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Synthesis and Biological Evaluation of Thiazolidine-2, 4-Dione Based Quinazolinone Derivatives for Antimicrobial and Antioxidant Activities

Swapna. D[1*] and Rajitha. G[2]

[1]Department of Pharmaceutical Chemistry, MNR College of Pharmacy, Fasalwadi, Sangareddy-502294, Telangana, India
[2]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati-517502, Andhra Pradesh, India

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Corresponding Author Email: swapna.dvnn@gmail.com

Abstract

A series of thiazolidine-2,4-dione based quinazolinone derivatives (**5a–5o)** were synthesized by reacting quinazolin-4(3H)-one in sodium hydroxide solution and 5-(substituted benzylidene)-3-(2-chloroacetyl) thiazolidine-2,4-dione. The chemical structure of synthesized compounds was established by IR, ^{1}H NMR and mass spectroscopy and screened for antibacterial and antioxidant activities. The antibacterial evaluation of synthesized compounds was carried out by cup plate method using streptomycin and griseofulvin as standard drugs. The compounds **5d, 5l and 5o** showed good activity against both gram positive and gram-negative bacteria with zone of inhibition ranging from 20-26 mm and the compounds **5b, 5i and 5o** showed good antifungal activity against *Aspergillus niger*, *Penicillium notatum* with zone of inhibition ranging from 20-23 mm. The antioxidant activity was performed by DPPH (2,2-diphenyl-1-picryl-hydrazyl), hydrogen peroxide and nitricoxide scavenging activity methods using ascorbic acid as standard drug. The compounds **5b, 5l and 5o** exhibited good antioxidant activity by DPPH, hydrogen peroxide methods and the compounds **5j and 5l** exhibited good antioxidant activity by nitricoxide method.

Keywords

Thiazolidine-2,4-dione, Quinazolinone, Antimicrobial activity and Antioxidant activity.

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INTRODUCTION

The development of antimicrobial agents is an important and challenging issue because acquisition of resistance by the microorganisms to present drugs available in market for treatment of infectious diseases [1,2]. Thiazolidine-2,4-diones (TZDs; also called as "glitazones") were one of the important class of heterocyclic compounds, show a wide range of biological applications such as antihyperglycemic [3], aldose reductase inhibition [4], anti-inflammatory [5], antitubercular [6], antimicrobial [7], anticancer [8] antiviral and anti-hyperlipidemic [9] etc. 4(3H)-quinazolinone fused with various heterocycles found to show wide range of pharmacological effects such as anti-inflammatory [10,11], anticonvulsant [12], antibacterial, antifungal [13], anti-viral [14], anti-HIV [15] and anticancer [16] activities etc.

In view of the above mentioned reported biological activities, we have planned to design and synthesise compounds containing both thiazolidine-2,4-dione and quinazolinone with antimicrobial and antioxidant activities.

MATERIALS AND METHODS

By using open capillary tubes melting points were determined and are uncorrected. Purity of the all synthesized compounds was checked by using precoated TLC plates and iodine was used as visualizing agent. IR spectra were recorded on FT-IR 8400S, Fourier Transform (Shimadzu) Infrared spectrophotometer using KBr pellet method. ^{1}H NMR spectra were recorded on Bruker AVANCE (400 MHz) spectrophotometer in dimethyl sulfoxide (DMSO) using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Shimadzu LCMS-8030 Mass Spectrometer. Elemental analyses (C, H, and N) were performed using Perkin Elmer model 240 C analyzer.

Experimental Procedure

The schematic representation of synthesized compounds (**5a–5o**) were summarized in Figure: 1. The physical data of synthesized compounds (**5a-5o**) were presented in Table: 1.

