March 2008 Volume 4 Issue 3

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 4(3), 2008 [227-234]

Synthesis and reactions of some new substituted thiazolidin-4-ones derivatives with antioxidant properties

Ahmed O.H.EL Nzhawy[1], Mostafa M.Ramla*[1], Nagy.M.Khalifa[2], Mohamed.M.Abdulla[3]

[1]Chemistry of Natural and Microbial Prod. Depart., NRC, Dokki, Cairo, (EGYPT)

[2]Medicinal Chemistry Department, NRC, Dokki, Cairo, (EGYPT)

[3]Research Units, Hi-Care Pharmaceutical Co., Cairo, (EGYPT)

E-mail: mostafa_ramla@yahoo.com

Received: 6th October, 2007 ; Accepted: 11th October, 2007

ABSTRACT

Several hetero substituted 2-(4-fluorophenyl) thiazolidin-4-one derivatives have been synthesized. A new series of substituted thiazolodinone derivatives were screened for their in vitro antioxidant activity. Compounds **(1-17)** exhibited the most active oxygen free-radical scavenger activity with percentage inhibitions of respectively. The detailed synthesis and antioxidant activity data are reported. Structures of the new compounds have been established by elemental analyses and spectral data.

KEYWORDS

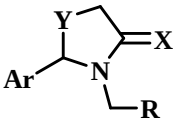
Thiazolidin-4-one;
Fluoro;
Phenyl;
Antioxidant activities.

INTRODUCTION

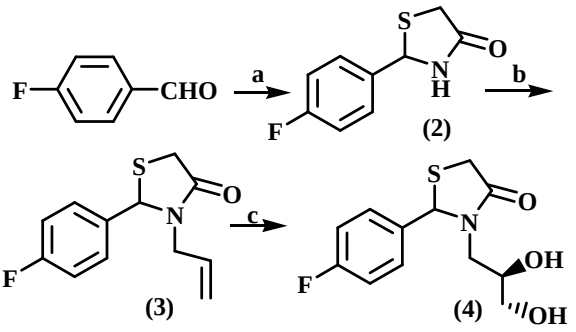
Antioxidants are of great interest because of their involvement in important biological and industrial processes. In general, compounds with antioxidant activity have been found to possess anticancer, anti-cardiovascular, anti-inflammation and many other activities[1-3]. Reactive oxygen species(ROS) and free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging[4–6]. ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radical, are thought to be generated subsequent to the reduction of molecular oxygen in aerobic organisms[7,8]. Under normal conditions, cells and tissues are protected against ROS by an array of enzyme defense systems, such as superoxide dismutase, catalase, and glutathione peroxidase, in addition to numerous non-enzymatic small molecules distributed widely in the biological system and capable of scavenging free radicals. These molecules include glutathione, α-tocopheral(vitamin E), vitamin C, β- carotene, and selenium[9]. In general, the cell is able to maintain an appropriate balance between oxidants and antioxidants under normal conditions. There has been considerable interest in the chemistry of thiazolidin-4-one ring systems, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity[10] such as antimycobacterial[11], anti-fungal[12], anti-cancer,[13] anti-tuberculosis[14], anti-convulsant[15], anti-edematous[16], Anti-diarrhea[17], anti-HIV[18,19], anti-platelet activating factor[20], antidiabetic[21], antihistaminic[22], cyclooxygenase inhibitors, lipoxygenase inhibitors[23], antiinflammatory, and analgesic[24] activities. Therefore, a general, simple, and efficient method for rapid synthesis of thiazolidine-4-ones would be greatly advantageous and warrants further investigations in drug dis-

Full Paper

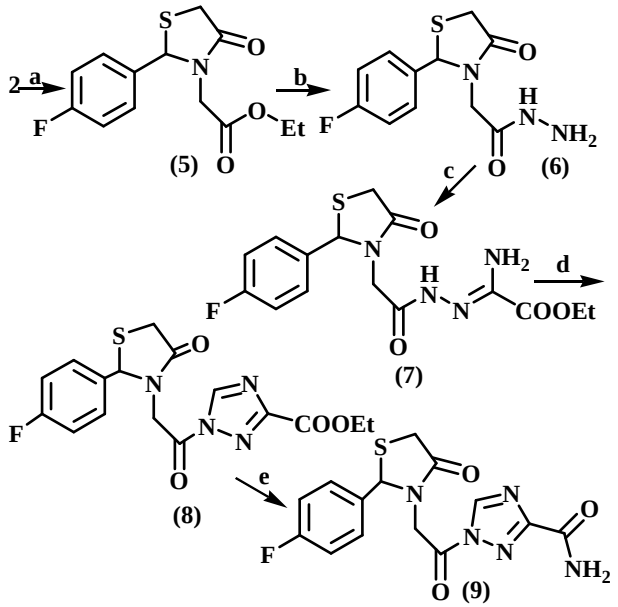
covery. Following reports of these activities, this study to synthesize a series of novel thiazolidin-4-one derivatives that contain heterocyclic ring also cyclic and acyclic sugar, as a part of our continuing work in the search of biologically active compounds with nitrogen and sulfur containing heterocyclic, we have synthesized substituted thiazoldin-4-one as a novel compound **(1)**.



