Antioxidant Activity of Heterocyclic Compounds Derived from 4-(4-Acetamidophenyl)-4-oxobut-2-enoic Acid

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Abstract: 4-(4-Acetamidophenyl)-4-oxobut-2-enoic acid 1 and / or itsaza Michael adduct 2 were efficiently utilized to construct novel various heterocycles including derivatives of furanones (3a-c & 14), pyridazinones (4-6), diazepine (7), piperazine (8), oxazine (9), oxazole (10a), thiazole (10b), benzo-, [1-diazin-, -oxazin, -thiaz]-ones (11a-c). Conduct (11c) with NH₂-NH₂.H₂O in boiling ethanol and / or NH₂.OH.HCl in refluxing pyridine provides the corresponding hydrazone and oxime derivatives 12a and 12b, respectively. The two latter compounds showed in vitro a high potent antioxidant activity, as well as, the aza Michael adduct 2e exhibited a moderate reducing antioxidant power.

Keywords: Pyridazinone, Diazepine, Benzothiazinone, Quinoxalione, Piperazine, Benzoxazinone, Furanone, Antioxidant activity

1. Introduction

Aryl- and heteroaryl substituted 4-oxobut-2-enoic acids and their derivatives represent an important class of compounds with interesting pharmacological medications including antiulcer and cytoprotective properties [1], kynurenine-3-hydroxylase [2] and human cytomegalovirus protease inhibition activity [3]. Several naturally occurring acryl acids show notable antibiotic activity [4, 5]. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals and they are effective in the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, cancer and Alzheimer’s disease, as well [6]. Flavonoids and phenolic compounds that are widely distributed in plants have been reported to exert multiple biological effects as antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic [7]. Accordingly, a great deal of research efforts has been devoted to the fields of natural and synthetic antioxidants. In addition, a large variety number of synthetic acetylindoles, furanones and quinazolines [8-11], have also been extremely exploited for similar antioxidant activity. From this view point and in continuation to our previous publications dealing with the pharmaceutical applications of this important class of compounds [12,13], the present work demonstrates the reactivity of 4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 and itsaza Michael adduct 2 towards various nucleophiles aiming to design new heterocycles and study their antioxidant power activity.

2. Result and Discussion

In continuation our contribution to study the reactivity of α,β-unsaturated of 2-enoic acids towards various nucleophiles, the authors decided to adopt 4-(4-acetamidophenyl)-4-oxobut-2-enoic acid 1 and itsaza Michael adduct 2. Traditionally, the acid 1 was prepared via Friedel-Crafts acylation of acetanilide using maleic anhydride in the presence of catalytic amount of anhydrous aluminum chloride [14]. The preparation of the latter derivative was achieved by submitting the acid 1 to react with benzimidazole and / or its 2-methyl derivative under different reaction conditions. Initially, when the reaction was carried out in boiling pyridine, anaza-Michael addition followed by decarboxylation has been occurred to afford (1H-benzo[d]imidazole-1-yl) propanoyl(phenyl) acetamide 2a (Scheme 1). The IR spectrum of 2a revealed the disappearance of absorption bands of both acidic carbonyl and hydroxylic group frequencies. Further, EI-fragmentation pattern showed the molecular ion peak at m/z: 307 due to the fragment M +

Probably, the foregoing reaction involved decarboxylation under the effect of elevated temperature, attained by the high boiling point of pyridine, a characteristic reaction for transformation of α-amino acids into amines under similar conditions [15].

On the other hand, when the reaction was conducted with benzimidazole, 2-methylbenzimidazole, benzylamine and / or p-anisidine in refluxing absolute ethanol, dry benzene and / or dry toluene, 4-oxobutanoic acid derivatives 2b-d and 4-oxobut-2-enoic acid 2e have been readily obtained, respectively. Prolonged heating in case of reaction with p-anisidine results in the formation of more thermodynamically stable configuration 2e. This result could be attributed to the higher stability gained by extended conjugation involving the presence of C2-C3 double bond. The 1H-NMR spectrum of product 2e revealed that it exists in solution, in dynamic equilibrium of (Z) and (E) isomers in a ratio of 37.78 % and 62.22% respectively.
dehetero-arylation, regardless the employed reaction medium. It was observed that the reaction undergoes deprotonation at the latter site and/or elimination of the same heterocycle moiety, again to provide open chain products. Interestingly, upon treatment with boiling acetic anhydride, the open chain products 2b-d underwent 5-exo-trig cyclization into the corresponding 5-oxo-furan-2-yl acetates 3a-c, respectively. The structure of the latter furanones was confirmed on the basis of the two absorption bands exhibited at 1748 and 1712 cm\(^{-1}\) assigned to the carbonyl functionalities of 5-membered lactone ring and ester group, respectively. Further, \(^1\)H NMR spectrum displayed two singlets at \(\delta\) 2.19 and 2.07 ppm due to two methyl protons of both ester (O-CO-CH\(_3\)) and acetamido (NH-CO-CH\(_3\)) groups, respectively.