Table 1 - Physical data of synthesized compounds 5a – 5o

Compounds	Molecular Formula	R_1	R_2	R_3	R	Molecular Weight (gms)	m.p (^{0}C)	% Yield
5a	$C_{21}H_{15}N_3O_4S$	H	H	H	CH_3	405	230-232	74
5b	$C_{21}H_{14}N_3O_4SCl$	H	Cl	H	CH_3	439	243-245	69
5c	$C_{21}H_{14}N_4O_6S$	H	NO_2	H	CH_3	450	261-263	78
5d	$C_{23}H_{20}N_4O_4S$	H	$N(CH_3)_2$	H	CH_3	448	225-227	75
5e	$C_{24}H_{21}N_3O_7S$	OCH_3	OCH_3	OCH_3	CH_3	495	253-255	76
5f	$C_{21}H_{14}N_4O_6S$	NO_2	H	H	CH_3	450	237-239	75
5g	$C_{22}H_{17}N_3O_5S$	H	OCH_3	H	CH_3	435	229-231	68
5h	$C_{23}H_{19}N_3O_6S$	OCH_3	OCH_3	H	CH_3	465	217-219	73
5i	$C_2H_{17}N_2O_4SBr$	H	Br	H	CH_3	484	225-227	67
5j	$C_{21}H_{15}N_3O_5S$	H	OH	H	CH_3	421	207-209	74
5k	$C_{20}H_{13}N_3O_4S$	H	H	H	H	391	227-229	78
5l	$C_{22}H_{18}N_4O_4S$	H	$N(CH_3)_2$	H	H	434	233-235	75
5m	$C_{20}H_{12}N_4O_6S$	H	NO_2	H	H	436	250-252	64
5n	$C_{20}H_{12}N_3O_4SCl$	H	Cl	H	H	425	261-263	78
5o	$C_{22}H_{17}N_3O_6S$	OCH_3	OCH_3	H	H	451	201-203	68

m.p: Melting point; %Yield: Percentage yield;

Synthesis of Thiazolidine-2,4-dione (1)

Chloroacetic acid (0.6 mol) in a 60 ml of water and of thiourea (0.6mol) dissolved in 60 ml of water and transferred into a 250 ml round bottomed flask. The reaction mixture was kept for 15 minutes to form a white precipitate with stirring. To content of the flask, added slowly 60 ml concentrated hydrochloric acid. The reaction mixture was stirred and refluxed for 8-10 hrs at 100-110^{o}C. On cooling the content of the flask solidified to mass of cluster of white needles. The product was filtered and washed with water to remove traces of HCl and dried. It was recrystallized from ethanol [17]. Yield: 73%; m.p: 124 – 126^{o}C.

Synthesis of 5-benzylidene thiazolidine-2,4-dione (2a)

Into a 250 ml round bottomed flask benzaldehyde (0.188 mol) and 2,4-thiazolidinedione (0.188 mol) were transferred and added dry toluene and 1 ml of piperidine. The reaction mixture was refluxed for 1 hr with stirring. On cooling, the product was precipitated. The compound was filtered and washed with cold, dry toluene and dry ethanol [18]. Yield: 68%; m.p: 236 – 238^{o}C.

Similarly compounds **2b–2o** were prepared by adopting similar procedure using appropriate substituted aldehydes.

