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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 its aza 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-, [-diazin, -oxazin, -thiaz-]-ones (11a-c). Conduct (11c) with NH_2-NH_2.H_2O in boiling ethanol and / or $NH_2OH.HCl$ 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, Quinoxalone, Piperazine, Benzoxazinone, Furanone, Antioxidant activity

1. Introduction

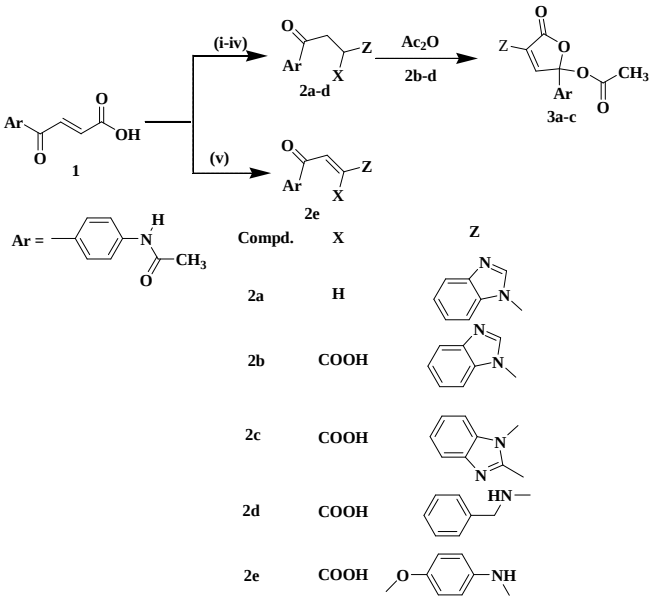
Aryl- and heteroaryl substituted 4-oxobut-2-enoic acids and their derivatives represent an important class of compounds with interesting pharmacological medications including antiulcer and cytoprotective properties [1], kynurenine-3-hydroxylase [2] and human cytomegalovirus protease inhibition activity [3]. Several naturally occurring acylacrylic 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 acetanilides, 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 its aza 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 its aza 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, an aza-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] $^{+\bullet}$.

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

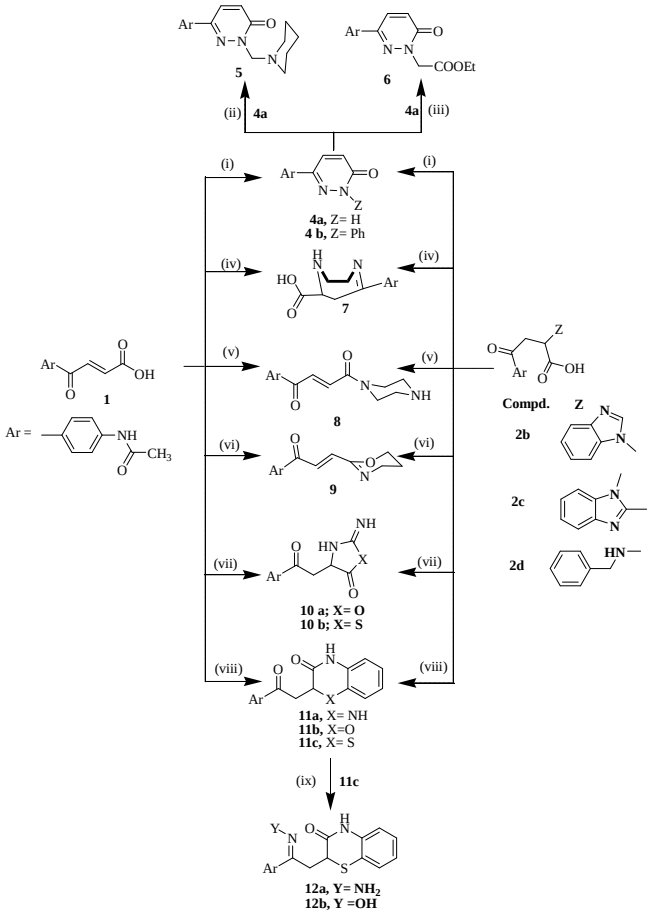
On the other hand, when the reaction was conducted with benzimidazole, 2-methylbenzimidazole, benzylamine and / or p-anisidine in refluxing absolute ethanol, dry benzene and / or dry toluene, 4-oxobutanoic acid derivatives **2b-d** and 4-oxobut-2-enoic acid **2e** have been readily obtained, respectively. Prolonged heating in case of reaction with p-anisidine results in the formation of more thermo-dynamically 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 ^{1}H-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.



Scheme 1: (i) Benzimidazole / pyridine;(ii) Benzimidazole/ EtOH or dry Benzene ;
(iii) 2-Methylbenzimidazole / EtOH or dry Toluene; (iv,v) Benzylamine
and / or p-Anisidine/ EtOH

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}HNMR spectrum displayed two singlets at δ 2.19 and 2.07 ppm due to two methyl protons of both ester (O-CO-$\underline{CH_3}$) and acetamido (NH-CO-$\underline{CH_3}$) 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 ν 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_2CO_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-acetamidophenyl)-6-oxo-pyridazin-1(6H)-yl)acetate **(6)** respectively.