A recent publication demonstrated that treatment of Michael adduct 2 with hydrazines afforded heteryl-pyridazinones [16, 17]. On contrary, herein, reinvestigation of this reaction type using hydrazine hydrate or phenylhydrazine with either the acid 1 or 2 under two alternative conditions involved dehetero-arylation to provide pyridazinones 4a and 4b. First, the reaction was carried out in absolute ethanol containing catalytic amount of glacial acetic acid, however, in the second methodology DMF was utilized as the sole reaction medium. It was observed that the reaction undergoes dehetero-arylation, regardless the employed reaction medium (Scheme 2). The structural features of 4a and 4b has been confirmed from their carbonyl absorption frequencies of pyridazinone rings at \(\nu\) 1685 and 1701 cm\(^{-1}\), respectively. A compelling evidence was received via elaboration of the former product with formaldehyde / piperidine under Mannich reaction conditions and/or ethyl chloroacetate in boiling dry acetone / anhydrous K\(_2\)CO\(_3\) to afford N-(4-(6-oxo-1-(piperidin-1-yl-methyl)-1,6-dihydro-pyridazin-3-yl)-phenyl)acetamide (5) and ethyl 2-(3-(4-acetamido phenyl)-6-oxo-pyridazin-1(6H)-yl)acetate (6) respectively.

Presumably, dehetero-arylation process in the former reaction occurred under the effect of protonation of the heteryl moiety influenced by the existing acetic acid. Consequently, progress of reaction was enhanced by the removal of heterocycle moiety and deprotonation at 4-position to give the thermodynamically more stable pyridazinones 4a and 4b (Route A). Alternatively, the second reaction underwent deprotonation at the latter site influenced by the base catalyst, DMF, followed by elimination of the same heterocycle moiety, again to provide similar latter products 4a and 4b (Route B). Indeed, we are uncertain about the type of elimination reaction mechanism of this suggested speculation (Scheme 3).

Our program was expanded to investigate the behavior of the acid 1 with some other binaucleophiles involving ethane-1,2-diamine, ethanolamine and 3-aminopropan-1-ol. It was fortunate that, when the reaction was performed in refluxing ethanol containing few drops of glacial AcOH, went smoothly to give the corresponding heterocycles, diazepine 7, piperazine 8, and oxazine 9, respectively in moderate yields (Scheme 2).
Prolonged heating of the given acid 1 with urea or thiourea under previous similar reaction conditions resulted in the formation of moderate yields of N-(4-(2-(2-imino-5-oxazolidin-4-yl)acetyl)phenyl)acetamide 10a and N-(4-(2-(2-imino-5-oxothiazolidin-4-yl)acetyl)phenyl)acetamide 10b respectively.

With the aim to improve this study to construct some fused annelated 6-membered heterocycles, the acid 1 or 2 was submitted to react with the bidentate nucleophiles, o-phenylendiamine, o-aminophenol and / or o-aminothiophenol in boiling ethanol / glacial AcOH mixture. It was surprising that, both reactions afforded good yields of similar products that, 11a, 1,4-benzoxazin-3-one (11b) and 1,4-benzothiazin-3-one (11c) respectively. These results have been confirmed by identity of TLC, mp and mixed mp of the obtained products, in addition to the compatibility of their IR, 1HNMR, as well as mass spectral data.

The existence of the aroyl group in the latter prepared products 11a-c was proved by conduct the compound, 1,4-benzothiazin-3-one 11c with hydrazine hydrate in boiling ethanol and / or hydroxylamine hydrochloride in boiling pyridine that provided moderate yields of the corresponding hydrazone 12a and oxime 12b, respectively. The structure of the former compound was identified on the basis of coupling band exhibited at ν 3311 and 3267 cm⁻¹ due to the amino =N-OH proton. In addition, these singlet peaks are recorded in integration of 43.38 % and 56.62% that refers to the existence the assigned compound in Z and E- isomers (cf. Experimental).

