, 1670 (C=O), 1618 (C=C), 1595, 1574 (C=N), 1485, 422 (deformation CH_3 , CH_2), 946, 917, 877 (aromatic rings). Anal.%Calcd for $C_{39}H_{37}N_9O_4$ (695.78): C, 67.32; H, 5.36; N, 18.12; O, 9.20 Found: C, 67.11; H, 5.18; N, 18.02; O, 9.00

9) 1,3-Di(3'-hydroxy-naphthalin-1'-sulfonic

acid-4'-amino)methyl-spiro-5-(fluoren-9-yl)-6-azauracil (10)

A mixture of **3** (0.01 mol), 3-amino-3-hydroxy-naphthaline-sulfonic acid (0.02 mol), HCHO (0.02 mol), MeOH (50 ml) refluxed 2 h, cooled. The yielded solid,

filtered off and crystallized from MeOH to give **10**, yield 59%; m.p. 289°C - 290°C, IR (vcm^{-1}): 3550 (OH), 3380, 3310, 3098 (NH), 2885 (aliphatic CH), 2640 (OH), 1685, 1655 (C=O), 1619 (C=C), 1576 (C=N), 1469, 1433 (deformation CH_2), 1182 (SO_2), 980, 939, 893, 860 (aromatic rings). $^1\text{HNMR}$ (DMSO-d_6) (δ): 8.30 - 8.11 (m, 4H aromatic protons), 7.91 - 7.72 (m, 4H, aromatic protons), 7.54 - 7.12 (m, 8H, phenyl protons), 6.89 (s, 4H, 2 NH_2), 6.3 (s, 1H, NH), 6.2 (s, 1H, NH), 5.55 (s, 1H, NH), 4.9, 4.52 (s, 4H, 2 CH_2). $^{13}\text{CNMR}$ (DMSO-d_6) (δ): 162.2, 156.4 (2C=O), 140.66 - 125.12 (aromatic carbons), 84.9 (CH_2), 77.9, 65.1 (2 CH_2 -N), 66.4, 52.8 (4 CH_2). Anal.%Calcd for $\text{C}_{37}\text{H}_{29}\text{N}_3\text{O}_{10}\text{S}_2$ (767.78): C, 57.88; H, 3.81; N, 9.12; O, 20.84; S, 8.35. Found: C, 57.59; H, 3.91; N, 9.02; O, 20.66; S, 8.15.

10) 1,3-Di(acetamido)-spiro-5-(fluoren-9-yl)-6-azauracil (11)

A mixture of **3** (0.01 mol) and acetamide (0.02 mol), in AcOH/EtOH (10 ml, 1:1) refluxed 2 h, cooled then poured onto ice. The produced solid, filtered off and crystallized from EtOH to give **11**, yield 68%; m.p. 233°C - 235°C. IR (vcm^{-1}): 3363 (NH), 3200 (NH), 1710, 1680, 1668 (C=O), 1596 (C=N), 1571 (C=N), 1486, 1459 (deformation CH_3), 1360 (NCON), 946, 918, 878, 781 (aromatic rings). $^1\text{HNMR}$ (DMSO-d_6) (δ): 9.30 - 9.11 (m, 4H aromatic protons), 8.93 - 8.82 (m, 4H, aromatic protons), 5.0 (s, 1H, NH), 2.40 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). $^{13}\text{CNMR}$ (DMSO-d_6) (δ): 175.2, 172.4 (2C=O), 152.8 (2C=O), 141.66 - 134.12 (aromatic carbons), 85.9 (spiro C), 25.3, 20.5 (2 CH_3). Anal.%Calcd for $\text{C}_{19}\text{H}_{15}\text{N}_3\text{O}_4$ (439.35): C, 65.32; H, 4.33; N, 12.03; O, 18.32. Found: C, 65.92; H, 4.83; N, 12.93; O, 18.02.

11) 1,3-Di(Anilido)-spiro-(5-fluoren-9-yl)-6-aza-uracil (12)

A mixture of **3** (0.01 mol), N-phenylurea (0.02 mol) in AcOH/EtOH (1:1, 10 ml) refluxed for 2 h, cooled, then poured onto ice. The solid obtained filtered off and crystallized from MeOH to give **12**, yield 70% m.p. 225°C - 227°C. IR (vcm^{-1}): 3300, 3202, 3128 (NH), 3060 (aromatic CH), 1680, 1668, 1630 (C=O, CONH), 1615 (C=C), 1595 (C=N), 1317 (NCON), 1176 (C-N), 946, 917, 880, 781 (aromatic rings). $^1\text{HNMR}$ (DMSO-d_6) (δ): 9.22 - 9.54 (m, 4H aromatic protons), 8.93 - 8.82 (m, 4H, aromatic protons), 7.54 - 7.12 (m, 8H, phenyl protons), 5.5 (s, 1H, NH). $^{13}\text{CNMR}$ (DMSO-d_6) (δ): 177.9, 172.33 (2C=O), 152.8 (2C=O), 144.66 - 134.12 (aromatic carbons), 88.1 (spiro C). Anal.%Calcd for $\text{C}_{29}\text{H}_{21}\text{N}_5\text{O}_4$ (503.52): C, 69.18; H, 4.20; N, 13.91; O, 12.71. Found: C, 69.00; H, 4.77; N, 13.99; O, 12.01.