Scheme 2: (i) Hydrazine hydrate and / or Phenyl hydrazine, EtOH, AcOH;(ii) Formaldehyde, piperidine, MeOH; (iii) Ethyl chloroacetate, K_2CO_3, dry acetone; (iv) Ethane-1,2-diamine, EtOH, drops AcOH; (v) Ethanolamine, and / or 3-Aminopropan-1-ol, EtOH, drops AcOH; (vi) Urea and / orThiourea , EtOH, drops AcOH; (vii) o- Phenylene diamine, o-Aminophenol and / or o-Aminothiophenol, EtOH, drops AcOH; (viii) Hydrazine hydrate, EtOH and / or Hydroxylamine hydrochloride, pyridine.

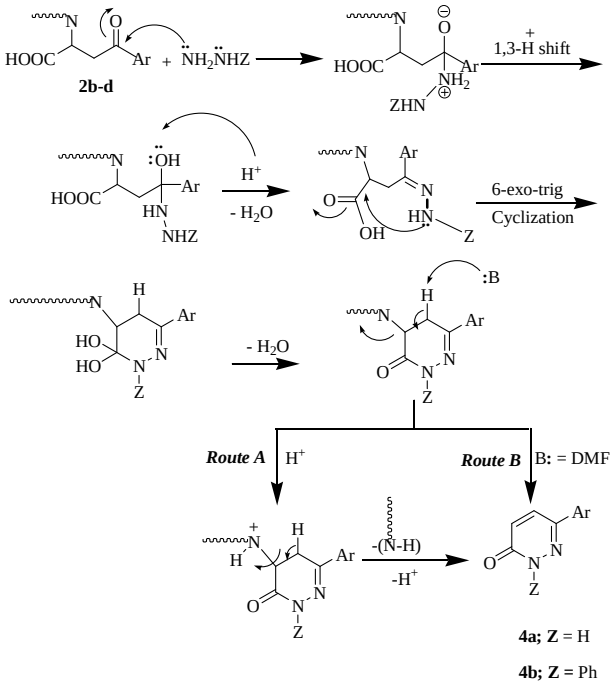
Presumably, dehetero-arylation process in the former reaction occurred under the effect of protonation of the heteryl moiety influenced by the existing acetic acid. Consequently, progress of reaction was enhanced by the removal of heterocycle moiety and deprotonation at 4-position to give the thermodynamically 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 binucleophiles 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)phenylacetamide **10b** respectively.

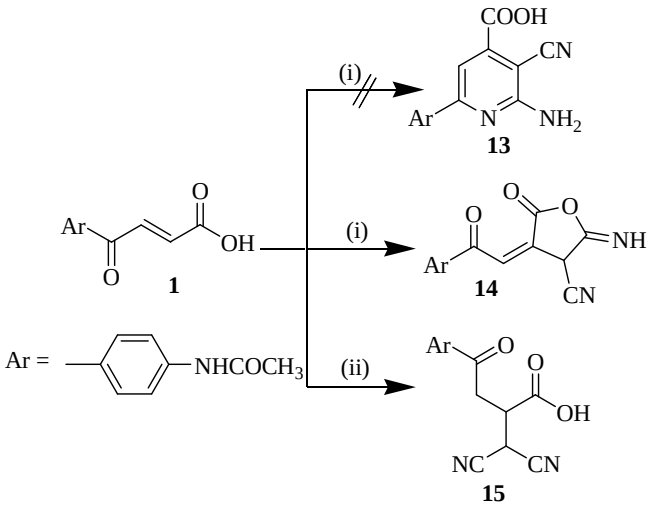
With the aim to improve this study to construct some fused annelated 6-membered heterocycles, the acid **1** or **2** was submitted to react with the bidentate nucleophiles, o-phenyl-enediamine, o-aminophenol and / or o-aminothiophenol in boiling ethanol / glacial AcOH mixture. It was surprising that, both reactions afforded good yields of similar products of quinoxalone **(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, ^{1}HNMR, 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-NH_2 functionality. On the other hand, the structure of the latter product **12b** was proved from ^{1}H-NMR spectrum that revealed two singlets at δ 11.31 and 10.88 ppm due to the oximino C=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).



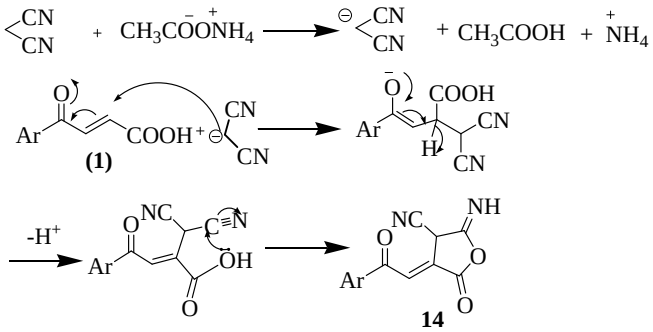
Scheme 3: Mechanistic Pathways for the Formation of Pyridazinones 4a and 4b

On contrary to one of our recent publications [12], that involved treatment the γ-keto acid with malononitrile in the presence of ammonium acetate as a base catalyst yielded the pyridine derivative **13**, herein, application of a similar reaction on **1** gave N-(4-(2-(4-cyano-5-imino-2-oxodihydro-furan-3(2H)-ylidene)acetyl)phenyl)acetamide **(14, Scheme 4)**.