Recently, when the base catalyst in the above reaction was replaced by piperidine, the isolated product has been identified as 4-(4-acetamidophenyl)-2-(dicyanomethyl)-4-oxo-butanoic acid (15). Presumably, the formation of compound 14 could be interpreted according to the following suggested mechanism (Scheme 5).

3. Antioxidant Evaluation

The antioxidant activities of the synthesized compounds were shown in table 1 and fig:1. The following points have been noticed:

(1) The results revealed that all compounds were found to be potent.
(2) Three compounds 2e and 12a,b were found to be the most potent levels of activity.
(3) Additionally, compounds 3, 11a,b and 15 were found to be moderate activity (RPAA).
(4) Compounds 1, 10a,b, 11c and 14 were found to be weak reducing power antioxidant activity (RPAA).
(5) The other compounds have very weak reducing power antioxidant activity (RPAA).
Table 1: Reducing power antioxidant activity of different compounds against ascorbic acid at concentration 200 µg/ml.

<table>
<thead>
<tr>
<th>Sample</th>
<th>OD value ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.312 ± 0.01</td>
</tr>
<tr>
<td>2a</td>
<td>0.178 ± 0.09</td>
</tr>
<tr>
<td>2b</td>
<td>0.159 ± 0.06</td>
</tr>
<tr>
<td>2c</td>
<td>0.166 ± 0.04</td>
</tr>
<tr>
<td>2d</td>
<td>0.183 ± 0.03</td>
</tr>
<tr>
<td>2e</td>
<td>1.73 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.433 ± 0.01</td>
</tr>
<tr>
<td>4a</td>
<td>0.156 ± 0.07</td>
</tr>
<tr>
<td>4b</td>
<td>0.174 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.223 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.205 ± 0.04</td>
</tr>
<tr>
<td>8</td>
<td>0.171 ± 0.01</td>
</tr>
<tr>
<td>9</td>
<td>0.198 ± 0.01</td>
</tr>
<tr>
<td>10a</td>
<td>0.296 ± 0.06</td>
</tr>
<tr>
<td>10b</td>
<td>0.258 ± 0.07</td>
</tr>
<tr>
<td>11a</td>
<td>0.883 ± 0.01</td>
</tr>
<tr>
<td>11b</td>
<td>0.641 ± 0.01</td>
</tr>
<tr>
<td>11c</td>
<td>0.275 ± 0.07</td>
</tr>
<tr>
<td>12a</td>
<td>2.243 ± 0.07</td>
</tr>
<tr>
<td>12b</td>
<td>2.16 ± 0.02</td>
</tr>
<tr>
<td>14</td>
<td>0.304 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>0.504 ± 0.09</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>2.50 ± 0.05</td>
</tr>
</tbody>
</table>

A mixture of 4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 (0.233 g, 10 mmol) and benzimidazole (0.118 g, 10 mmol) in pyridine (10 ml) was refluxed for 20 h. The reaction mixture was cooled, filtered off, washed with petroleum ether (40-60 °C) and recrystallized from acetic acid to give 2a as yellow crystals, mp 234-236 °C, yield 60%. FT-IR (KBr, ν cm⁻¹): 3240 (NH), 1694 (C=O ketone), 1667 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 10.24 (s, 1H, D₂O-exchange, -NHCOCH₃), 8.23 (s, 1H, C-H imidazole), 7.94-7.17 (m, 8H, Ar-H), 4.59 (t, 2H, -COCH₂CH₂-N-, J= 6.9 Hz), 3.61 (t, 2H, -COCH₂CH₂-N-, J= 6.6 Hz), 2.07 (s, 3H, CH₃). MS (m/z, %): 307 (M⁺, 2), 292 (4), 189 (25), 147 (34), 120 (100), 91 (41), 64 (32).

4.1 Chemistry

N-(4-(3-(1H-Benzimidazol-1-yl)propanoyl)phenyl) acetamide 2a:

A mixture of 4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 with benzimidazole, 2-methylbenzimidazole and/or benzylamine; Formation of 2b-d:

Method A:

To a solution of 4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 (2.33 g, 10 mmol) in dry toluene (30 ml), benzimidazole and/or 2-methylbenzimidazole (10 mmol) was added. The reaction mixture was refluxed for 20 h. The precipitated solid so formed, was filtered off, dried and recrystallized from the appropriate solvent to give 2b.c respectively.

