Synthesis of Some Novel Compounds of Saccharinyl Acetic Acid Containing Nucleus and Evaluation of Their Biological Activities as Antimicrobial

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ABSTRACT

A new series of Compounds of Saccharinyl Acetic acid Containing nucleus have been prepared via an improved synthetic procedure. Where saccharinyl moiety have been introduced to 4-benzyldiene-2-methyl-1,3-oxazole-5-one in position 2, compound (3) which has been reacted with nitrogen nucleophiles as hydrazine hydrate, phenyl haydrazine, aniline, p-toludine, m,p-aminobezoic acid to get compounds from (4-6). Also the reaction of compound (3) with aromatic substrate in presence of anhydrous AlCl₃ (Friedel – Crafts reaction) affordedacetamide derivative (7) via the elimination of arylidine group. Moreover saccharinyl acetic acid hydrazide (8) was refluxed in acetic anhydride to give benzisothiazole derivative (9), which reacted with carbon nuleophiles (Grignard reagent) to afford compound (10). But when compound (9) reacted with PCl₅/POCl₃ it gave compound (11) which reacted with urea and thiourea to give compound (12(a, and b)). Also the condensation of compound (9) with aromatic aldehyde gave compound (13). Structures of all synthesized compounds were elucidated from I.R., ¹HNMR, mass-spectroscopy, and elemental analysis.

Key words: Saccharin, hydrazinehydrate, oxazole, imidazole, benzisothiazole.

INTRODUCTION

The reported pharmaceutical properties of saccharin and its derivatives¹⁴ and in connection with our ongoing interest to developing new synthetic strategies for construction of heterocyclic systems involving saccharin due to its significant biological and pharmacological activities. Also imidazole derivatives are of interest to medical chemists for many years because of their biological activities such as anticancer, anti tubercular, antibacterial, and antifungal activities¹³-²² In view of the above facts we report in the present work the synthesis and antimicrobial activities of Saccharinyl derivatives.

METHODS AND EXPERIMENTS

Chemistry

Melting points were determined in open capillaries using Gallen Kamp melting point
apparatus and are uncorrected F.T.-IR spectra (KBr disc) were recorded on a Perkin Elmer 1720 F.T. – IR spectrometer. 1H – NMR spectra were recorded on varin Gemini NMR spectrometer 300 MHz using TMS as internal standard. All reactions were monitored by TLC using aluminum silica gel plates 60 F 245. Elemental analysis and antimicrobial activity were carried out at micro-analytical center, faculty of science, cairo university, Egypt. Elemental analysis of all synthesized compounds is in agreement with the structure elucidated.

4-Benzylidene-2-Saccharinyl methyl-1,3-Oxazole-5-one.(3)
A mixture of Saccharinyl acetic acid chloride (10mmol) and glycine (10mmol) were refluxed in boiling ethanol for 1hr. The spectral solid was then refluxed for 4 hr. in freshly distilled acetic anhydride (30ml) and anhydrous sodium acetate in the presence of benzaldehyde. The reaction mixture was poured onto water and solid that separated was re-crystallized from ethanol to give (3). Orange (68%) mp 140-142 °C, IR (KBr, cm⁻¹): ν 1700 (10 azlactone), 1610 (CN), 1337 and 1120 (SO₂). EI- MS: m/z 368 [M+1]. Analytical calculation for C₂₈H₁₉O₆N₅S: C 75.6; H 3.3; N 7.6 found C 75.65; H 3.3; N 7.55.

5-Benzylidene–3–Saccharinyl methyl-1, 2, 4–triazin–6–one (4a,4b)
A mixture of 3 (10 mmol) and hydrazine hydrate or phenyl hydrazine (10 mmol) in absolute ethanol (30 ml) was refluxed for 3hr. The solid that obtained was recrystallized from ethanol to afford 4a: orange (75%), mp 163-165°C; IR (KBr, cm⁻¹): 1700 (CO), 1615 (C=N), 3350-3450 (br NH – NH) and 1337 and 1138 (SO₂). EI- MS: m/z 382 (M⁺). And 4b: brown (80%), mp 95-97; IR (KBr, cm⁻¹): 1680 (CO), 1610 (C=2), 3250 (NH), 1330 and 1168 (SO₂) MF C₂₅H₁₉O₇N₅S. EI- MS: m/z 460 (M⁺)

General procedure for the preparation of cinnamamide derivatives (5a-d) and imidazole one derivatives (6a-d).
A mixture of 3(10 mmol) and aromatic amine oaminobenzoic acid) was refluxed for 3hr. in absolute ethanol (30 ml). For compounds (5a-d) and in n-nutanol (30ml) for compounds (6a-d). The solid that obtained was recrystallized from proper solvent to afford (5a-d) and / or (6a-d).30

(α-Saccharinyl- N- acetamido) cinnamamide (5a)
Yellowish (70%), mp 210 -212 ; IR( KBr, cm⁻¹): 1670 and 1660 (CO), 3200-3200 (NH), 1330 and 1170 (SO₂). MF C₂₅H₁₉O₅N₅S' . EI- MS : m/z 461 (M⁺), crystallized from benzene (5b) dark brown (75%), mp 219-221; IR( KBr cm⁻¹).1670 (CO), 3230, 3250 (NH) 1616 (C=N), 1320 and 1170 (SO₂).