Scheme 4: (i) Malononitrile, AcONH4, EtOH; (ii)Malononitrile, EtOH, piperidine

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**).



Scheme 5: A plausible mechanism of the formation of compound 14

3. Antioxidant Evaluation

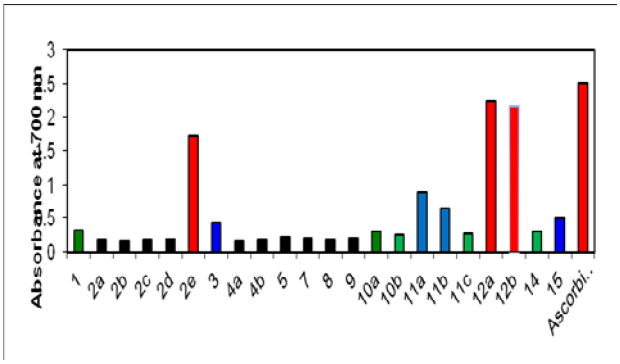
The antioxidant activities of the synthesized compounds were shown in table 1 and fig.1. The following points have been noticed:

(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.

Sample	*OD value ± S.D.*
1	0.312 ± 0.01
2a	0.178 ± 0.09
2b	0.159 ± 0.06
2c	0.166 ± 0.04
2d	0.183 ± 0.03
2e	1.73 ± 0.01
3	0.433 ± 0.01
4a	0.156 ± 0.07
4b	0.174 ± 0.08
5	0.223 ± 0.01
7	0.205 ± 0.04
8	0.171 ± 0.01
9	0.198 ± 0.01
10a	0.298 ± 0.06
10b	0.258 ± 0.07
11a	0.883 ± 0.01
11b	0.641 ± 0.01
11c	0.275 ± 0.07
12a	2.243 ± 0.07
12b	2.16 ± 0.02
14	0.304 ± 0.04
15	0.504 ± 0.09
Ascorbic acid	2.50 ± 0.05

Figure 1: Reducing power antioxidant activity of different compounds against ascorbic acid at concentration 200 µG/ML



4. Experimental

All melting points were measured on Gallenkamp electric melting point apparatus and are uncorrected. The infrared spectra were recorded using KBr disks on a Pye Unicam SP-3-300 infrared spectrophotometer. ^{1}H-NMR spectra were run at 300 MHz on a Varian Mercury VX-300 NMR spectrometer using TMS as internal standard in $CDCl_3$ or DMSO d_6. Chemical shifts are quoted δ. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometer at 70 eV. All the spectral measurements as well as elemental analyses were car-ried out at Micro analytical Center of Cairo University and Main Defense Chemical Labora- tory, Egypt. All the newly synthesized compounds gave satisfactory elemental analyses. 4-(4-Acetamido-phenyl)-4-oxobut-2-enoic acid **1** was prepared according to the literature [14].

4.1 Chemistry

N-(4-(3-(1H-Benzo[d]imidazol-1-yl)propanoyl)phenyl) acetamide 2a:

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 ^{o}C) and recrystallized from acetic acid to give **2a** as yellow crystals, mp 234-236 ^{o}C, yield 60%. FT-IR (KBr, ν cm^{-1}): 3240 (NH), 1694 (C=O $_{ketone}$), 1667 (C=O_{amide}). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.24 (s, 1H, D_2O-exchange., -NHCOCH$_3$), 8.23 (s, 1H, C-H $_{imidazole}$), 7.94-7.17 (m, 8H, Ar-H), 4.59 (t, 2H, -COCH$_2$CH$_2$-N-, J= 6.9 Hz), 3.61 (t, 2H, -COCH$_2$CH$_2$-N-, J= 6.6 Hz), 2.07 (s, 3H, CH_3). MS (m/z, %): 307 ($M^{\ddagger}$, 2), 292 (4), 189 (25), 147 (34), 120 (100), 91 (41), 64 (32).

Aza-Michael Addition Reaction of Amines to the Enoic Acid 1:

Reaction of 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. left to cool. 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-methyl-benzimidazole 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 ^{o}C (methanol), yield 65%. FT-IR (KBr, ν cm^{-1}): 3320 (br., OH), 3183 (NH), 1708 ($C=O_{acid}$), 1698 ($C=O_{ketone}$), 1661 ($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 12.65 (s, 1H, D_2O-exchange.,COOH), 10.27 (s, 1H, D_2O-exchange.,-NHCOCH$_3$), 8.38 (s, 1H, Ar-H $_{imidazole}$), 8.22-7.17 (m, 8H, Ar-H), 5.86-5.81 (dd, 1H, -COCH$_2$CH-N-, J= 6.9 & 6.6 Hz), 4.07-4.03 (dd, 2H, -COCH$_2$CH-N-, J= 6.3 & 6.6 Hz), 2.09 (s, 3H, CH$_3$). MS (m/z, %): 350 (M-1]$^{+\bullet}$, 1.9), 289 (5), 233 (19), 191 (29), 147 (13), 120 (100), 92 (24), 65 (24).