Method B:

A mixture of 4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 (2.33 g, 10 mmol) and benzimidazole 2-methylbenzimidazole and/or benzylamine (10 mmol), in absolute ethanol (30 ml) was refluxed for 2 h and/or 5 min. The reaction mixture was left to cool, filtered off and then crystallized from the appropriate solvent to give 2b-d respectively.

Method C:

A mixture of 4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 (2.33 g, 10 mmol) and benzylamine (10 mmol) in absolute ethanol (20 ml) was stirred for 30 min. at room temperature. The solid product was filtered off, dried and crystallized from dioxane to give 2d.

4-(4-Acetamidophenyl)-2-(1H-benzo[d]imidazol-1-yl)-4-oxobutanoic acid 2b:
Yellow crystals, mp 199-201 °C (methanol), yield 65%. FT-IR (KBr, ν cm⁻¹): 3320 (br., OH), 3183 (NH), 1708 (C=O acid), 1698 (C=O ketone), 1661 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 12.65 (s, 1H, D₂O-exchange...COOH), 10.27 (s, 1H, D₂O-exchange...NHCOCH₃), 8.38 (s, 1H, Ar-H imidazole), 8.22-7.17 (m, 8H, Ar-H), 5.86-5.81 (dd, 1H, -COCH₂CH=N-, J = 6.9 & 6.6 Hz), 4.19 (dd, 2H, CH₂CH=J = 6.6 & 6.9 Hz), 2.63 (s, 3H, -CH₃), MS (m/z, %): 350 (M⁺ 1, 1.9), 289 (5), 233 (19), 191 (29), 147 (13), 120 (100), 92 (24), 65 (24).

4-(4-Acetamidophenyl)-2-(2-methyl-1H-benzod[d]imidazol-1-yl)-4-oxo-butan-2-one acid 2c

Yellow crystals, mp 215-217 °C (methanol), yield 63%. FT-IR (KBr, ν cm⁻¹): 3430 (br., OH), 3268 (NH), 1679 (C=O acid & ketone), 1635 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 12.80 (s, 1H, D₂O-exchange...COOH), 10.26 (s, 1H, D₂O-exchange...NHCOCH₃), 8.03-7.08 (m, 8H, Ar-H), 5.78 (dd, 1H, CH₂CH=J = 6.6 & 6.9 Hz), 4.19 (dd, 2H, CH₂CH=J = 6.6 & 6.9 Hz), 2.63 (s, 3H, -CH₃), 2.05 (s, 3H, -COCH₂). MS (m/z, %): 3414 (br., OH), 3251 (NH), 1698 (C=O acid), 1678 (C=Oketone), 1642 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 11.24 (s, 1H, D₂O-exchange...NHCOCH₃), 7.96-7.30 (m, 9H, Ar-H), 4.07-4.03 (dd, 2H, -NHCH₂J = 6.9 & 6.6 Hz), 3.97 (s, 3H, CH₃), 2.63 (s, 3H, -CH₂). MS (m/z, %): 354 (M⁺, 1), 311 (100), 293 (20), 267 (68), 252 (93), 176 (65), 120 (73).

Reaction of 4-oxobutanoic acid derivatives 2b-d with acetic anhydride; Formation of 3a-c

A solution of acid 1 (1 g, 4 mmol) and p-anisidine (0.52 g, 4 mmol) in absolute ethanol (20 ml) was refluxed for 2 h. The reaction mixture was cooled, filtered off and recrystallized from the appropriate solvent to give 4a-b in 80% yield and 4b in 75% yield, respectively.

Method A:

A solution of acid 1 and/ or 4-oxobutanoic acid derivatives 2b-d with hydrazine hydrate and/or phenylhydrazine; Formation of 4a,b respectively

Method B:

A solution of acid 1 and/ or 4-oxobutanoic acid derivatives 2b-d (5 mmol) and hydrazine hydrate and/or phenyl hydrazine (5 mmol) in dimethylformamide (20 ml) containing drops of glacial acetic acid was refluxed for 4 h. The reaction mixture was cooled, filtered off and recrystallized from the appropriate solvent to give 4a in 80% yield and 4b in 75% yield, respectively.