I, X=O, NH, S ;Y=CH, S ; R=Aliphatic chain, heterocyclic ring



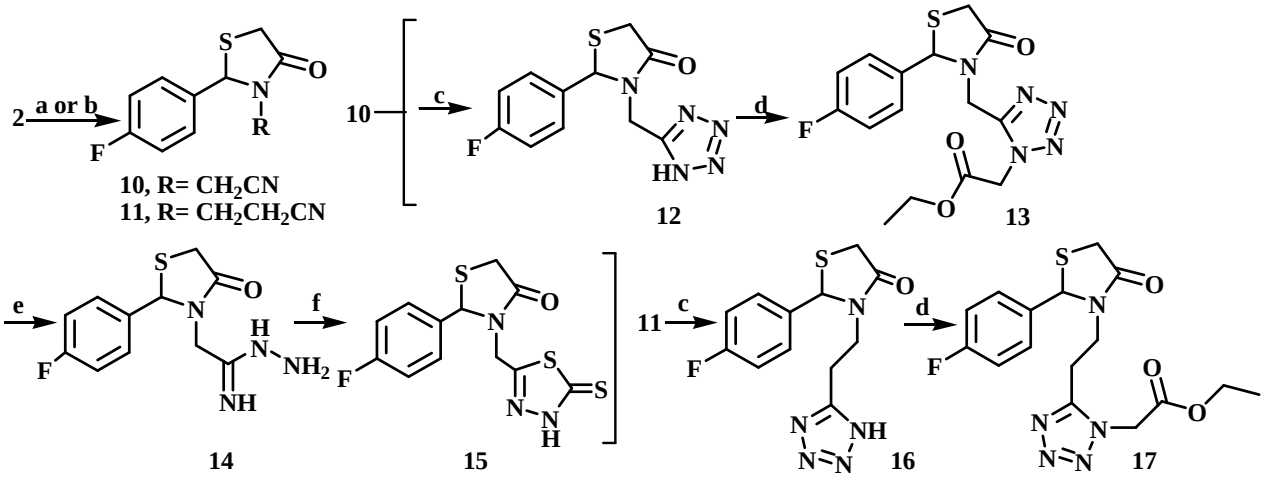
SCHEME 1: (a) $SHCH_2COOH$,$(NH_4)_2CO_3$, Toluene, reflux. (b) allyliodide, NaH, DMF, r.t. 3h. (c) $KMNO_4$, H_2O, r.t. 6h.



SCHEME 2 : (a) $BrCH_2COOC_2H_5$, NaH, DMF, r.t. 2h. (b) NH_2NH_2, ethanol, TEA, reflux, 6h. (c) ethylthiooxamate, toluene, reflux, 9h. (d) triethylorthoformate, toluene reflux, 6h. (e) ammonia in methanol, stirred overnight at room temperature

RESULTS AND DISCUSSION

The synthesis of the 2-(4-fluorophenyl) thiazolidin-4-one **(2)** was carried out, according to reported procedures[25], by reacting 4-fluorobenzaldehyde with thioglycollic acid and ammonium carbonate(molar ratio, 1:1.30:5.20). The cyclocondensation was carried out by refluxing the reaction mixture in dry toluene for 18 h with azeotropic removal of water. The structures of 2 have been confirmed by both analytical and spectral data(^{1}H NMR) Compound**(3)** namely, 3-Allyl-2-(4-fluorophenyl) thiazolidin-4-one was synthesized in an excellent yield by electrophilic substitution on 2-(4-fluorophenyl) thiazolidin-4-one **(2)** by allyl bromide on presence of dimethylformamide and sodium hydride. Treatment of **(3)** with potassium permanganate in water afforded the diol **(4)**. The structures of compounds **(3,4)** were confirmed on the basis of elemental analysis and spectral data. The ^{1}H NMR showed signal at δ 5.32m, 1H, NCH_2-CH= CH_2 which disappear after treatment of **(3)** with $KMnO_4$ (SCHEME 1). Electrophilic substitutions on **(2)** by ethyl bromoacetate in presence of anhydrous potassium carbonate in dry acetone afford**(5)**. Amination of **(5)** with hydrazine hydrate afforded 2-(2-(4-fluoro-phenyl)-4-oxothiazolidin-3-yl) acetohy drazide **(6)**. Reaction between **(6)** and ethylthio oxamate under reflux condition afford **(7)**. Treatment of **(7)** with trimethylortho formate afford ethyl 1-(2-(2-(4-fluoro phenyl)-4-oxothiazo-lidin-3-yl)acetyl)-1H-1, 2,4-triazole-3-carboxylate **(8)**. Hydrolysis of ester **(8)** with ammonia in methanol gives carboxamide **(9)**. Compound **(2)** being a secondary amine was cyanoethylated to **(11)** by acrylonitrile and NaH in dimethylformamide. To afford **(11)**, it was readily converted to 1,2,3, 4-tetrazole by treating with sodium azide and ammonium chloride in dimethylformamide affords **(16)**. Compound **(10)** was obtained by treatment of **(2)** with chloroacetonitrile in presences of base, which converted to 1,2,3,4-tetrazole by treating with sodium azide and ammonium chloride in dimethylformamide to give **(12)**. The secondary amino group at position 1 of tetrazoles **(16)** and **(12)** is free and hence electrophilic substitution with ethylbromoacetate in presence of triethylamine and ethanol afford **(13)** and **(17)**. Addition of hydrazine hydrate to cyano group of **(10)** afford **(14)**, which treated with carbon disulphide in methanol to give **(15)** (SCHEME 3). The structures of compounds**(10-**



SCHEME 3: (a) $ClCH_2CN$, acetone, TEA, reflux, 8h. (b) CH_2=CHCN, NaH, DMF, 80^{0}C, 6h. (c) NaN_3, NH_4Cl, DMF, 120^{0}C, 10h. (d) $BrCH_2COOC_2H_5$, ethanol, TEA, reflux, 5h. (e) NH_2NH_2, ethanol, TEA, reflux12h. (f) CS_2, KOH, methanol, reflux, 7h

17) were confirmed on the bases of elemental analysis and spectral data(see experimental section).

