Synthesis and Biological Evaluation of New 1,2,3,5-Tetrasubstituted-1,2,4-triazine-6-ones

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ABSTRACT

5-(P-Florobenzylidene) - 3- (P-Chlorophenyl )-2-substituted-1,2,4-triazine-6-ones (2 and 3) were prepared via cyclocondensation of 1,3-oxazolinon derivative (1) with hydrazine hydrate and thiosemicarbazide. Treatment of compound (3) with phenacyl bromide and P-hydroxy benzalhyde yeilded the corresponding to 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2-(5-phenyl-1,3-thiazol-2-yl) - 1,2,4-triazine-6-one (5) and 5-(p-florobenzylidene) -3- (p-chlorophenyl)-2(p-hydroxy benzylidene amino) thiocarbonyl-1,2,4-triazine-6-one(7). Acetylation of compound (2, 5 and 7) with acetic anydriede led to the formation of monoacetyl and diacetyl derivatives (4, 6 and 8). The structure of synthesized compounds were established with IR, NMR, MS and elemental analysis.

Some of the new 1,2,4-triazine derivatives were evaluated for cytotoxicity activities.

Keywords: Synthesis, Biological Evaluation, 1,2,3,5-Tetrasubstituted-1,2,4-triazine-6-ones

1. INTRODUCTION

Substituted 1,2,4-triazines play a vital role in many biological processes and as synthetic drugs. 1,2,4-triazine derivatives have attracted considerable pharmaceutical interest due to their antitumor, antimicrobial, antifungal, antibacterial, anticancer, anti-HIV, antiinflammatory, antituberculosis and antimalarial, etc(6,7,8,9,10,11,12,13,14).

The synthesis of 1,2,4-triazines and their derivatives are well documented(15) and their methods of preparation are manifold and varied. A survey of the literature revealed that 1,3-oxazolinone compounds are the most common reagents used for the preparation of 1,2,4-triazines and their derivatives. El-deen and Coworkers(16,17,18), reported the condensation of carbazole derivatives with 1,3-oxazolinone in acetic acid to give 1,2,4-triazines with various arylidene, aryl and aminothiocarbmyl groups attaced at positions 5,3 and 2.

Herein we descried a similar method was also applied for the preparation and in vitro biological activity of novel tetra substituted 1,2,4-triazines. The some new prepared 1,2,4-triazines derivatives were screened for anticancer activity and some of them were found active both in vitro.
2. RESULTS AND DISCUSSION

Chemistry

1,3-oxazolinon (1) was obtained via condensation of p-florobenzaldehyde with n-(p-chlorobenzoyl)-glycine in presence of fused sodium acetate and acetic anhydride under fusion as a key starting material according to the literature method(19). 5-(p-florobenzylidene)-3-(p-chlorophenyl)-1,2,4-triazine-6-one(2) and 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2(aminothiocarbonyl)-1,2,4-triazine-6-one(3) were prepared by the reaction of 1,3-oxazolinone(1) with nitrogen nucleophilic reagents (such as hydrazine hydrate and thiosemicarbazide) in different conditions under reflux(20). Acylation of 1,2,4-triazine-6-one(2) with acetic anhydride under reflux led to the formation of 5-(p-florobenzylidene)-3-(p-chlorophenyl)-1,2-diacetyl-1,2,4-triazine-6-one(4) .

Treatment of 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2-(aminothiocarbonyl)-1,2,4-triazine-6-one(3) with phenacyl bromide in the presence of fused sodium acetate in ethanol under reflux afforded the corresponding to 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2-(5-phenyl-1,3-thiazol-2-yl)-1,2,4-triazine-6-one(5) . Heating of 1,2,4-triazine derivative(5) with acetic anhydride gave the corresonding 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2-(5-phenyl-1,3-thiazol-2-yl)-1-acetyl-1,2,4-triazine-6-one(6 , scheme 1).

Condensation of 1,2,4-triazine-6-one(3) with 4-hydroxybenzaldehyde in acetic acid yielded the corresponding 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2-(p-hydroxybenzylidene amino)thiocarbonyl-1,2,4-triazine-6-one(7).

1,2,4-triazine derivaveive(7) which acylated with boiling acetic anhydride led to the formation of 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2(p-acetoxybenzylidene amino)thiocarbonyl-1-acetyl-1,2,4-triazine-6-one (8,scheme 1).

Mass spectrometry of substituted 1,2,4-triazine drevateives

The mass spectral decomposition modes of some substituted 1,2,4-triazines (2,3,4,5 and 6) have been suggested and investigated(21,22).