N-(4-(6-Oxo-1,6-dihydropyridazin-3-yl)phenyl)acetamide 4a

Yellow crystals, mp >300 °C (dioxane), yield 53 %. FT-IR (KBr, ν cm⁻¹): 3294, 3266 (NH), 1789 (C=O lactone), 1713 (C=Oester), 1672 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 11.24 (s, 1H, D₂O-exchange...NHCOCH₃), 7.96-7.30 (m, 9H, Ar-H), 4.07-4.03 (dd, 2H, -NHCH₂J = 6.9 & 6.6 Hz), 3.97 (s, 3H, CH₃), 2.63 (s, 3H, -CH₂). MS (m/z, %): 3430 (br., OH), 3268 (NH), 1698 (C=O acid), 1678 (C=O ketone), 1642 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 11.24 (s, 1H, D₂O-exchange...NHCOCH₃), 7.96-7.30 (m, 9H, Ar-H), 4.07-4.03 (dd, 2H, -NHCH₂J = 6.9 & 6.6 Hz), 3.97 (s, 3H, CH₃), 2.63 (s, 3H, -CH₂). MS (m/z, %): 350 (M⁺ 1, 1.9), 289 (5), 233 (19), 191 (29), 147 (13), 120 (100), 92 (24), 65 (24).

4-(4-Acetamidophenyl)-2-(2-methyl-1H-benzod[d]imidazol-1-yl)-4-oxo-butan-2-one acid 2d

4-(4-Acetamidophenyl)-2-(benzylamino)-4-oxobutanoic acid 2d

Yellow crystals, mp 190-192 °C (dioxane), yield 81 %. FT-IR (KBr, ν cm⁻¹): 3414 (br., OH), 3251 (NH), 1684(C=O acid & ketone), 1649 (C=Oamide). ¹H-NMR (300 MHz, DMSO-d₆): 12.65 (br. s, 1H, D₂O-exchange...COOH), 10.35 (s, 1H, D₂O-exchange...NHCOCH₃), 7.96-7.30 (m, 9H, Ar-H), 3.97 (s, 2H, -NHCH₂Ph), 3.60-3.17 (m, 4H, CH₂CH and NHCH₂D₂O-exchange...), 2.02 (s, 3H, CH₃). MS (m/z, %): 340 (M⁺ 1, 7), 331 (41), 316 (36), 305 (47), 261 (24), 120 (100), 91 (86), 65 (16).

4-(4-Acetamidophenyl)-2-(4-methoxyphenylamino)-4-oxobut-2-enoic acid 2e

A solution of acid 1 (1 g, 4 mmol) and p-anisidine (0.52 g, 4 mmol), in absolute ethanol (20 ml) was refluxed for 8 h, filtered on hot and recrystallized from ethanol to give 2e as yellow crystals, mp 225-227 °C, yield 64%. FT-IR (KBr, ν cm⁻¹): 3396 (br., OH), 3260 (NH), 1667, 1630(C=O). ¹H-NMR (300 MHz, DMSO-d₆): 12.10 (br. s, 1H, D₂O-exchange...COOH), 10.18 (s, 1H, D₂O-exchange...NHCOCH₃), 8.00-6.90 (m, 8H, Ar-H), 6.54 (d, -CH=COOH), N-isomer, J = 12.6 Hz), 6.02 (d, -CH=COOH), E-isomer, J = 7.8 Hz), 3.73 (s, 3H, -OCH₃), 2.08 (s, 3H, CH₃), MS (m/z, %): 354 (M⁺ 1, 1), 311 (100), 293 (20), 267 (68), 252 (93), 176 (65), 120 (73).

Reaction of 4-oxobutanoic acid derivatives 2b-d with acetic anhydride; Formation of 3a-c:

A solution of 4-oxobutanoic acid derivatives 2b-d (1 g, 3 mmol) in freshly distilled acetic anhydride (10 ml) was refluxed for 3 h. then poured onto ice cold water. The precipitated solid was filtered off and recrystallized from the proper solvent to afford 3a-c.
A solution of pyridazine derivative 4a (1 g, 4 mmol), formaldehyde (1.09 ml, 4 mmol) and piperidine (0.68 ml, 8 mmol) in methanol (25 ml) was refluxed for 9 h. the excess solvent was evaporated under vacuum. The resulting residue was recrystallized from ethanol to give 5 as pale yellow crystals, mp 211-213 °C, yield 81%. FT-IR (KBr, v cm⁻¹): 3312 (NH), 1691 (C=O amide), 1653 (C=O amide). ¹H-NMR (300 MHz, DMSO-d₆): 10.10 (s, 1H, D₂O-exchangeable, -NHCOCH₃), 7.99 (d, 1H, CO-CH=CH₂, J = 9.3 Hz), 7.84-7.68 (m, 4H, Ar-H), 7.01(d, 1H, CO-CH₂-CH=CH₂, J = 9.3 Hz), 4.97(s, 2H, N=CH₂), 2.64 (br.s, 4H, piperidine moiety), 2.07 (s, 3H, CH₃), 1.48-1.32 (m, 6H, piperidine moiety). MS (m/z, %): 326 (M⁺, 14), 244 (4), 229 (92), 187 (100), 130 (75), 70 (18), 55 (24).