12) 1,3-Di(thiaanilido)-spiro-(5-fluoren-9'-yl)-6-aza-uracil (13)

A mixture of **3** (0.01 mol), N-phenylthiourea (0.02 mol), in HCHO (0.02 mol) in MeOH (50 ml) refluxed for 2 h, cooled, then poured onto ice. The solid produced filtered off and crystallized from EtOH to give **13**, yield 71% m.p. 235°C - 237°C. IR (vcm^{-1}): 3310, 3205, 3057 (NH), 1712, 1669 (C=O), 1610 (C=C), 1597 (C=N), 1357 (NCSN), 1171 (C-S), 946, 917, 877, 812, 782 (aromatic rings). $^1\text{HNMR}$ (DMSO-d_6) (δ): 8.90 (s, 2H, 2NH), 8.56 - 8.11 (m, 4H aromatic protons), 7.94 - 7.55 (m, 4H, aromatic protons), 5.0 (s, 1H, NH), 2.40 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). $^{13}\text{CNMR}$ (DMSO-d_6) (δ): 175.2, 172.4 (2C=O), 152.8 (2C=O), 141.66 -

134.12 (aromatic carbons), 85.9 (spiro C), 25.3, 20.5 (2CH₃). Anal.%Calcd for C₂₉H₂₁N₅O₂S₂ (535.64): C, 65.03; H, 3.95; N, 13.08; O, 5.97; S, 11.97. Found: C, 64.89; H, 3.77; N, 12.79; O, 5.97; S, 11.89.

13) 1,3-Diformyl-spiro-(5-fluoren-9'-yl)-6-aza-uracil (14)

To compound **3** (0.01 mol), in dry ethylether, 100 ml; formylacetate (0.02 mol) added with stirring at room temperature for 12 h, The solid obtained filtered off and crystallized from dioxan to give **14**. [Ac₂O + HCOOH (1:1) were refluxed 20 min then cooled to give formyl acetate]. Compound **14**, yield 80%; m.p. 280°C - 282°C, IR (vcm⁻¹): 3100 (NH), 1710, 1680 (C=O), 920, 860, 810 (aromatic rings). ¹HNMR (DMSO-d₆) (δ): 8.21 (s, 1H, CH), 8.05 - 7.95 (m, 4H aromatic protons), 7.82 - 7.55 (m, 4H, aromatic protons), 5.9 (s, 1H, NH). ¹³CNMR (DMSO-d₆) (δ): 168.7, 152.4 (2C=O), 161.8 (C=O), 144.66 - 124.12 (aromatic carbons), 88.1 (spiro C). Anal.%Calcd for C₁₇H₁₁N₃O₄ (321.29): C, 63.55; H, 3.45; N, 13.08; O, 19.92. Found: C, 63.40; H, 3.15; N, 12.89; O, 19.00.

14) 1,3-Dischiff base-spiro-(5-fluoren-9'-yl)-6-aza-uracil (15)

A mixture of **14** (0.01 mol) and anhydrous aniline (0.02 mol) in abs. EtOH (20 ml) warmed for 1 h, cooled. The solid produced filtered off and crystallized from EtOH to give **15**, yield 75%; m.p. 150 - 152. IR (vcm⁻¹): 3222(NH), 1709, 1660 (2C=O) 1670 (C=C), 1190 (P=O), 814 (substituted phenyl). ¹HNMR (DMSO-d₆) (δ): 8.91 (s, 1H, CH), 7.95 (d, 2H aromatic protons), 7.54 (d, 2H aromatic protons), 7.32 - 7.25 (m, 6H, aromatic protons), 6.65 (d, 2H aromatic protons) 5.34 (s, 2H, NH₂), 5.09 (s, 1H, NH). Anal.%Calcd for C₂₉H₂₁N₅O₂ (471.5): C, 73.87; H, 4.49; N, 14.85; O, 6.79. Found: C, 73.07; H, 4.79; N, 14.55; O, 6.69.

15) 1,3-Di(hydrazono)-spiro-(5-fluoren-9'-yl)-6-aza-uracil (16)

A mixture of **14** (0.01 mol) and hydrazine hydrate (100%. 0.022 mol) in abs. EtOH (100 ml) warmed under reflux for 1 h, cooled. The resultant solid filtered off and crystallized from EtOH to give **16**, yield 60%; m.p. 100°C - 102°C. IR (vcm⁻¹): 3202 (NH), 1719, 1630 (2C=O) 1690 (C=C), 1208 (C=N), 930, 850, 830 (aromatic rings). Anal.%Calcd for C₁₇H₁₅N₇O₂ (349.35): C, 58.45; H, 4.33; N, 28.07; O, 9.16. Found: C, 58.05; H, 4.90; N, 29.07; O, 9.81.