Determination of new heterocyclic compounds as superoxide radical scavenging

Superoxide radicals were generated by xanthine/ xanthine oxidase(XO) and measured by the nitroblue tetrazolium(NBT) reduction method. A test sample was mixed in a 100Mm phosphate buffer solution(pH 7.0) containing XO(1.65×10^{-2} unites/mL) and NBT (133μM) at 25^{0}C in 96-well flat- bottomed microassay plates. The measurement was started by adding xanthine(164μM). Production of superoxide radical was followed spectrophotometerically at 560nm at 25^{0}C for 10min. superoxide scavenging activity was calculated according to the following formula:

$$\text{Superroxide scavenging activity(\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbace}_{\text{control}}} \times 100$$

Where Absorbance $_{control}$ and Absorbance $_{sample}$ represent the increased absorbance in the absence and presence of samples, respectively[26].

Cytotoxicity in HT-29 cells

A-cell cultures

HT-29 cells were obtained from the Korean cell line Bank(KCLB, Seoul, Korea) and cultured in DMEM supplemented 10% FBS, 100U/ml penicillin and 100μg/ml streptomycin at 37^{0}C under 5% CO_2 atmosphere. Cells(10^6ml^{-1}) were cultured in 35 mm culture dishes and in 96-well plates(Becton Dickinson Lab ware), respectively. The final volumes of culture media were 2ml for the 35mm culture dish and 100μl for each well on the 96-well plate.

B- Neutral red assay

All experiments were performed on the cultured HT-29 cells. Briefly, cells(10^6ml^{-1}) were exposed to 100mU/ml Go in culture media containing 0.5% D-glucose without fetal bovme serum and then incubated in the presence of various concentrations of heterocyclic compounds of thiazoldinone derivatives. After 2 days of culture, a neutral red uptake assay was performed according to the method of Wadsworth and koop (1999). Cells(10^6ml^{-1}) were cultured in a 96-well plate for 12 and 24h. Following the various treatments, the medium was removed and the cells were incubated in 100μl of new medium containing 10μg/ml neutral red for 90min at 37^{0}C. After neutral red treatment, the medium was removed, and the wells were washed three times with 100μl PBS. One hundred micro liters of 50% ethanol containing 50mM sodium citrate(pH 4.2) was added into each well on well on the 96-well multiple plate. After 20min, the absorbance was measured at 510nm using a spectra count TM(Packard Instrument Co., Downers Grove, USA) ELISA reader.

RESULTS

All the newly synthesized tested compounds

TABLE 1: Cytotoxic effect on HT-29 at a conc. 50, 100, 150ug/ml after time in hours

Comp. no.	Cytotoxic effect on HT-29at a conc. 50ug/ml after time in hours			Cytotoxic effect on HT-29 at a conc. 100ug/ml after time in hours			Cytotoxic effect on HT-29 at a conc. 150ug/ml after time in hours	
	12h	24h	48h	12h	24h	48h	12h	24h
2	28	32	33	30	52	55	34	73
3	15	16	19	18	42	52	26	58
4	27	31	40	29	41	52	40	52
5	24	27	39	31	43	57	42	47
6	20	31	35	27	51	66	35	67
7	19	27	32	29	53	66	37	62
8	21	25	31	31	43	57	42	57
9	23	33	42	32	62	77	37	74
10	22	40	44	31	65	74	43	72
11	17	19	22	23	46	62	37	69
12	22	24	30	24	53	62	27	62
13	16	14	22	20	36	55	42	61
14	16	20	35	26	42	47	32	50
15	30	32	40	37	42	56	47	55
16	16	22	27	22	52	68	35	67
17	21	32	25	35	42	53	40	50

TABLE 2 : The % scavenging effect of compounds on super oxide at a dose 50ug/ml

Comp. no.	The % scavenging effect of compounds on super oxide at a dose 50ug/ml	Relative antioxidant potencyto vitaminC
2	2.12	0.09
3	62.57	2.82
4	7.88	0.35
5	3.81	0.71
6	39.88	1.81
7	25.95	1.17
8	20.65	0.93
9	31.3	1.415
10	13.16	0.599
11	31.96	1.452
12	33.33	1.515
13	26.92	1.22
14	37.98	1.726
15	7.79	0.35
16	32.2	1.463
17	17.99	0.819

showed potent cytotoxic activities against HT-29 cell line. The cytotoxic activities increases as the dose increase as we regard that 150μg/ml doses induced more cell death rate than that induced by 100mg/ml and the later induced more cell death rate than that induced with a dose of 50μg/ml. Also the cytotoxic activities increases by increase the time of contact between cell line and cytotoxic agents at any fixed dose level so the cell death fate after 48 hours is greater than that after 24 hours and the cell death rate after 24hours is higher than after 12 hours. The high cell death killing activities after 12hours at small starting dos(50μg/ml) indicated potent cytotoxic activities. The most potent compounds are **(10,9,2,11,12,6,17,7,13,14,3,8,4,15)** and **(5)** they arranged in descending order of activities.