Ethyl 2-(3-(4-acetamidophenyl)-6-oxopyridazin-1(6H)-yl)acetate 6:
A mixture of pyridazine derivative 4a (0.46 g, 2 mmol), ethyl chloroacetate (0.24 g, 2 mmol) and anhydrous potassium carbonate (0.55 g, 4 mmol) in dry acetone (30 ml) was refluxed for 24 hrs. The excess solvent was evaporated. The residue was washed with water, filtered off and recrystallized from toluene to give 6 as yellow crystals, mp 197-199 °C, yield 70%. FT-IR (KBr, v cm⁻¹): 3312 (NH), 1748 (C=O amide), 1690 (C=O amide), 1659 (C=O amide). ¹H-NMR (300 MHz, DMSO-d₆): 10.12 (s, 1H, D₂O-exchangeable, -NHCOCH₃), 8.05 (d, 1H, CO-CH=CH₂, J =10.2Hz), 7.82 (d, 2H, Ar-H, J = 9 Hz), 7.69 (d, 2H, Ar-H, J = 9 Hz), 7.10 (d, 1H, CO-CH₂-CH=CH₂, J = 9.6Hz), 4.92 (s, 2H, -N=CH₂), 4.18 (q, 2H, -CH₂-CH₃, J = 7.2 Hz), 2.07 (s, 3H, CH₃), 1.21 (t, 3H, -CH₂-CH₃, J = 7.2 Hz). MS (m/z, %): 315 (M⁺, 100), 273 (84), 242 (32), 200 (69), 172 (44), 130 (50), 91 (18), 57 (6).

5-(4-Acetamido-phenyl)-2,3,6,7-tetrahydro-1H-1,4-diazepine-7-carboxylic acid 7:
A mixture of acid 1 and/or 4-oxobutanoic acid derivatives 2b-d (5 mmol) and ethylenediamine (0.3 g, 5 mmol) in ethanol (20 ml) and catalytic amount of glacial acetic acid was refluxed for 10 hrs. The precipitated solid on hot was filtered off and recrystallized from the suitable solvent. Yellow crystals, mp 182-184 °C (dioxane), yield 83%. FT-IR (KBr, v cm⁻¹): 3267 (NH), 1674 (C=O), 1600 (C=N). ¹H-NMR (300 MHz, DMSO-d₆): 10.38 (s, 1H, D₂O-exchangeable, -NHCOCH₃), 8.05 (d, 1H, CO-CH=CH₂, J =10.2Hz), 7.82 (d, 2H, Ar-H, J = 9 Hz), 7.69 (d, 2H, Ar-H, J = 9 Hz), 7.10 (d, 1H, CO-CH₂-CH=CH₂, J = 9.6Hz), 4.92 (s, 2H, -N=CH₂), 4.18 (q, 2H, -CH₂-CH₃, J = 7.2 Hz), 2.07 (s, 3H, CH₃), 1.21 (t, 3H, -CH₂-CH₃, J = 7.2 Hz). MS (m/z, %): 315 (M⁺, 100), 273 (84), 242 (32), 200 (69), 172 (44), 130 (50), 91 (18), 57 (6).
Yellow crystals, mp 192-194 °C (ethanol), yield 69%. FT-IR (KBr, v cm⁻¹): 3262, 3186 (NH), 1755(C=O azoaxidine), 1675(C=O ketone), 1642 (C=O amide). MS (m/z, %): 275 (M⁺ + , 4), 261 (20), 227 (13), 162 (35), 120 (100), 92 (27), 65 (17).