16) 5H-Spiro-6-(5-fluoren-9'-yl)-1,2,4-Triazololo [3,4-b]-1,2,4-triazolo [5,4-d] [1,2,4] triazene (17)

a) A mixture of **14** (0.01 mol) and hydrazine hydrate (0.022 mol) in abs.EtOH (100 ml) refluxed for 4 h, cooled. The solid obtained filtered off and crystalized from THF to give **17**, yield 70%; m.p. > 300.

b) Compound **16** was heated above its melting point and mixed melting point not change.

Anal.%Calcd for C₁₇H₁₁N₇ (313.31): C, 65.17; H, 3.54; N, 31.29 Found: C, 65.98; H, 3.45; N, 31.02.

5. The Anti-Oxidant Evaluation

Antioxidants are substances that can prevent or slow damage to cells caused by unstable free radicals [15] [16] [17]. Also, the molecules that can neutralize free

radicals by accepting or donating electrons to eliminate the unpaired condition of the radicals. Oxidation reactions can produce a free radical, which starts the chain reactions that damage cells. The oxidation damage to DNA, proteins, and other macromolecules, have been implicated in the pathogenesis of a wide variety of diseases, mostly notably heart, cancer, inflammatory and renal diseases [18] [19]. When skin is exposed to high levels of UV, Photo-oxidative damage is induced by the formation of different types of reactive species of oxygen, super oxide, radicals and peroxide radicals. These forms of reactive oxygen damage cellular lipids, proteins and DNA and cause premature aging of the skin, photo dermatoses and skin cancer [20], based upon these observations, and results, and in search for new antioxidants substances as 6-azauracil derivatives have been synthesized in view of their antioxidant effects.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was used to produce and reducing the odd electron stable-free radical which showed a strong UV-absorption maximum at $\lambda = 517$ nm. The new systems obtained dissolved in DMSO/EtOH at 50 and 300 mmol·L⁻¹ added to DPPH at 100 mmol·L⁻¹.

The tube kept at room temperature for 20 minutes and the absorption measured at λ 517 nm. The difference between the test and the control taken as the present scavenging of the DPPH radical by the formula

$$\% \text{ inhibition} = \frac{AB - AA}{AB} \times 100$$

where AB = Absorption of blank, AA = Absorption of the tested compound.

The radical scavenging activity of ascorbic acid also measured and compared with that of the difference synthesized compound AA [21]-[26]. The results obtained as shown in **Table 2**. From this data, we can conclude that:

Table 2. The DPPH radical scavenging activity of novel N-substituted 6-azauracils at 150 and 300 mmol·L⁻¹.

| Compound No. | DPPH % inhibition anti-oxidant \pm SD | |
|---------------|---|--------------------------|
| | 150 mmol·L ⁻¹ | 300 mmol·L ⁻¹ |
| 6 | 52.09 \pm 0.06 | 55.5 \pm 0.18 |
| 7 | 5.80 \pm 0.21 | 51.99 \pm 0.80 |
| 8 | 55.11 \pm 0.05 | 60.55 \pm 0.15 |
| 9 | 50.76 \pm 0.25 | 50.78 \pm 0.01 |
| 10 | 52.85 \pm 0.21 | 59.01 \pm 0.04 |
| 12 | 45.08 \pm 0.05 | 46.66 \pm 0.05 |
| 13 | 48.85 \pm 0.11 | 49.00 \pm 0.01 |
| 16 | 44.33 \pm 0.14 | 45.55 \pm 0.01 |
| 17 | 43.89 \pm 0.13 | 45.99 \pm 0.015 |
| Ascorbic acid | 43.00 | 50.70 |

- 1) The activity higher in the order $8 > 10 > 6 > 7 > 9 > 13 > 12 > 16$.
- 2) In view of the activity of a new compound which attribute to compounds **8** to sulfanilamide drug **6** and **10** to sulfonic acid while compounds **6** and **8** to presence of Mannich bases. Also, the activity of compound **9** attribute to presence of 4-aminoantipyrine drug, which compounds **13** and **12** due to bonded with thiourea and urea moieties.
- 3) It is clear that the antioxidant activities of these compounds depend on the distribution of electrons nature over all the 6-azauracil derivatives.
- 4) All the results obtained agree with the other study on the substituted 6-azauracil.

6. Conclusion

In search for new antioxidant probs, some new 1,3-disubstituted-5-spiro(florin-9'-yl)-6-azauracils have been obtained via a simple and safe alkylation and formation followed by condensation with amines. Most of the new targets exhibit a degree of antioxidant activity in the compare with ascorbic acid by using DPPH to produce and reduce the stable odd-electron free radical.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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