SAR of the cytotoxic activities

- The presence of extra alicyclic systems appended to the thiazolidone moiety greatly increase cytotoxicity activities, this probably due to its comparability of DNA interchelating property.
- As the number of nitrogen atoms attached to the N-allyl moiety increases the cytotoxicity activity decreases.
- Heterocyclic ring systems appended to the thiazolidone markedly reduces cytotoxic activity

The antioxidant activities of the newly synthesized compounds were recorded via measuring the scavenging effects of the synthesized compounds on the super oxide that produced by HX/XO system. We measure the scavenging activities of the tested compounds at a dose level of 50ug/ml {which is the C-50 of vitamin C}. The relative potency to Vitamin C was determined.

It was found that all compounds showed antioxidant via inhibition of NBT reduction in various degrees and compounds **(3,6,15,13,17,12,9,14)** and **(7)** are more potent than vitamin C and are arranged in descending order . It is worth to mention that compound 3 nearly three and two times active as vitamin C. The remaining compounds have less activities and are arranged in the following descending order, 8, 10,5, 11,{16,4}and 2

SAR of the antioxidant activities

- Thiazolidin-4-one is essential for antioxidant activities
- N-allyl attached to thiazolidin-4-one moiety sharply increases antioxidant activities
- As the number of nitrogen atoms attached to the N-allyl moiety increases the antioxidant activity decreases
- Further appending to the N-allyl greatly reduces the antioxidant activities

EXPERIMENTAL

Melting points are uncorrected and were taken on

an open glass capillaries Melting point apparatus. Analytical data were obtained from the microanalytical unit, Cairo University, Egypt. IR spectra(KBr discs) were recorded on a Perkin Elmer 1430 spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were determined on Joel 270MHz in DMSO-d_6 and the chemical shifts were recorded in ppm relative to TMS. The mass spectra were run 70ev with afinnigan SSQ GC/ MS spectrometer using EI technique for ionization. All reactions were followed by TLC(silica gel, aluminum sheets 60 F_{254}, Merck) and Merck Silica gel(0.040-0.063mm) was used for column chromatography.

2-(4-Fluorophenyl) thaizolidin-4-one(2)

A mixture of 4-fluorobenzaldehyde(12.4g, 1mmol), thioglycollic acid(11.96g, 1.3 mmol) and ammonium carbonate(49.92g, 5.2 mmol) in dry toluene(150ml) were refluxed for 18h with stirring and collecting the generated water in azeotropic collector. The solution was then cooled, evaporated under reduced pressure and the oily residue was recrystallized from pet. Ether(40-60^0)-acetone to give **(2)**(Yield 86%), m.p. (141-142^0) as white solid.^{1}H-NMR(DMSO-d_6) δ 3.64-3.73(AB system, J=15.4Hz, 2H, 5CH_2), 5.82(s, 1H, 2CH), 7.20-7.40(m, 4H, aromatic protons), 9.00(s, 1H, NH). MS: m/z(%): 199[M^{+2}](16.6), 197 (100), 150(62), 124(75). Anal.Calcd. For C_9H_8FNOS: C, 54.81; H, 4.09; F, 9.63; N, 7.10; S, 16.26. Found: C, 54.71; H, 4.12; F, 9.73; N, 7.19; S, 16.40.

3-Allyl-2-(4-Fluorophenyl) thaizolidin -4-one(3)

To a solution of **(2)**(1.97g, 10mmol) in DMF(20ml) was added NaH(0.48g, 20 mmol) and the mixture was stirred for 30min., and then allyl iodide(1.68g, 10mmol) was added. The reaction mixture was stirred at room temperature for 12h. The mixture was then poured into water and the obtained oil residue was recrystallized from pet. Ether(40-60^0)-acetone to give **3**(Yield 75 %), m.p. (186-188^0)as white solid.^{1}H-NMR(DMSO-d_6) δ2.80(dd, J=5.8, 9.3Hz, 1H, NCH), 3.40-3.53(AB system, J=15.2Hz, 2H, 5CH_2), 4.10(dd, J=5.8, 9.3Hz, 1H, NCH) 4.82(m, 2H, NCH_2CH=CH_2) 5.25(s, 1H, 2CH), 5.32(m, 1H, NCH_2CH=CH_2) 6.70-6.90(m, 4H, aromatic protons). MS: m/z(%): 237[M^+](80), 191(75), 162(100), 139(70), 109(84). Anal. Calcd. for $C_{12}H_{12}$ FNOS: C,60.74; H,5.10; F,8.01; N,5.90; S, 13.51. Found: C, 54.59; H, 4.23; F, 9.71; N, 7.40; S, 16.24.