MF C₂₅H₁₉O₅N₅S. EI- MS: m/z 476 (M+1) recrystallized from toluene.

(5c) yellow (65%), mp 90-92 , IR(5Br, cm⁻¹):1710 , ( CO), 1680 , (CO amide), 3350-3400 (broud NH, OH), 1310 and 1120 (SO₂). MF C₂₅H₁₉O₅N₅S. EI-MS: m/z 505 (M⁺), crystallized from benzene.

(5d) brown (70%), mp 110-112 , IR (KBr, cm⁻¹):1710 (CO), 1670 (CO amide), 3300-3450(broud NH, OH), 1300 and 1110 (SO₂).

MF C₂₅H₁₉O₅N₅S. EI – MS : m/z 505 (M⁺). recrystallized from benzene.

(6a) brown (75%) ,mp 180-182, IR( KBr, cm⁻¹):1660 (CO), 1600 (CO amidazolone) 1580 (C=N),1330 and 1160 (SO₂). MF C₂₅H₁₉O₅N₅S. EI- MS : m/z 443 (M⁺) recrystallized from toluene.

(6b). brown (65%) ,mp 202-204 , IR(KBr, cm⁻¹): 1670 (CO), 1630 (CO amidazole), 1580( C=N), 1320 and 1170 (SO₂). MF C₂₅H₁₉O₅N₅S. EI- MS: m/z 457 (M⁺).

(6c) brown (70%), mp 150-152 , IR (KBr , cm⁻¹), 1680 (coimidazoline), 1610 (C=N), 1320 and 1110 ( SO₂), 3450 (OH). MF C₂₅H₁₉O₅N₅S EI-MS: m/z 487 (M⁺), recrystallized from benzene.

(6d) yellowish (77%), mp 155-157, IR (KBr, cm⁻¹): 1690 (Coimidazoline), 1620 (C=N), 1280 and 1120 (SO₂) 3440 (OH).

MF C₂₅H₁₉O₅N₅S. EI- MS : m/z 487 (M⁺) recrystallized from toluene.
**General procedure for the preparation of imidazolone derivatives (6a-d) From (saccharinyl-N- acetamido ) cinnamide derivatives (5a-d) by cyclization.**

A solution of compounds 5a-d (10 m mole) in acetic anhydride (15ml) was boiled under reflux for 2h. The resulting solution was poured onto crushed ice, and the product that separated out was filtered off, washed with solution of sodium hydrogen carbonate followed by water and then dried. The products were recrystallized from a proper solvent.

**Saccharinyl (N'- benzoyl methyl) acetamide (7)**

A solution of oxazolonedervative 3 (10 m mole) in dry benzene was treated with anhydrous \(\text{AlCl}_3\) (30m mole) with continuously stirring on water bath for 3h.

The reaction mixture was decomposed with ice-cold hydrochloric acid. Then the ethereal layer was separated and dried over anhydrous Na\(_2\)SO\(_4\). The excess solvent was evaporated then the separated compound was recrystallized from benzene to give (7) : buff (60%) , mp 115-117 , IR (KBr, cm\(^{-1}\) ) , 1700 (CO ketone) , 1680 (CO imide) , 3340 (NH) , 1330 and 1140 (SO\(_2\)) MF C\(_{17}\)H\(_{14}\)O\(_5\)N\(_2\)S. EI-MS: m/z 358 (M\(^+\)).

**6- Oxo -1, 2 ,4 – triazino (4,3-b) (1,2) benzoisothiazole (9)**

A mixture of compound 8 (10 m mole) in redistilled acetic anhydride 20ml was refluxed for 1h. the reaction mixture was cooled and poured onto ice – cold water. The solid product separated was filtered and recrystallized from ethanol to afford compound (9), yellowish brown (65%) , mp 145-147, IR (KBr, cm\(^{-1}\) ) : 1710 (CO) , 3432 (OH) , 3100 (NH) 1634 (C=\(\text{N}\)) 1363 and 1133 (SO\(_2\)) , HNMR (DMSO-d\(_6\)) S: 2.5 (s, 2H, cH\(_2\)), 4.4 (s,1H, NH) , 5.7 (s. 1H, OH of lactam- lactim dynamic equilibrium) and 7.8-8.1 (m, 4H, ArH), MF C\(_9\)H\(_7\)N\(_3\)O\(_3\)S, EI-ms, m/z 251 (M\(^+\)).