N-4(2-(2-Imino-5-oxothiazolidin-4-yl)acetyl)phenyl)acetamide 10b:

Yellow crystals, mp 253-255 °C (ethanol), yield 73%. FT-IR (KBr, v cm⁻¹): 3350, 3291 (NH), 1700 (C=O thiazolidone), 1683 (C=O amide). IR (KBr, ν cm⁻¹): 3371, 3308, 3188 (NH), 1700 (C=O ketone), 1664 (C=O amide). ¹H-NMR (300 MHz, DMSO-d₆): 10.18 (s, 1H, D₂O-exchangeable, -NHCOCH₃). 8.65 (s, 2H, D₂O-exchangeable, -NHCOCH₃), 7.92 (d, 2H, Ar-H, J= 6 Hz), 7.71 (d, 2H, Ar-H, J= 6 Hz), 4.39 (d, 1H, CHCH₂, J= 8.4 Hz), 3.88(dd, 1H, 1H, CHCH₂, Jgem=15.3 Hz, Jvic cis = 3.3Hz), 3.40 (dd, 1H, CHCH₂, Jgem= 15.3 Hz, Jvic cis = 10.8 Hz), 2.09 (s, 3H, CH₃). MS (m/z, %): 291 (M⁺ + , 48), 236 (28), 163 (37), 135 (67), 120 (100), 83 (52), 57 (73).

Reaction of acid 1 and/or 4-oxobutanoic acid derivatives 2b-d with o-phenylenediamine, o-aminothiophenol; Formation of 11a-c

A solution of acid 1 (1 g, 3 mmol) and o-phenylenediamine (5 mmol) in absolute ethanol (20 ml) containing few drops of glacial acetic acid was refluxed for 2 hrs., The precipitated solid on hot was filtered off and recrystallized from the suitable solvent.

N-(4-(2-(3-Oxo-1,2,3,4-tetrahydroquinoxalin-2-yl)acetyl)phenyl)acetamide 11b:

Yellow crystals, mp 240-242 °C (methanol), yield 69%. FT-IR (KBr, v cm⁻¹): 3303, 3188 (NH), 1672 (br., C=O). ¹H-NMR (300 MHz, DMSO-d₆): 10.66 (s, 1H, D₂O-exchangeable, NH₂imin). 10.27 (s, 1H, D₂O-exchangeable, -NHCOCH₃). 7.93 (d, 2H, Ar-H, J= 8.7 Hz), 7.70 (d, 2H, Ar-H, J= 8.7 Hz), 7.32-6.96 (m, 4H, Ar-H), 4.04 (t, 1H, CHCH₂, J= 6.6 Hz), 3.62 (dd, 1H, CHCH₂, Jgem=17.4 Hz, Jvic = 6.6 Hz), 3.20 (dd, 1H, CHCH₂, Jgem=17.7 Hz, Jvic = 6.3 Hz), 2.09 (s, 3H, CH₃), CO), MS (m/z, %): 340 (M⁺ + , 25), 298 (4), 297 (5), 263 (4), 239 (5), 178 (83), 150 (71), 135 (71), 120 (100), 109 (25), 92 (70), 91 (28), 77 (14), 65 (67), 51 (13).

N-(4-(1-Hydrazono)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)ethyl)phenyl)acetamide 12a:

A solution of 11c (1 g, 3 mmol) and hydrazine hydrate (0.15 g, 3 mmol) in dioxane (20 ml) was refluxed 6 hrs. The reaction mixture was poured onto ice cold water. The precipitated solid was filtered off and crystallized from benzene to give 12a as yellow crystals, mp 206-208 °C, yield 63%. FT-IR (KBr, v cm⁻¹): 3311, 3268 (NH₂). 3215 (NH), 1676 (C=O), 1595 (C=N). ¹H-NMR (300 MHz, DMSO-d₆): 10.66 (s, 1H, D₂O-exchangeable, NH₂imin). 10.27 (s, 1H, D₂O-exchangeable -NHCOCH₃), 7.95-6.99 (m, 8H, Ar-H), 5.46 (s, 2H, D₂O-exchangeable, NH₂imin), 4.05 (dd, 1H, CHCH₂J= 3.2 & 3.5 Hz), 3.64 (dd, 1H, CHCH₂J= 3.2 & 3.9 Hz), 3.20 (dd, 1H, CHCH₂J= 3.5 & 3.9 Hz), 2.06 (s, 3H, CH₃). MS (m/z, %): 354 (M⁺ + , 3), 340 (100), 292 (47), 263 (14), 150 (66), 135 (90), 120 (74), 92 (35).