2-(4-Fluorophenyl)-3-(2,3-dihydroxypropyl) thiazolidin-4-one(4)

To a solution of **(3)**(9.85g, 5.12mmol) in 50ml H_2O, stirred for 6h. at room temperature,(18.2g, 10.25mmol) of $KMnO_4$ was added. The solution was extracted with diethyl ether. The ether extracts were collected, dried and evaporated. The residue was loaded onto a column of silica gel and eluted with(8:2) pet. ether–ethyl acetate to give **(4)(**Yield 78%), as oily products.^{1}H-NMR(DMSO-d_6) δ3.75-3.96(AB system, J=15.5Hz, 2H, 5CH_2), 5.67(s, 1H, 2CH), 6.70-6.90(m, 4H, aromatic protons). MS: m/z(%): 271[M^+](91), 237(23) 196(85), 180(100). Anal.Calcd. for $C_{12}H_{14}FNO_3S$: C, 53.12; H, 5.20; F, 7.00; N, 5.16; S, 11.82 Found: C, 54.59; H, 5.23; F, 7.71; N, 5.40; S, 11.24.

Ethyl 2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) acetate(5)

A mixture of compound **(2)**(11.6 g, 0.1 mol), anhydrous potassium carbonate(13.8 g, 0.1 mol) and ethyl bromoacetate(18 ml, 0.1 mol) in dry acetone(150 ml) was refluxed for 15h. The reaction mixture was filtered, washed with acetone(50 ml). The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethanol to give **(5)**(Yield 91 %), m.p. 47º as orange solid.^{1}H-NMR(DMSO-d_6) ? 1.32(t, 3H, CH_3), 3.37(s, 2H, NCH_2C=O), 3.53-3.72(AB system, J=15.2 Hz, 2H, 5CH_2), 3.94(q, 2H, CH_2), 5.90(s, 1H, 2CH), 7.20-7.43(m, 4H, aromatic protons). MS: m/z(%): 283[M^+](16), 238(19), 196(100), 136(23), 109(38). Anal. Calcd. For $C_{13}H_{14}FNO_3S$: C, 55.11; H, 4.98; F, 6.71; N, 4.94; S, 11.32. Found: C, 55.29; H, 5.03; F, 6.80; N, 4.92; S, 11.25.

2-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl) acetohydrazide(6)

Compound **(5)**(8.1g, 10mmol), hydrazine hydrate (20mmol) and triethylamine(5 ml) were refluxed in absolute ethanol(50 ml) for 7h. The reaction mixture was cooled, white precipitate was formed after filtration the solid was recrystallized from ethanol to give **6**(Yield 94 %), m.p.(110-112^0) as white solid. ^{1}HNMR(DMSO-d_6) δ3.10(br s, 2H, NH_2), 3.41(s, 1H, CH_2C=O), 3.57-3.79(AB system, J=15.1 Hz, 2H, 5CH_2), 5.91(s, 1H, 2CH), 7.20-7.42(m, 4H, aromatic protons),

9.00(s, 1H, NH). MS: m/z(%): 269.1[M^+](8), 238(20), 210(18), 196(100), 109(43). Anal.Calcd. for $C_{11}H_{12}FN_3O_2S$: C, 49.06; H, 4.49; F, 7.05; N, 15.60; S, 11.91.Found: C, 49.19; H, 4.30; F, 6.80; N, 15.45; S, 11.75.

Ethyl-2-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)acetoylimino)-2-amino- acetate(7)

A mixture of **(6)**(3.5g, 13mmol), ethylthiooxamate (1.73g, 13mmol) in toluene(30 ml) was refluxed for 3 h. The excess solvent was removed under reduced pressure and solid product obtained was recrystallized from ethanol to give **(7)**(Yield 74%), m.p. 207º as white powder. ^{1}H-NMR(250 MHz, DMSO-d_6) 1.19(t, 3H, CH_3), 2.91(br. S, 2H, NH_2), 3.34(s, 1H, CH_2C=O), 3.73-3.92(AB system, J=15.4 Hz, 2H, $5CH_2$), 4.15(q, 2H, CH_2), 5.93(s, 1H, 2CH), 7.22-7.47(m, 4H, aromatic protons), 9.85(s, 1H, NH). ^{13}C NMR(DMSO-d_6); δ13.9, 31.7, 43.4, 58.0, 61.6, 115.8, 129.6, 135.5, 140.2, 161.3, 163.2, 167.7, 171.5. MS: m/z(%): 368.1 [M^+](8), 351(3), 305(8), 252(10), 208(43), 173(100), 109(96). Anal. Calcd. for $C_{15}H_{17}FN_4O_4S$: C,48.91; H, 4.65; F, 5.16; N, 15.21; S, 8.70. Found: C,48.71; H, 4.62; F, 5.36; N, 15.11; S, 8.79

Ethyl-1-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)acetyl)-1H-1,2,4-triazole-3-carboxylate(8)

A mixture of **(7)**(2.5g, 6.8 mmol), trimethylorthofor mate(2.0g, 20 mmol) in toluene(20 ml) was refluxed for 5h. The excess solvent was removed under reduced pressure. The obtained residue was chromatographed on silica gel with pet. Ether-AcOEt(2:1) to give **(8)**(Yield 63%). m.p. (180-183^0) as white powder. ^{1}H-NMR (DMSO-d_6) δ1.17(t, 3H, CH_3), 3.30(s, 1H, CH_2 C=O), 3.75-3.96(AB system, J=15.5 Hz, 2H, $5CH_2$), 4.20(q, 2H, CH_2), 5.84(s, 1H, 2CH), 7.32-7.57(m, 4H, aromatic protons), 8.85(s, 1H, CH). ^{13}C NMR (DMSO-d_6); δ14.2, 35.4, 45.2, 59.1, 61.2, 115.5, 130.4, 134.8, 157.4, 161.2, 161.8, 171.5, 175. MS: m/z(%): 378.1[M^+](12), 333(43), 305(38), 238(15), 210(43), 173(10), 109(100). Anal. Calcd. For $C_{16}H_{15}FN_4O_4S$: C, 50.79; H, 4.00; F, 5.02; N, 14.81; S, 8.47. Found: C, 50.77; H, 4.10; F, 5.22; N, 14.89; S, 8.37.