The mass spectra of substituted 1,2,4-triazines (2,3,4,5 and 6) show relatively weak and strong molecular ions and peaks typical of a cleavage and rearrangement processes type fragmentation. Thus compounds 2,3,4,5 and 6 showed an intense molecular ion peak at 315,374,399,474 and 516 corresponding to the molecular formulas C16H11N3ClF0, C17H12N4ClF0S, C20H15N3ClF03, C25H16N4ClOFS and C27H18N4ClO2FS respectively.

All the spectra of compounds 2,3 and 4 showed characteristic common fragmentation pathways, as shown in scheme 2. The molecule ion of compound 3 and 4 (Fig 2) underwent fragmentation to produce peak at m/z 315, corresponding to the molecule ion of compound 2 by losing HN=C=S group and two molecule from ketene (CH2=C=S) .the loss of nitrogen atom from the ion with m/z 315 resulted in an ion at m/z 301 , the atom of m/z 301 underwent loss of p-florophenyl , acetylene ,carbon monoxide ,nitrogen atom and CH=NH group to give peaks at m/z 181 , m/z...
153 and two stable peaks at m/z 139, m/z 111, respectively.

Also, the ion of m/z 301 (molecular ion peak of compound 2, (fig 1) underwent loss of nitrogen molecule to give peak at m/z 287. The ion of m/z 287 underwent fragmentation to produce peak at m/z 149. It further underwent loss of formyl group (CHO) and acetylic molecule (CH = C) to give peaks at m/z 120 and m/z 95.

The molecular ion of 5-(p-florobenzylidene)-3-(p-chlorophenyl)-2-(5-phenylthiazole-2-yl)-1-acetyl-1,2,4-triazine-6-one (6, fig 4) underwent loss of ketene molecule (CH2CO) to give stable. Peak at m/z 474, corresponding to the molecular ion of 5-(p-florobenzylidene)-2-(p-chlorophenyl-imidazolidin-5-one).

The fragmentation of ion of m/z 300 led to different fragment ions at m/z 152, m/z 148, m/z 138, m/z 120, m/z 111 and m/z 95 (scheme 3).

The molecular ion of compound 5 (m/z 474) was also found to undergo fragmentation to produce the ion of m/z 175, corresponding to 5-phenyl-2-aminothiazol radical cation. The ion of m/z 175 broke to give an ion at m/z 134 which lost of N=C=NH group. It further underwent loss of sulphur atom and two carbon atom (C2) to give peaks at m/z 102 and m/z 77, respectively.

The M+2 of compound of 2-6 were observed along with the molecular ion peaks due to the presence of isotopes chlorine atom present in this compounds.
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**Fig 2:** Mass spectrum of compound 3

**Fig 4:** Mass spectrum of compound 5
Antitumour evaluation

The in-vitro anticancer of the test of some 1,2,4-triazine derivatives (3,4,6 and 7) was achieved in the cell culture lab. (cancer biology department and pharmacology unit, National cancer institute, cairo university, cairo, Egypt). Compounds 3-7 were tested for their in-vitro antitumor activity against human liver cancer cells (HEPG-2). Doxorubicin was used as a reference standard drug.

Inhibitory activity against liver carcinoma cells (HEPG-2) was tasted using different concentrations of the samples (0.00, 5.00, 12.50, 25.00 and 50.00 ug/ml) and surviving fraction was determined via color intensity with ELISA reader. The 50% inhibitory concentration (IC50) of the (HEPG-2) cell line was calculated from Table 1 and figure 5 and 6.

Table 1: Evaluation of cytotoxicity of 1,2,4-triazine derivatives (3-7) against (HEPG-2) cell line

<table>
<thead>
<tr>
<th>Sample conc. (ul/ml)</th>
<th>surviving fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>5.000</td>
<td>0.837</td>
</tr>
<tr>
<td>12.50</td>
<td>0.593</td>
</tr>
<tr>
<td>25.00</td>
<td>0.361</td>
</tr>
<tr>
<td>50.00</td>
<td>0.262</td>
</tr>
</tbody>
</table>
The results of 50% inhibitory concentration (IC\textsubscript{50}) of tested 1,2,4-triazine derivatives (3-7) showed a great variable activity compared to reference drug as shown in table 2.

**Table 2: IC\textsubscript{50}(ug/ml) values of tested compounds (3-7)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cell tumor</th>
<th>3</th>
<th>4</th>
<th>8</th>
<th>9</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPG-2</td>
<td>9.50</td>
<td>16.70</td>
<td>16.2</td>
<td>9.50</td>
<td>5.18</td>
<td></td>
</tr>
</tbody>
</table>

Compounds 3 and 7 had the most prominent activity against the human liver tumour cells (HEPG-2) (IC50 = 9.5 ug/ml).