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**Table 1: Antimicrobial activity (in vitro) of some synthesized compounds**

<table>
<thead>
<tr>
<th>Compd no.</th>
<th>Zone of inhibition mm/ mg of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Eschericka coli</em></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4a</td>
<td>0</td>
</tr>
<tr>
<td>4b</td>
<td>18</td>
</tr>
<tr>
<td>5a</td>
<td>0</td>
</tr>
<tr>
<td>5b</td>
<td>0</td>
</tr>
<tr>
<td>5g</td>
<td>0</td>
</tr>
<tr>
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<td>9</td>
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</tr>
<tr>
<td>10a</td>
<td>19</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>12b</td>
<td>0</td>
</tr>
<tr>
<td>13a</td>
<td>18</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
</tr>
<tr>
<td>Amphotericin</td>
<td>0</td>
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</tbody>
</table>
General procedure for the preparation of 6-alkyl–6-hydroxyl–1,2,4-triazino (4,3-b) (1,2) benzisothiazole (10a-c).

To Mg metal (10m mole) in dry ether (40ml) an alkyl halide namely, ethyl iodide, methyl iodide or benzyl chloride (30m mole) in dry ether (20ml) was added dropwisely. The reaction mixture was refluxed and compound a (10m mole) in dry ether (40ml) added portion wise within 1h. The reaction mixture was further refluxed for 3h, left over night and then decomposed with dil cold HCl. The ethereal layer was washed with NaHCO₃ solution then water and dried over analydrous Na₂SO₄ and evaporated to give compound 10a-c which was recrystallized form benzene (10a) : ball yellow (6690), mp (195-197, IR(KBr, cm⁻¹)) 3420-3390 (OH and NH), 1610 (C=N), 1310 and 1130 (SO₂), MF C₁₀H₁₁N₃O₃S, EI –MS: m/z 3(M⁺).

(10b) : yellowish (70%), mp 172-174 ; IR (KBr, cm⁻¹) : 3410 – 3380 (OH and NH), 1600 (C=N), 1310 and 1130 (SO₂), 'HNMR(DMSO-d₆): S: 1.7 (t,3H,CH₃), 2.5 (q,2H,CH₂), 4(S,1H,OH), 4.5 (S,2H, CH₂, N-CH₃), 7.8 (m, 4H, A,H), 9.1 (S, 1H, NH). MF C₁₁H₁₃N₃O₄S. El – MS: m/z 267 (M⁺).

(10c) yellow (60%) ,mp 205-207 , IR (KBr, cm⁻¹) 3400-3370 (OH, and NH), 1630 (C=N) 1320 and 1120 (SO₂). MF (C₁₆H₁₃N₃O₃S). El – MS : m/z 329 (M⁺).

6-chloro -1,2,4-triazino – (4,3-b) (1,2) benzisothiazole(11)

A mixture of compound 3(10m mole) , phosphorous oxychloride ( 20m mole) and PCl₅ (1qm) was refluxed on a steam bath for 3h.

Then poured slowly into ice – cold water. The solid that separated was washed several times with water, dried and recrystallized from benzene to give compound (11); brown (70%) ,mp 105-107. IR(KBr( cm⁻¹), γ1630 (C=N), 1330 and 1120 (SO₂) and no absorption for γCO and γNH.

MF C₉H₆N₃O₂S C₁. El – MS⁺: m/z 255 (M⁺) and 257 (M⁺+2).

Formation of compounds (12 a and b)

A mixture of compound 11 ( 10m mole) was refluxed with urea and / or thio- urea ( 10 m mole) in ( 40ml) sodium ethoxide for 3h, then cooled and poured into water. The solid that separated was dried and recrystallized from benzene – ethanol (1:1) to give 12a and for 12b.

Compound (12a) : yellow (72%) , mp 72-74 . IR (KBr, cm⁻¹) 3350 -3320 (NH), 1610 (C=N) , 1310 and 1120 (SO₂). MF C₁₀H₉N₅O₃S₂. EI- MS: m/2 279 (M⁺).

Compound (12b) : yellowish (65%) , mp 235- 237. IR ( KBr, cm⁻¹) 3370 -3330 (NH), 1630 (C=N), 1310 and 1120 (SO₂). MF C₁₆H₁₃N₃O₃S. EI- MS: m/z 279 (M⁺).

Scheme 1:

\[ R - C\equiv O/Na acetate \]

\[ \text{benzaldehyde} \]

\[ \text{Ac}_2O/Na acetate \]

\[ \text{ph-HC} \]

\[ 3 \]

\[ +2\text{HNC}_2\text{COOH} \]
6-OXO-5-arylidene – 1,2,4-triazino – (4,3-b) (1,2)-benzisothiazole (13a-c).