4-(4-Acetamidophenyl)-2-(2-methyl-1H-benzo[d]imidazol-1-yl)-4-oxo-butanoic acid 2c:

Yellow crystals, mp 215-217 ^{o}C (methanol), yield 63%. FT-IR (KBr, ν cm^{-1}): 3430 (br., OH), 3268 (NH), 1679 (C=O $_{acid\ \&\ ketone}$), 1635 ($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 12.80 (s, 1H, D_2O-exchange., COOH), 10.26 (s, 1H, D_2O-exchange.,-NHCOCH$_3$), 8.03-7.08 (m, 8H, Ar-H), 5.78 (dd, 1H, CH$_2$CH, J= 6.6 & 6.9 Hz), 4.19 (dd, 2H, CH$_2$CH, J= 6.6 & 6.9 Hz), 2.63 (s, 3H, -CH$_3$), 2.05 (s, 3H, -COCH$_3$). MS (m/z, %): 364 (M-1]$^{+\bullet}$, 5), 233 (8), 191 (10), 168 (10), 132 (100), 104 (24), 120 (4), 92 (18), 65 (23), 64 (32).

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

Yellow crystals, mp 190-192 ^{o}C (dioxane), yield 81 %. FT-IR (KBr, ν cm^{-1}): 3414 (br., OH), 3251 (NH), 1684(C=O $_{acid\ \&\ ketone}$), 1649 ($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 12.65 (br. s, 1H, D_2O-exchange., COOH), 10.35 (s, 1H, D_2O-exchange.,-NHCOCH$_3$), 7.96-7.30 (m, 9H, Ar-H), 3.97 (s, 2H, -NHCH$_2$Ph), 3.60-3.17 (m, 4H, CH$_2$CH and NHCH$_2$, D_2O-exchange.), 2.02 (s, 3H, CH$_3$). MS (m/z, %): 340 (M$^{+\bullet}$, 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 ^{o}C, yield 64%. FT-IR (KBr, ν cm^{-1}): 3396 (br., OH), 3260 (NH), 1667, 1630(C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 12.10 (br. s, 1H, D_2O-exchange., COOH), 10.18 (s, 1H, D_2O-exchange.,-NHCOCH$_3$), 8.00-6.90 (m, 8H, Ar-H), 6.34 (d, -CH=C(COOH)-NH-, Z-isomer, J = 12.6 Hz), 6.02 (d, -CH=C(COOH)-NH-, E-isomer, J = 7.8 Hz), 3.73 (s, 3H, -OCH$_3$), 2.08 (s, 3H, CH$_3$). MS (m/z, %): 354 (M$^{+\bullet}$, 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**.

2-(4-Acetamidophenyl)-4-(1H-benzo[d]imidazol-1-yl)-5-oxo-2,5-dihydrofuran-2-yl acetate 3a:

Brown crystals, mp > 300 ^{o}C (dioxane), yield 64%. FT-IR (KBr, ν cm^{-1}): 3318, 3127 (NH), 1784 ($C=O_{lactone}$), 1748 ($C=O_{ester}$), 1642 ($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.14 (s, 1H, D_2O-exchange.,-NHCOCH$_3$), 7.85-7.11 (m, 10H, Ar-H), 2.19 (s, 3H, CH$_3$), 2.07 (s, 3H, -COCH$_3$).

2-(4-acetamidophenyl)-4-(2-methyl-1H-benzo[d]imidazol-1-yl)-5-oxo-2,5-dihydrofuran-2-yl acetate 3b:

Brown crystals, mp >300 ^{o}C (dioxane), yield 53 %. FT-IR (KBr, ν cm^{-1}): 3294, 3266 (NH), 1789 ($C=O_{lactone}$), 1713($C=O_{ester}$), 1672 ($C=O_{amide}$).

2-(4-acetamidophenyl)-4-(benzylamino)-5-oxo-2,5-dihydro-furan-2-yl acetate 3c:

Yellow crystals, mp 187-189 ^{o}C (dioxane), yield 60 %. FT-IR (KBr, ν cm^{-1}): 3322 (NH), 1780 ($C=O_{lactone}$), 1715 ($C=O_{ester}$), 1678 ($C=O_{amide}$).

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

Method A:

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 absolute ethanol (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.

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) was stirred for 8 h and/or refluxed for 2 h. The reaction mixture was poured into ice cold water. The obtained solid was filtered off to give **4a** in 65% yield and **4b** in 73% yield, respectively.

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

Yellow crystals, mp > 300 ^{o}C, (methanol/dioxane). FT-IR (KBr, ν cm^{-1}): 3305, 3190 (NH), 1685 ($C=O_{pyridazine}$), 1652 ($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 13.08 (s, 1H, D_2O-exchange., $NH_{pyridazine}$), 10.08 (s, 1H,-NHCOCH$_3$), 8.00-7.97 (d, 1H, CH=CH-CO, J=10.2 Hz), 7.81-7.66 (m, 4H, Ar-H), 6.98-6.94 (d, 1H, CH=CH-CO, J=9.9 Hz), 2.07 (s, 3H, CH$_3$). MS (m/z, %): 229 (M$^{+\bullet}$, 83), 187 (100), 130 (67), 93 (40), 60 (57).