The structure activity relationship (SAR) of compounds 3 and 7 demonstrates that the present of thiocarboxamide group as shown in structure as follows:

**fig 5: survival curve of compounds (3 and 4)**

**fig 6: survival curve of compounds (6 and 7)**

3. **EXPERIMENTAL**

Melting points were determined on MEL-TEMP II melting point apparatus and uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1420 spectrometer and a biorad FT57 (KBr). NMR spectra were recorded on a Jeol instrument and chemical shifts were given with respect TMS. Mass spectra were recorded on Probe Agilent MSD 5979. With CI (chemical ionization) and a Hewlett-Packard MS Engine thermospray and ionization by electron impact to 70 Ev. Microanalyses were conducted using an elemental analyzer 1106.

5-(p-florobenzylidene)-3-(p-chlorophenyl)-1,2,4-triazine-6-one (2)

To a mixture of oxazolinon (1), (0.01 mol) and the hydrazine hydrate (0.02 mole) in ethanol (30 ml) was heated under reflux for 4h. The reaction mixture was cooled and then poured on water and acidified with dilute hydrochloric acid (IN). Te solid obtained was collected by filtration off and crystallized from ethanol to give compound 2 as yellow crystals, yield 63%, m.p. 1820c. IR (KBr) : 3225 (NH), 1695 (C=O), 1635 (C=N), 1605,1583 (C=C), 1613,1097 (C-O) cm\textsuperscript{-1}. 1H-NMR (DMSO-d6): \(\sigma 7.32-8.4 \text{ (m, 9H, Ar-H and H-olefinic) } , \text{10.21 (S, 1H, NH) , 10.43 (br-S, 1H, NH) ppm. MS: M/Z=317 (M++2, 56.1), 316 (M++1, 41.80), 315 (M+, 100 ), 303 (3.20), 302 (5.50), 301 (9.30), 300 (8.00), 299 (3.80), 288 (4.60), 287 (4.90), 286 (12.10), 273 (1.4), 272 (1.10), 271}
A mixture of oxazolinone (1, 0.01 mole) and thiohydroxybenzaldyde (0.01 mole) in acetic acid (30 ml) was heated under reflux for 3 h. The reaction mixture was cooled and poured on cooled water. The resulting solid was filtered off, washed with water, dried and purified from ethanol to give 3 as yellow crystals, yield 67%, m.p, 218°C. IR (KBr): \( 3327, 3161, 3159, 3150 \) cm\(^{-1}\). NMR (DMSO-d\(_6\)) : \( \delta 7.27 - 8.46 \) (m, 11H, Ar-H), \( 8.12 \) (m, 15H, Ar-O), \( 10.77 \) (br, 1H, NH) ppm. 13C NMR (DMSO-d\(_6\)) : \( \delta 129.66, 116.074, 116.69, 116.57 \) (C-aromatic), \( 37.47, 37.44, 35.39, 35.80, 31.71, 31.68, 31.6, 30.32, 30.01, 30.23 \) (26.30), \( 30.01, 30.23, 25.34, 25.24, 25.2 \) (11.20), \( 221, 221 (2.40), 181 (1.30), 180 (1.60), 179 (1.20), 178 (3.80), 163 (7.50), 162 (11.30), 141 (10.00), 139 (36.50), 138 (26.50), 137 (20.50), 135 (39.9), 134 (23.80), 121 (2.10), 120 (10.90), 116 (2.8), 115 (7.70), 113 (26.50), 112 (7.20), 111 (100), 107 (11.90), 103 (1.90), 102 (9.40), 101 (3.30), 97 (1.30), 95 (5.60), 90 (1.18), 87 (5.70), 86 (1.10), 83 (1.80), 77 (2.30), 76 (4.20), 75 (5.70), 74 (2.60). Anal. calc. for C\(_{17}\)H\(_{12}\)N\(_4\)ClFOS: C, 54.55; H, 3.86; N, 8.66; S, 7.27. Found: C, 54.32; H, 3.03; N, 14.69.