N-(4-(2-(3-Oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)ethyl)phenyl)acetamide 12b:

A solution of 11c (1 g, 3 mmol) and hydroxylamine hydrochloride (0.2 g, 3 mmol) in pyridine (20 ml) was refluxed 7 hrs. The reaction mixture was poured onto ice/HCl. The precipitated solid was filtered off and crystallized from ethanol to give 12b as yellow crystals, mp 240-242 °C, yield 66%. FT-IR (KBr, v cm⁻¹): 3473 (OH), 3363, 3295 (NH), 1662 (C=O). ¹H-NMR (300 MHz, DMSO-d₆): 11.31, 10.88 (2s, 2H, OHC=Ximine exchange, CH₂O, Z- and E-isomers), 10.60 (2s, 2H, OHC=Ximine exchange, -NHCOCH₃), 7.56-6.48 (m, 16H, Ar-H, E and Z isomers), 3.80 (dd, 2H, 2(=CHCH₂), J= 3.3 & 3.6 Hz), 3.31-3.13 (m, 4H, 2(=CHCH₂), 2.04 (s, 3H, CH₃). MS (m/z, %): 355 (M⁺ + , 11), 338 (52), 313 (27), 282 (28), 268 (33), 226 (39), 177 (72), 108 (100), 91 (17).

N-(4-(2-(4-Cyano-5-imino-2-oxodihydrofuran-3(2H)-ylidene)acetyl)phenyl)acetamide 14:

A mixture of acid 1 (1 g, 4 mmol), malononitrile (0.28 g, 4 mmol) and ammonium acetate (0.32 g, 4 mmol) in absolute ethanol (20 ml) was refluxed on a water bath for 4 hrs., Filtered on hot and recrystallized from dioxane to give 14 as brown crystals, mp 291-293 °C, yield 73%. FT-IR (KBr, v cm⁻¹): 3309, 3252 (NH), 2215 (C≡N), 1737 (C=O ketone), 1673...
A solution of acid 1 (1 g, 4 mmol) and malononitrile (0.28 g, 4 mmol) in absolute ethanol (20 ml) in the presence of drops of piperidine was refluxed on water bath for 9 hrs. The excess solvent was evaporated under vacuum, the resultant residue was triturated with ether, filtered and recrystallized from benzene/ethanol to give 15 as brown crystals, mp 134-136 ºC, yield 66%. FT-IR (KBr, ν cm⁻¹): 3480 (br., OH), 2970, 2932, 2918, 2820 (26), 2580 (49), 2290 (40), 1620 (67), 1200 (100), 1170 (65), 72 (100), 71 (52), 64 (69).

4-(4-Acetimidophenyl)-2-(dicyanomethylene)-4-oxobutanoic acid 15:

A variety of heterocyclic systems have been synthesized from the reaction of (4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 with different nucleophiles. All the synthesized compounds are potent antioxidants. In particular the 4-oxobutanoic acid derivatives showed the most reducing power antioxidant activity (RPAA) expressed in 1.73 ± 0.01, 2.243 ± 0.07 and 2.16 ± 0.02 OD value ± S.D. using ascorbic acid as standard.

4.2 Reducing Power Antioxidant Assay (RPAA)

The spectrophotometric method described [18]; was used for the measurement of reducing power. For this 2.5 ml of each of the sample was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min, then rapidly cooled, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (2.5 ml) of the supernatant was mixed with 2.5 ml of 1% potassium ferricyanide (10 mg/ml) and 2.5 ml of 1% potassium ferricyanide (10 mg/ml) and then ferric chloride (0.5 ml, 0.1%) was added and allowed to stand for 10 min. The absorbance was read spectrophotometrically at 700 nm, ascorbic acid used as standard. Three replicates were made for each test sample. The percentage of reducing power was calculated using the formula:

\[
\text{Reducing power} \% = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Where, \(A_{\text{control}}\) was the absorbance of solution without extract and \(A_{\text{sample}}\) was the absorbance with different dilutions of sample and the reducing power was reported as ascorbic acid equivalent per gm of dry sample [18].

Statistical Analysis

All data were presented as mean ± S.D. using SPSS 13.0 program [19].

5. Conclusion

A variety of heterocyclic systems have been synthesized from the reaction of (4-(4-acetamido-phenyl)-4-oxobut-2-enoic acid 1 with different nucleophiles. All the synthesized compounds are potent antioxidants. In particular the 4-oxobutanoic acid 2e and benzothiazinone 12ab showed the most reducing power antioxidant activity (RPAA) expressed in 1.73 ± 0.01, 2.243 ± 0.07 and 2.16 ± 0.02 OD value ± S.D. using ascorbic acid as standard.

References