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

Pale yellow crystals, mp 263-265 °C (ethanol). FT-IR (KBr, ν cm^{-1}): 3272(NH), 1701 (C=O $_{pyridazine}$), 1657 (C=O_{amide}). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.11 (s, 1H, D_2O-exchange., -NHCOCH_3), 8.49 (d, 1H, CO-CH=CH, J=10.2 Hz), 7.88-7.44 (m, 9H, Ar-H), 7.16 (d, 1H, CO-CH=CH, J=10.2 Hz), 2.07 (s, 3H, CH_3). MS (m/z, %): 305 ($M^{+\bullet}$, 83), 187 (100), 130 (67), 93 (40), 60 (57).

N-(4-(6-Oxo-1-(piperidin-1-ylmethyl)-1,6-dihydropyridazin-3-yl)phenyl)acetamide 5.

A solution of pyridazinone 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, ν cm^{-1}): 3312 (NH), 1691 (C=$O_{pyridazinone}$), 1653 (C=O_{amide}). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.10 (s, 1H, D_2O-exchangeable, -NHCOCH_3), 7.99 (d, 1H, CO-CH=CH, J=9.3 Hz), 7.84-7.68 (m, 4H, Ar-H), 7.01(d, 1H, CO-CH=CH, J=9.3 Hz), 4.97(s, 2H,-NCH_2-), 2.64 (br.s, 4H, piperidine moiety), 2.07 (s, 3H, CH_3), 1.48-1.32 (m, 6H, piperidine moiety). MS (m/z, %): 326 ($M^{+\bullet}$, 14.4), 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 pyridazinone 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, ν cm^{-1}): 3312 (NH), 1748 (C=O_{ester}), 1690 (C=$O_{pyridazinone}$), 1659 (C=O $_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.12 (s, 1H, D_2O-exchangeable, -NHCOCH_3), 8.05 (d, 1H, CO-CH=CH, 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-CH=CH, J= 9.6Hz), 4.92 (s, 2H, -N-CH_2), 4.18 (q, 2H, -CH_2CH_3, J = 7.2 Hz), 2.07 (s, 3H, CH_3), 1.21 (t, 3H, -CH_2CH_3, J = 7.2 Hz). MS (m/z, %): 315 ($M^{+\bullet}$, 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 was separated by filtration on hot and recrystallized from dioxane to give **7** as yellow crystals, mp 210-212 °C, yield 57%. FT-IR (KBr, ν cm^{-1}): 3427 (br., OH), 3260, 3181 (NH), 1710 (C=O_{acid}), 1674 (C=O_{amide}). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.37 (s, 1H, D_2O-exchange., COOH), 10.07 (s, 1H, D_2O-exchange., -NHCOCH_3), 7.78-7.43 (m, 4H, Ar-H), 5.19 (dd, 1H, CH-COOH, J = 8.7 & 4.5 Hz), 3.76-3.22 (m, 4H, -NH-CH_2-CH_2N=), 2.94 (s, 1H, D_2O-exchange., NH), 2.21-1.88 (m, 5H, CH_2-C=N and CH_3). MS (m/z, %): 275 ($M^{+\bullet}$, 29), 257 (100), 245 (64), 233 (80), 231 (24), 229 (26), 189 (33), 159 (55), 117 (58), 95 (65), 68 (45).

Reaction of acrylic acid 1 and/or 4-oxobutanoic acid derivatives 2b-d with ethanolamine and/or propanolamine; Formation of 8 and 9 respectively:

A solution of acid **1** and/or 4-oxobutanoic acid derivatives **2b-d** (5 mmol) and ethanolamine (0.3 g, 5 mmol) and/or propanolamine (0.375 g, 5 mmol) in absolute ethanol (20 ml) containing few drops of glacial acetic acid was refluxed for 15 min. the reaction mixture was filtered on hot and recrystallized from the suitable solvent.

N-(4-(4-Oxo-4-(piperazin-1-yl)but-2-enoyl)phenyl)acetamide 8:

Yellow crystals, mp 187-189 °C (dioxane), yield 80%. FT-IR (KBr, ν cm^{-1}): 3306, 3264 (NH), 1676 (C=O), 1600 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.38 (s, 1H, D_2O-exchangeable, -NHCOCH_3), 7.91 (d, 2H, Ar-H, J = 7.5 Hz), 7.72 (d, 2H, Ar-H, J = 6.9 Hz), 7.47 (d, 1H, CH=CH, J= 15.6 Hz), 6.67 (d, 1H, CH=CH, J= 15.3 Hz), 3.71-3.47 (m, 4H, -CH_2-N-CH_2-), 2.97(t, 2H, -CH_2-NH-, J= 5.4 Hz), 3.16 (s, 1H, D_2O-exchange., -NH piperazine moiety), 2.86 (t, 2H, -CH_2-NH-, J= 5.4 Hz), 2.06 (s, 3H, CH_3). MS (m/z, %): 301 ($M^{+\bullet}$, 00.0), 258 (M-C_2H_5N-]$^{+\bullet}$, 61), 242 (43), 198 (52), 201 (41), 213 (42), 188 (65), 162 (62), 155 (54), 147 (50), 129 (44), 120 (94), 119 (55), 105 (44), 92 (42), 91 (60), 65 (76), 64 (100), 51 (62).