1-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)acetyl)-1H-1,2,4-triazole-3-carboxamide(9).

To a solution of **(8)**(2.0g, 5.27mmol) in methanol (15ml) was added(20ml) ammonia in methanol and the mixture was stirred overnight at room temperature and concentrated under reduced pressure. The obtained residue was chromatographed on silica gel with pet. Ether-AcOEt(1:1) to give **(9)**(Yield 83%). m.p. (168-170^0) as white powder. ^{1}H-NMR(DMSO-d_6) δ3.75-3.96(AB system, J=15.5Hz, 2H, $5CH_2$), 4.12(s, 1H, CH_2C=O), 5.84(s, 1H, 2CH), 6.20(br. S, 2H, NH_2), 7.22-7.43(m, 4H, aromatic protons), 8.92(s, 1H, CH). ^{13}C NMR(DMSO-d_6); δ33.6, 43.6, 57.8, 115.7, 131.6, 136.3, 158.5, 161.3, 164.3, 169.5, 171.4, 178.3.MS: m/z(%): 349.1[M^+](18), 333(23), 304(58), 238(35), 109(100). Anal. Calcd. For $C_{14}H_{12}FN_5O_3S$: C, 48.13; H, 3.46; F, 5.44; N, 20.05; S, 9.18. Found: C, 48.33; H, 3.43; F, 5.34; N, 20.25; S, 9.10.

2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) acetonitrile(10)

To a solution of compound **(2)**(10g, 56.5mmol) in (75ml) acetone, chloroacetonitrile(4.25g, 56.6mmol) was added dropwise and the reaction mixture was refluxed for 5h., The reaction mixture was cooled, a colorless crystals was formed after filtration the solid was recrystallized from ethanol to give **(10)**(Yield 85%). m.p. as a pale yellow powder. ^{1}H-NMR(, DMSO-d_6) δ3.75-3.96(AB system, J=15.5Hz, 2H, $5CH_2$), 4.33(s, 2H, CH_2CN), 5.90(s, 1H, 2CH), 7.20-7.43(m, 4H, aromatic protons). MS: m/z(%): 236[M^+](56), 196(100), 141(33), 109(30). Anal. Calcd. For $C_{11}H_9FN_2OS$: C, 55.92; H, 3.84; F, 8.04; N, 11.86; S, 13.57. Found: C, 55.09; H, 3.03; F, 7.80; N, 11.92; S, 13.25.

3-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)propanenitrile(11)

To a solution of **(2)**(1.00g, 10mmol) in DMF(20ml) was added NaH(0.00g, 20mmol) and the mixture was stirred for 30min., and then acrylonitrile(0.00g, 10mmol) was added. The reaction mixture was stirred at 80^{0}C for 5h. The mixture was then poured into water and the obtained oil residue was recrystallized from pet. Ether(40-60^0)-acetone to give **(11)**(Yield 90%), as oily. ^{1}H-NMR(DMSO-d_6) δ3.75-3.96(AB system, J=15.5 Hz, 2H, $5CH_2$), 5.84(s, 1H, 2CH), 7.32-7.57(m, 4H, aromatic protons). MS: m/z(%): 250[M^+](100), 236

(12), 196(100), 137(27), 109(55). Anal.Calcd. For $C_{12}H_{11}FN_2OS$: C, 57.58; H, 4.43; F, 7.59; N, 11.19; S, 12.81. Found: C, 58.09; H, 4.03; F, 7.60 ; N, 10.98; S, 12.70.

3-((1H-tetrazol-5-yl)methyl)-2-(4-fluorophenyl) thiazolidin-4-one(12).

A mixture of compound **(10)**(4g, mmol), sodium azide(1.2g, mmol) and ammonium chloride(0.98g, mmol) in dimethylformamide(10ml) was refluxed for 7h at 125^{0}C. The solvent was removed under reduced pressure; the residue was dissolved in(100 ml) water and carefully acidified with conc. Hydrochloric acid to pH 2 The solution was cooled to 5^{0}C in ice bath. Recrystallized from aqueous methanol to give **(12)(**Yield 75%). m.p. as a white powder. ^{1}H-NMR(DMSO-d_6) δ3.75-3.96(AB system, J=15.5 Hz, 2H, $5CH_2$), 4.33(s, 2H, CH_2), 5.90(s, 1H, 2CH), 7.20-7.43(m, 4H, aromatic protons). MS: m/z(%): 279[M^+](100), 265(19), 251(68), 236(16), 196(100), 141(9), 109(44). Anal. Calcd. For $C_{11}H_{10}FN_5OS$: C, 47.30; H, 3.61; F, 6.80; N, 25.08;S, 11.48. Found: C, 45.99; H, 4.03; F, 6.80; N, 24.92; S, 11.25.

Ethyl-2-(5-((2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)methyl)-1H-tetrazol-1yl)acetate(13).