A mixture of compound 9 (10m mole) and aromatic aldehyde (10m mole) namely Benz aldehyde, p- chloro- Benz aldehyde and anisaldehyde in (40 ml).

Acetic anhydride – acetic acid (1: 1) was refluxed for 3h. After concentrated, the solid that separated was cooled and recrystallized from benzene – ethanol (1: 1) to give (13a –c).

Compound 13a yellow (75%), mp 105-107.

IR (KBr, cm⁻¹) 3400 -3360 (br. NH, OH), 1720 (CO), 1560 , 1480 (C=N, C=C), 1350 and 1150 (SO₂). MF C₁₆H₁₁N₃O₃. EI –MS : m/z 325 (M⁺).
Compound 13b: yellow (75%) , mp 200-202. IR (KBr, cm⁻¹) 3420-3380(br. OH and NH), 1700 (CO), 1580, 1460 (C=N, C=C), 1330 and 1120 (SO₂), MF C₁₆H₁₀N₃O₃SC₁. EI- MS: m/z 359 (M⁺) AND 361 (2).

Compound 13c: ball yellow (65%) , mp 188-190. IR (KBR, cm⁻¹) 3450-3400 (br: OH and NH), 1720 (CO), 1610, 1450 (C=N, C=C), 1300 and 1110 (SO₂). MF C₁₇H₁₀N₃O₄S. EI- MS: m/z 355(M⁺).

Antimicrobial

The newly synthesized compounds (3,4a-b, 5a-b, 5d, 5g, 6c, 7, 9, 10a, 11, 12b, and 13a) were tested for their in vitro growth inhibitory activity against a panel of standard strains of the Institute of fermentation of Osaka (IFO) namely; the Gram-positive bacteria (Staphylococcus aureus IFO3060 and Bacillus subtilis IFO 3007), the Gramnegative bacteria (Escherichia coli IFO 3301 and Proteus vulgaris IFO 3851).

The primary screening was carried out using the agar disc-diffusion method using Müller-Hinton agar medium.24-28

RESULTS AND DISCUSSION

Chemistry

In the present investigation, saccharinyl acetic acid chloride ⁹ reacted with glycine to give N-carboxymethylsaccharinylacetamide(2), which
reacted with benzaldehyde in the presence of acetic anhydride and sodium acetate to give 4-benzylidene-2-saccharinyl-methyl-1,3-oxazole-5-one (3). Treatment of (3) with hydrazine and/or phenylhydrazine in refluxing ethanol afforded 5-benzylidene-3-saccharinyl-methyl-1,2,4-triazine-6-one (4a) and/or 5-benzylidene-3-saccharinyl-methyl-2-N-phenyl-1,2,4-triazine-6-one (4b). Furthermore when compound (3) reacted with aromatic amines (aniline, p-toluidine) and aminobenzoic acids (m, and p-aminobenzoic acid) in refluxing ethanol it gave cinnamimide derivatives (5 a-d). But when the same reaction was carried out in n-butanol it gave imidazolone derivatives (6a-d) which were also obtained by refluxing compounds (5a-d) in acetic anhydride. Moreover compound (3) undergoes acid catalyse ring opening reaction with dry benzene in presence of anhydrous AlCl₃ (Fridel – Crafts reaction) to give saccharinyl – (N-benzoyl-methyl) acetamide (7), whereas the reaction product obtained via elimination of aryldiene group. On the other hand, when saccharinyl acetic acid hydrazide (8) was refluxed in acetic anhydride it cyclized to 6-oxo-1,2,4-triazino-[4,3-b]benzisothiazole (9).

Also the behavior of compound (9) towards carbon nucleophiles (Grignard reagent) was investigated. Thus when compound (9) submitted to react with ethyl magnesium iodide, methyl magnesium chloride then refluxed in dry benzene, it afforded 6-alkyl-6-hydroxy-1,2,4-triazino-[4,3-b]1,2-benzisothiazole (10). Treatment of compound (9) with mixture of POCl₃/PCl₅ afforded 6-Chloro-1,2,4-triazino[4,3-b][1,2]benzisothiazole (11) which reacted with urea and/or thiourea to give compound (12 a and b) respectively.

Also when compound (9) was allowed to condense with aromatic aldehyde namely benzaldehyde, anisaldehyde, and p-chlorobenzaldehyde in the presence of pyridine in a mixture of acetic anhydride and acetic acid (1:1) it afforded 5-arylidene-6-oxo-1,2,4-triazino-[4,3-b] benzisothiazole (13a-c).

Antimicrobial Activity

The antimicrobial activity (in vitro) of some synthesized compounds was determined against some bacteria and fungi using tetracycline and amphotericin B as standard antimicrobial agents by using the agar diffusion method. The obtained zones of inhibition were presented in table 1, which indicated that most of the synthesized derivatives have moderate to good antimicrobial activities.

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