N-(4-(3-(5,6-Dihydro-4H-1,3-oxazin-2-yl)acryloyl)phenyl)-acetamide 9:

Yellow crystals, mp 182-184 °C (ethanol), yield 83%. FT-IR (KBr, ν cm^{-1}): 3267 (NH), 1674 (C=O), 1600 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.38 (s, 1H, D_2O-exchangeable, -NHCOCH_3), 7.93-7.90 (dd, 2H, Ar-H, J= 9 Hz), 7.73 (dd, 2H, Ar-H, J= 9 Hz), 7.43 (d, 1H, COCH=CH, J= 15 Hz), 6.67 (d, 1H, COCH=CH, J= 15 Hz), 3.67 (t, 2H, O-CH_2, J= 5.7 Hz), 2.98-2.81 (m, 2H, CH_2-N), 2.09 (s, 3H, CH_3), 1.78-1.67 (m, 2H, $OCH_2CH_2CH_2$). MS (m/z, %): 272 ($M^{+\bullet}$, 43), 245 (42), 214 (30), 212 (37), 186 (31), 121 (33), 109 (36), 104 (30), 93 (33), 69 (100).

Reaction of acid 1 and/or 4-oxobutanoic acid derivatives 2b-d with urea and/or thiourea; Formation of 10a and 10b:

A mixture of acid **1** and/or 4-oxobutanoic acid derivatives **2b-d** (5 mmol), urea and/or thiourea (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-(2-Imino-5-oxooxazolidin-4-yl)acetyl)phenyl) acetamide 10a:

Yellow crystals, mp 192-194 °C (ethanol), yield 69%. FT-IR (KBr, ν cm^{-1}): 3262, 3186 (NH), 1755(C=O oxazolidine), 1675(C=O ketone), 1642 (C=O amide). MS (m/z, %): 275 ($M^{+\bullet}$, 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, ν cm^{-1}): 3350, 3291 (NH), 1700 ($C=O_{thiazolidinone}$), 1683 ($C=O_{ketone}$), 1662 ($C=O_{amide}$), 1602 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.18 (s, 1H, D_2O-exchangeable, -$NHCOCH_3$), 8.65 (s, 2H, D_2O-exchangeable, 2NH), 7.92 (d, 2H, Ar-H, J= 6 Hz), 7.71 (d, 2H, Ar-H, J= 6 Hz), 4.39 (d, 1H, $\underline{CH}CH_2$, J= 8.4 Hz), 3.88(dd, 1H, $CH\underline{CH_2}$, $J_{gem.}$=15.3 Hz, $J_{vic.tr}$ =3.3Hz), 3.40 (dd, 1H, $CH\underline{CH_2}$, $J_{gem.}$ = 15.3 Hz, $J_{vic.cis}$ = 10.8 Hz), 2.09 (s, 3H, CH_3). MS (m/z, %): 291 ($M^{+\bullet}$, 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-aminophenol and/or o-aminothiophenol; Formation of 11a-c

A solution of acid **1** and/or 4-oxobutanoic acid derivatives **2b-d** (5 mmol) and/or o-phenylenediamine, o-aminothiophenol and/or o-aminophenol (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 11a:

Yellow crystals, mp 242-244 °C (methanol), yield 64%. FT-IR (KBr, ν cm^{-1}): 3371, 3308, 3188 (NH), 1700 ($C=O_{ketone}$), 1664 ($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.31 (s, 1H, $NHCO_{pyrazine}$, exchangeable with D_2O), 10.28 (s, 1H, , D_2O-exchangeable, -$NHCOCH_3$), 7.95(d, 2H, Ar-H, J=9 Hz), 7.71(d, 2H, Ar-H, J=9 Hz), 6.79-6.60 (m, 4H, Ar-H), 5.92 (s, 1H, , D_2O-exchangeable, $NH_{pyrazine}$), 4.32 (m, 1H, $\underline{CH}CH_2$), 3.54-3.40 (m, 2H, $CH\underline{CH_2}$), 2.08 (s, 3H, CH_3). MS (m/z, %): 324 (M+1]$^{+\bullet}$, 40), 220 (40), 135 (47), 120 (47), 91 (73), 69 (100).

N-(4-(2-(3-Oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-yl)acetyl)phenyl)acetamide 11b:

Brown crystals, mp 213-215 °C (toluene/ethanol), yield 53%. FT-IR (KBr, ν cm^{-1}): 3314, 3113 (NH), 1677 (br., C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.68 (s, 1H, D_2O-exchangeable, $NH_{oxazine}$), 10.28 (s, 1H, D_2O-exchangeable, -$NHCOCH_3$), 7.95 (d, 2H, Ar-H, J= 9 Hz), 7.72(d, 2H, Ar-H, J= 9 Hz), 7.45-6.83 (m, 4H, Ar-H), 5.07 (t, 1H, $\underline{CH}CH_2$, J= 5.4 Hz), 3.60 (d, 2H, $CH\underline{CH_2}$, J= 5.1 Hz), 2.09 (s, 3H, CH_3CO). MS (m/z, %): 324 ($M^{+\bullet}$, 100), 282 (45), 240 (37), 210 (34), 120 (99), 92 (45), 65 (26).