To a solution of compound **(12)**(g, 6.4mmol) in absolute ethanol(50ml),(0.8ml, 6.4mmol) ethyl bromo acetate and a few drops of triethylamine were added. The reaction mixture was refluxed on a water bath for 5h. he reaction mixture was filtered, washed with acetone(50 ml). The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethanol to give **(13)**(Yield 91%), m.p. (47^0) as orange solid.^{1}H-NMR(DMSO-d_6) δ1.32(t, 3H, CH_3), 3.53-3.72(AB system, J=15.2Hz, 2H, $5CH_2$), 3.91(q, 2H, CH_2), 4.57(s, 2H, NCH_2C=O), 5.94(s, 1H, 2CH), 7.20-7.43(m, 4H, aromatic protons). MS: m/z(%): 365 [M^+](76), 336(29), 292(9), 265(100), 196(88). Anal.Calcd. For $C_{15}H_{16}FN_5O_3S$: C, 49.31; H, 4.41; F, 5.20; N, 19.17; S, 8.78. Found: C, 50.29; H, 4.39; F, 5.30; N, 18.92; S, 8.75.

2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl) ethaneiminohydrazide(14)

Compound **(10)**(3.1g, 10mmol), hydrazine hydrate (20mmol)(99%) and triethylamine(5ml) were refluxed in absolute ethanol(50ml) for 12 h. The reaction mixture was cooled, white precipitate was formed after filtration the solid was recrystallized from ethanol to give **(14)** (Yield 74 %), m.p. (161-165^0) as yellow solid. ^{1}H-NMR(DMSO-d_6) δ2.90(br s, 2H, NH_2), 3.21(s, 1H, CH_2C=NH), 3.31-3.49(AB system, J=15.1Hz, 2H, $5CH_2$), 5.80(s, 1H, 2CH), 7.31-7.44(m, 4H, aromatic protons), 9.00(s, 1H, NH). MS: m/z(%): 268.08[M^+](28), 237(12), 210(33), 196(80), 140(13). Anal. Calcd. for $C_{11}H_{13}FN_4OS$: C, 49.24; H, 4.88; F, 7.08; N, 20.88; S, 11.95. Found: C, 49.39; H, 4.70; F, 6.88; N, 20.45; S, 11.75.

2-(4-fluorophenyl)-3-((4,5-dihydro-5-thioxo-1,3,4-thiadiazol-2-yl)methyl) thiazolidin-4-one(15)

To a solution of compound **(14)**(2g, mmol) in absolute methanol(50ml),(5ml, mmol) of carbon disulfide were added. The reaction mixture was refluxed on a water bath for 5 h. the solvent was removed under reduced pressure, The precipitate was crystallized from methanol to give **(15)**(Yield 91%), oil orange. ^{1}H-NMR (DMSO-d_6) δ3.70-3.92(AB system, J=15.5Hz, 2H, $5CH_2$), 4.43(s, 2H, CH_2), 5.87(s, 1H, 2CH), 7.19-7.39(m, 4H, aromatic protons). MS: m/z(%): 327[M^+](10), 297(59), 256(48), 224(76), 196(100), 141(9), 109(44). Anal. Calcd. For $C_{12}H_{10}FN_3OS_3$: C, 44.02; H, 3.08; F, 5.80; N, 12.83; S, 29.38. Found: C, 44.99; H, 3.03; F, 5.70; N, 12.92; S, 29.25.

3-(2-(1H-tetrazol-5-yl)ethyl)-2-(4-fluorophenyl) thiazolidin-4-one(16)

A mixture of compound **(11)**(4g, mmol), sodium azide(1.2g, mmol) and ammonium chloride(0.98g, mmol) in dimethylformamide(10ml) was refluxed for 7 h at 125^{0}C. The solvent was removed under reduced pressure; the residue was dissolved in(100 ml) water and carefully acidified with conc. hydrochloric acid to pH 2 The solution was cooled to 5^{0}C in ice bath. Recrystallized from aqueous methanol to give **(16)**(Yield 75%). m.p. as a red crystals . ^{1}H-NMR(DMSO-d_6) δ3.75-3.96(AB system, J=15.5Hz, 2H, $5CH_2$), 4.33(s, 2H, CH_2), 5.90(s, 1H, 2CH), 7.20-7.43(m, 4H, aromatic protons MS: m/z(%): 293.07[M^+](65), 279(25), 251(20), 238(22), 196(100), 141(9), 109(14). Anal. Calcd. For $C_{12}H_{12}FN_5OS$: C, 49.14; H, 4.12; F, 6.48; N, 23.88; S,10.93. Found: C, 49.99; H, 4.10; F, 6.50;

Full Paper

N, 23.92; S, 10.85.