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A mixture of 3 (0.01 mole) and phenacyl bromide (0.01 mole) in ethanol (30 ml) was added fused sodium acetate (0.03 mole), and the reaction mixture was heated under reflux for 4 h, and then the mixture was poured on water. The solid produced was collected by filtration and crystallized from ethanol to give as yellow crystals, yield 71%, m.p, 2350c. IR (KBr)= \( 3225 \) (NH), \( 1695 \) (C=O), \( 1642 \) (C=N) cm\(^{-1}\). MS: M/Z (%)=746 (M++2, 7.40), 475 (M++1, 7.10), 474, (18.00), 401 (1.40), 400 (1.00), 399 (3.80), 369 (1.20), 368 (2.80), 329 (1.20), 317 (3.80), 316 (1.90), 315 (7.7), 313 (2.50), 312 (3.10), 303 (43.30), 302 (31.60), 301 (100), 258 (2.30), 257 (2.0), 256 (3.20), 234 (2.20), 230 (3.60), 223 (2.70), 223 (1.50), 216 (2.80), 207 (3.90), 197 (1.30), 195 (5.60), 194 (2.00), 183 (2.00), 181 (30.40), 163 (7.40), 162 (3.90), 161 (3.90), 156 (8.40), 155 (12.70), 154 (26.60), 152 (32.00), 149 (13.70), 148 (3.60), 147 (2.60) 141 (50.30), 140 (17.80), 139 (100), 138 (17.30), 137 (20.70), 135 (32.10), 134 (13.80), 125 (19.90), 123 (21.30), 122 (24.0), 121 (21.80), 120 (15.10), 113 (18.10), 112 (6.60), 111 (55.90), 109 (30.60), 108 (15.90), 107 (18.30), 103 (2.60), 102(13.80), 101(19.50), 97 (6.90), 95 (26.60), 85 (7.10), 83 (15.50), 77 (4.60), 76 (10.70), 75 (41.50), 74 (11.20). Anal. calc. for C\(_{17}\)H\(_{12}\)N\(_4\)ClFOS: C, 54.55; H, 3.86; N, 8.66; S, 7.27. Found: C, 54.32; H, 3.03; N, 11.81. Found: C, 54.32; H, 3.03; N, 11.81. Found: C, 54.32; H, 3.03; N, 11.81.

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5-(p-fiorobenzylidene)-3-(p-chlorophenyl)-2(p-acetoxyl benzylidene amino) thiocarboxyl-1-acetyl-1,2,4-triazine-6-one (8).

A solution of 2, 5 and 7 (0.01mole) in acetic anhydride (25 ml) was heated under reflux for 2-3 hr, then cooled and poured onto ice water. The resulting product was filtered off, washed with water, dried and purified by recrystallization from the appropriate solvent to give the corresponding compounds 4, 6 and 8.

Compound 4 as pale yellow crystals, yield 61%, m.p 1700°C. IR(KBr): 1710, 1693, 1605, 1586 (C=C) cm⁻¹. MS : m/z (%) = 400 (M++1, 5.40), 399 (M++2, 7.40), 400 (M++1, 5.40), 399 (M++2, 7.40). Anal calcd for C₂₀H₁₅N₃ClFO₃: C, 60.15; H, 3.56; N, 9.69.

Compound 5 as pale yellow crystals, yield 51%, m.p 1400°C. IR(KBr): 1751, 1697 (C=O) cm⁻¹. MS : m/z (%) = 518 (M++2, 10.40), 517 (M++1, 9.20), 516 (M+, 24.80), 476 (56.50), 475 (51.20), 474 (100), 369 (1.30), 368 (2.70), 367 (1.30), 340 (1.0), 339 (1.10), 315 (2.70), 314 (6.60), 313 (6.50), 312 (15.50), 303 (4.60), 302 (22.00), 301 (15.800), 300 (63.00), 258 (1.40), 257 (1.20), 256 (3.60), 255 (2.70), 236 (3.70), 235 (1.20), 222 (1.70), 221 (30.00), 203 (1.50), 202 (2.50), 177 (2.80), 176 (7.00), 175 (22.80), 174 (12.80) ppm.

Compound 6 as pale yellow crystals, yield 71%, m.p 1700°C. IR(KBr): 1608, 1586 (C=C) cm⁻¹. 1H-NMR (DMSO-d6): σ 2.35 (S, 3H, COCH₃), 7.33 (S, 1H, CH=N) ppm. Anal calcd for C₂₇H₁₈N₄ClFO₂S: C, 62.79; H, 3.49; N, 10.85. Found: C, 62.53; H, 3.31; N, 10.63.

Compound 8 as pale yellow crystals, yield 63%, m.p 1400°C. IR(KBr): 1715, 1697 (C=O), 1638 (C=N), 1608, 1592 (C=C), 1356 (C=S), 1125, 1083 (C-O) cm⁻¹. 1H-NMR (DMSO-d6): σ 2.35 (S, 3H, CH=N), 7.33 (S, 1H, CH=N), 2.41 (S, 3H, COCH₃), 7.23 - 8.13 (m, 13 H, Ar-H and H-olefinic) ppm. Anal calcd for C₂₈H₂₀N₄ClFO₄S: C, 59.79; H, 3.56; N, 9.96. Found: C, 59.59; H, 3.33; N, 9.69.

4. CONCLUSION

This paper containing the synthesis of 1,2,4-triazine derivatives (2-8) and the antitumor evaluation of some novel compounds.

Compounds 3 and 7 has the most potent activity against the human liver tumor cells (HEPG-2 Cell lines) than another compounds with compared to Doxorubicine drug as reference. This potency could be attributed to the presence of thio carboxamide group.

REFERENCES


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20. B.S. Dawane, N.S. Kadam, M.B. Shaikh; Der Pharma Chemica Let., 2010, 2, 126.