N-(4-(2-(3-Oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetyl)phenyl)acetamide 11c:

Yellow crystals, mp 240-242 °C (methanol), yield 69%. FT-IR (KBr, ν cm^{-1}): 3303, 3188 (NH), 1672 (br., C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.66 (s, 1H, D_2O-exchangeable, $NH_{thiazine}$), 10.27 (s, 1H, , D_2O-exchangeable, -$NHCOCH_3$), 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, $\underline{CH}CH_2$, J= 6.6 Hz), 3.62 (dd, 1H, $CH\underline{CH_2}$, $J_{gem.}$=17.4 Hz, J_{vic} = 6.6 Hz), 3.20 (dd, 1H, $CH\underline{CH_2}$, $J_{gem.}$=17.7 Hz, J_{vic} = 6.3 Hz), 2.09 (s, 3H, CH_3CO). MS (m/z, %): 340 ($M^{+\bullet}$, 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, ν cm^{-1}): 3311, 3268 (NH_2), 3215 (NH), 1676 (C=O), 1595 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.66 (s, 1H, D_2O-exchange., $NH_{Thiazine}$), 10.27 (s, 1H, D_2O-exchange., -$\underline{NH}COCH_3$), 7.95-6.99 (m, 8H, Ar-H), 5.46 (s, 2H, D_2O-exchangeable, NH_2), 4.05 (dd, 1H, $\underline{CH}CH_2$, J= 3.2 & 3.5 Hz), 3.64 (dd, 1H, $CH\underline{CH_2}$, J= 3.2 & 3.9 Hz), 3.20 (dd, 1H, $CH\underline{CH_2}$, J= 3.5 & 3.9 Hz), 2.06 (s, 3H, CH_3). MS (m/z, %): 354 ($M^{+\bullet}$, 3), 340 (100), 292 (47), 263 (14), 150 (66), 135 (90), 120 (74), 92 (35).

N-(4-(1-(Hydroxyimino)-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 204-206 °C, yield 66%. FT-IR (KBr, ν cm^{-1}): 3473 (OH), 3363, 3295 (NH), 1662 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 11.31, 10.88 (2s, 2H, $2OH_{Oxime}$, exchange., D_2O, Z- and E- isomers), 10.60 (2s, 2H, D_2O-exchange., 2(-NHCO-), Z- and E- isomers), 10.00 (s, 1H, D_2O-exchange., $NH_{Thiazine}$), 7.56-6.48 (m, 16H, Ar-H, E and Z isomers), 3.80 (dd, 2H, 2(-$\underline{CH}CH_2$-), J= 3.3 & 3.6 Hz), 3.31-3.13 (m, 4H, 2(-$CH\underline{CH_2}$-), 2.04 (s, 3H, CH_3). MS (m/z, %): 355 ($M^{+\bullet}$, 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, ν cm^{-1}): 3309, 3252 (NH), 2215 (C≡N), 1737 ($C=O_{lactone}$), 1673

($C=O_{amide}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.46 (s, 1H, D_2O-exchangeable, -$NHCOCH_3$), 8.09-7.57 (m, 4H, Ar-H), 6.99 (s, 1H, CH=C), 6.82 (s, 1H, CH-CN), 6.69 (s, 1H, NH, exchangeable with D_2O), 2.09 (s, 3H, CH_3). MS (m/z, %): 297 ($M^{\bullet+}$, 63), 282 (89), 233 (60), 196 (80), 188 (62), 145 (59), 117 (65), 72 (100), 71 (52), 64 (69).

4-(4-Acetamidophenyl)-2-(dicyanomethyl)-4-oxobutanoic acid 15:

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 resulted 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), 3320 (NH), 2252, 2220 (C≡N), 1705 ($C=O_{acid}$), 1681 ($C=O_{ketone}$). ^{1}H-NMR (300 MHz, DMSO-d_6): 10.27 (s, 1H, D_2O-exchange., COOH), 10.02 (s, 1H, , D_2O-exchange., -NHCOCH$_3$), 7.94-7.63 (m, 4H, Ar-H), 3.90 (d, 1H, $CH(CN)_2$, J= 12 Hz), 2.29 (m, 1H, CH_2CHCOOH), 2.08 (m, 2H, CH_2CHCOOH), 2.00 (s, 3H, CH_3). MS (m/z, %): 299 ($M^{\bullet+}$, 9), 282 (26), 258 (49), 229 (40), 162 (67), 120 (100), 84 (58).