Ethyl-2-(5-(2-(2-(4-fluorophenyl)-4-oxothiazolidin-3-yl)ethyl)-1H-tetrazol-1-yl)acetate(17)

To a solution of compound **(16)**(3g, mmol) in absolute ethanol(50ml),(5ml, mmol) ethyl bromoacetate and a few drops of triethylamine were added. The reaction mixture was refluxed on a water bath for 5 h. he reaction mixture was filtered, washed with acetone(50ml). The filtrate was evaporated under reduced pressure and the residue was recrystallized from ethanol to give **(17)**(Yield 73%), as oil orange solid.^{1}H-NMR(DMSO-d_6) δ1.22(t, 3H, CH_3), 3.53-3.72(AB system, J=15.2 Hz, 2H, $5CH_2$), 3.91(q, 2H, CH_2), 4.57(s, 2H, NCH_2 C=O), 5.94(s, 1H, 2CH), 7.20-7.43(m, 4H, aromatic protons). MS: m/z(%): 379.1[M^+](36), 350(79), 306 (13), 293(45), 279(15), 251(50), 238 (86), 196(100), 141(11), 109(44).. Anal. Calcd. For $C_{16}H_{18}FN_5O_3S$: C, 50.65; H, 4.78; F, 5.01; N, 18.46; S, 8.45. Found: C, 50.09; H, 4.79; F, 5.10; N, 18.52; S, 8.45.

REFERENCES

[1] C.A.Rice-Evans, A.T.Diplock; Free Radic.Biol. Med., **15**, 77 **(1993)**.
[2] E.Cadenas, L.Packer; 'Handbook of Antioxidants', Marcel Dekker, Inc., New York, **(1996)**.
[3] C.A.Rice-Evans, L.Packer; 'Flavonoids in Health and Disease', Marcel Dekker, Inc., New York, **(1998)**.
[4] H.Kappus; Arch.Toxicol., **60**, 144 **(1987)**.
[5] C.G.Cochrane; Am.J.Med., **91**, 23S **(1991)**.
[6] J.M.C.Gutteridge; Free Radic.Res.Commun., **19**, 141 **(1993)**.
[7] J.M.N.Mccord; Engl.J.Med., **312**, 159 **(1985)**.
[8] R.A.Clark, K.G.Leidal, D.W.Pearson, W.M. Nauseef; J.Biol.Med., **262**, 4065 **(1987)**.
[9] R.A.Jacob, B.J.J.Burri; Clin.Nutr., **63**, 985S **(1996)**.
[10] B.C.C.Cantello, M.A.Cawthorne, G.P.Cottam, P.T. Du, D.Haigh, R.M.Kindley, C.A.Lister, S.A.Smith, P.L.J.Thurlby; Med.Chem., **37**, 3977 **(1994)**.
[11] S.G.Kucukguzel, E.E.Oruc, S.Rollas, F.Sahin, A.Ozbek; Eur.J.Med.Chem., **37**, 197 **(2002)**.
[12] G.Capan, N.Ulusoy, N.Ergenc, M.Kiraz; Monatshefte Fur.Chemie., **130**, 1399 **(1999)**.
[13] J.J.Bhatt, R.R.Shah, H.P.Shah, P.B.Trivedi, N.K. Undavia, N.C.Desai; Indian J.Chem., **33B**, 189 **(1994)**.
[14] A.R.Bhat, S.J.Shetty; Indian.Pharm.Sci., 194 **(1987)**.
[15] F.A.Ragab, N.M.Eid, H.A.El-Tawab; Pharmazie, **52**, 926 **(1997)**.
[16] J.G.De Lima, M.Perrissin, J.Chantegrel, C.Luu-Duc, A.Rousseau, G.Narcisse; Arzneim. Forsch. Drug.Res., **44**, 831 **(1994)**.
[17] Andreani, M.Rambaldi, A.Locatelli, A.Leoni, R. Bossa, M.Chiericozzi, I.Galatulas, G.Salvatore; Eur. J.Med.Chem., **28**, 825 **(1993)**.
[18] M.L.Barreca, A.Chimirri, L.De Luca, A.M. Monforte, P.Monforte, A.Rao, M.Zappala, J. Balzarini, E.De Clercq, C.Pannecouque, M. Witvrouw; Bioorg.Med.Chem.Lett., **11**, 1793 **(2001)**.
[19] A.Rao, A.Chimirri, E.De Clercq, A.M.Monforte, P. Monforte, C.Pannecouque, M.Zappala; Farmaco., **57**, 747 **(2002)**.
[20] Y.Taanabe, H.Yamamoto, M.Murakami, K.Yanagi, Y.Kubota, H.Okumura, Y.Sanemitsu, G.Suzukamo; J.Chem.Soc.Perkin.Trans., **1**, 935 **(1995)**.
[21] B.C.C.Cantello, M.A.Cawthorne, G.P.Cottam, P.T. Duff, D.Haigh, R.M.Hindley, C.A.Lister, S.A. Smith, P.L.Thurlby; J.Med.Chem., **37**, 3977 **(1994)**.
[22] M.V.Diurno, O.Mazzoni, G.Correale, I.G.Monterrrey, A.Calignano, G.La Rana, A.Bolognese; Il Farmaco, **54**, 579 **(1999)**.
[23] D.H.Boschelli, D.T.Connor, P.J.Kuipers, C.D. Wright; Bioorg.Med.Chem.Lett., **2**, 705 **(1992)**.
[24] M.G.Vigorita, R.Ottana, F.Monforte, R.Maccari, A. Trovato, M.T.Monforte, M.F.Taviano; Bioorg.Med. Chem.Lett., **11**, 2791 **(2001)**.
[25] R.S.Alexander, A.C.Royal; J.Am.Chem.Soc., **76**, 578-580 **(1954)**.
[26] C.Joo Eun, K.Motiochi, K.U.Young-Jin, K.Shiro; Biomacromolecules, **5**, 113-118 **(2004)**.