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^{o}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 diluted with distilled water (2.5 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:

Reducing power (%) = $A_{control}$ - A_{sample} / $A_{control}$
Where, $A_{control}$ was the absorbance of solution without extract and A_{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 **12a,b** 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

[1] M. Bianchi, A. Butti, Y. Christidis, J. Perronnet, F. Barzaghi, R. Cesana, A. Nencioni, “Gastric Anti-Secretory, Anti-Ulcer and Cytoprotective Properties of Substituted(E)-4-Phenyl- and Heteroaryl-4-Oxo-2-Butenoic Acids”, Eur. J. Med. Chem. 23, pp. 45–52, 1988.
[2] A. Giordani, P. Pevarello, C. Speciale, M. Varasi, “4-Phenyl-4-oxo-2-butenoic acid derivatives with kynurenine-3-hydroxylase inhibiting activity”, US Pat. 6, 048, 896, 2000.
[3] I. L. Pinto, R.L. Jarvest, B. Clarke, C. E. Dabrowski, A. Fenwick, M. M. Gorczyca, L. J. Jennings, P. Lavery, E. J. Sternberg, D. G. Tew, A. West: “Inhibition of human cytomegalovirus protease by enedione derivatives of thieno[2,3-d]oxazinones through a novel dual acylation/alkylation mechanism”, Bioorg. Med. Chem. Lett., 9, pp. 449-452, 1999.
[4] C. Pfefferle, C. Kempter, J.W. Metzger and H.P. Fiedler: “(E)4-Oxonon-2-enoic acid, an antibiotically active fatty acid produced by Streptomyces olivaceus Tu 4018”, J. Antibiot., 49, pp. 826–828,1996.
[5] A. Teichert, T. Lubken, J. Schmidt, A. Porzel, N. Arnold, L. Wessjohann: “Unusual bioactive 4-oxo-2-alkenoic fatty acids from Hygrophorus eburneus”, Z. Naturforsch. A, 60, pp. 25–32, 2005.
[6] T. P. A. Devasagayam, J. C. Tilak,; K. K. Boloor, K. S. Sane, S. S. Ghaskadbi, R. D. Lele. J. Assoc. Phys. India., 52, pp.794–804, 2004.
[7] A. L. Miller. "Antioxidant flavonoids: structure, function and clinical usage" Alt. Med. Rev., 1, pp.103–111, 1996.
[8] F. Karata, A. Cansiz, H. Kara, M. Karatepe, M. Koparir "Synthesis of Two New Acetanilide Derivatives and Their Effect on the Serum Antioxidant Vitamins (A, E, and C) and the MDA Level in Rats", Russian Journal of Bioorganic Chemistry, 31(5), pp. 499–501, 2005.
[9] Ch. Suresh, J. V. Rao, K. N. Jayaveera, G. J. Reddy "Synthesis of 2-Hydrazino Benzothiazoles-2-Amino-(4-Substituted)-Acetanilides for Antioxidant Activity" International Journal of Pharmacy and Biological Sciences (IJPBS), 1(4), pp.409-413, 2011.
[10] S. Singh, P. K. Sharma, N. Kumar, R. Dudhe, "Furanone Derivatives: Diverse Biological Activities" An International Journal of Pharmaceutical Sciences" 2(3), pp. S-51- S-61, 2011.
[11] S. Rajasekaran, G. Rao, P. P. N. Sanjay, G. S. Sodhi "Synthesis, Antibacterial and in vitro Antioxidant Activity of 2,3-Substituted Quinazolin-4(3H)-ones" J. Chem. Pharm. Res., 2(1), pp.482-488, 2010
[12] M. S. Salem, M. A. E. M. El-Hashash, A. O. A. M., Hassan Mohamed Fawzy Madkour, "Facile synthesis of new fused and non-fused heterocyclic systems from a γ-ketoacid" European Journal of Chemistry, 5 (1), pp. 33-40, 2014.
[13] H. M. F. Madkour, K. A. Kandeel, M. S. Salem, S. M. Haroun "Antioxidant Activity of Novel Pyrimidines Derived from Arylidenes" International Journal of Pharmaceutical Science and Health Care 4(2), pp. 102-

115, 2014.
[14] D. Papa, E. Schwenk, F. Villani, E. Klingsberg, β-aroylacrylic acids J. Am. Chem. Soc., 70 (10), pp. 3356–3360, 1948.
[15] G. Laval, B. T. Golding "One-pot Sequence for the Decarboxylation of α-Amino Acids" Synlett, pp. 542-546, 2003.
[16] A. A. F. Wasfy, M. M. H. Arief, M. S. Amine, S. G. Donia, A. A. Aly " γ-Oxo Carboxylic Acids in Heterocyclic Synthesis, III. Synthesis of Biologically Active 4-Benzylamino-6-(5,5-dioxodibenzothiophen-2-yl)-2,3,4,5-tetrahydro-pyridazin-3-ones" Z. Naturforsch. 57b, pp. 668-676, 2002.
[17] N. F. Abd El-Ghaffar, M. Kh. Mohamed, M. S. Kadah, A. M. Radwan, G.H. Said, S. N. Abd el Al "Synthesis and anti-tumor activities of some new pyridazinones containing the 2-phenyl-1H-indolyl moiety" J. Chem. Pharm. Res., 3(3), pp. 248-259, 2011.
[18] I. C. F. R. Ferreira, M. Baptista, V. Boas, L. Barros, "Free radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal; Individual cap and stipe activity", Food Chemistry, 100, pp.1511-1516, 2007.
[19] H. V. Annegowda, C. W. Nee, M. N. Mordi, S. Ramanathan, S. M. Mansor, "Evaluation of phenolic content and antioxidant property of hydrolysed extracts of Terminalia catappa (L.) leaf." Asian Journal of Plant Sciences, 9, pp. 479-485, 2010.