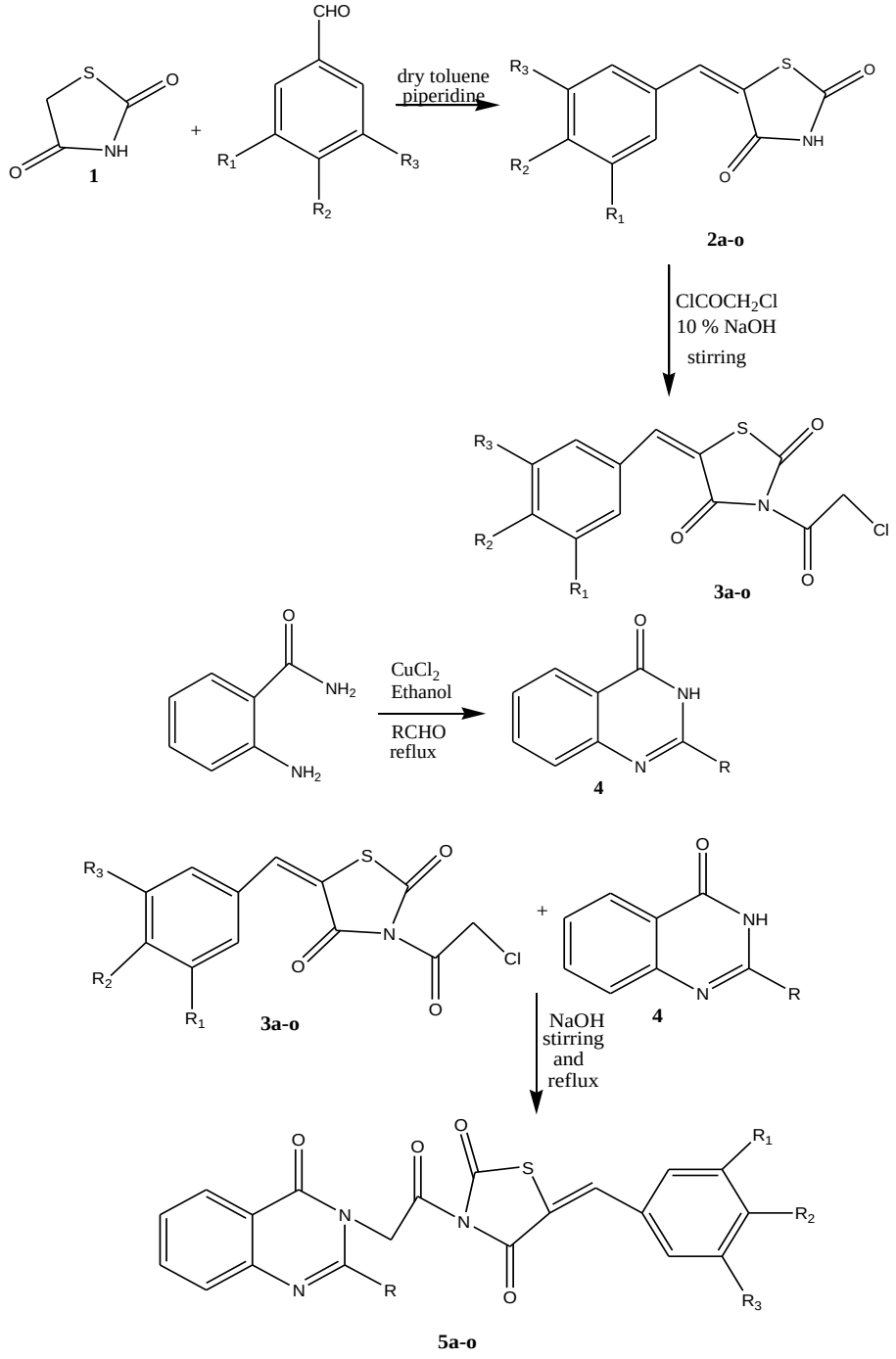
Synthesis of 5-benzylidene-3-(2-chloroacetyl) thiazolidine-2,4-dione (3a)

5-benzylidene-thiazolidine-2,4-dione (0.01 mol) dissolved in 50 ml of 10% sodium hydroxide solution and transferred into a conical flask. To this added

chloroacetyl chloride (0.01 mol) dropwise and the reaction mixture was stirred for 3hrs. The product obtained was washed with water, separated by filtration and dried. It was recrystallized from absolute ethanol [19].

Yield :76%; m.p: 191-193°C.

Similarly compounds **3b–3o** were prepared by adopting similar procedure using appropriate 5-(substituted benzylidene) thiazolidine-2,4-diones.



R = H, CH_3; R_1 = H, OCH_3, NO_2; R_2 = H, Br,Cl, NO_2, $N(CH_3)_2$, OCH_3, OH; R_3 = H, OCH_3

Figure 1: Schematic representation of synthesis of compounds 5a-5o

Synthesis of 2-methyl quinazolin-4(3H)-one (4a)

13.6 g of anthranilamide (0.1 mol), acetaldehyde (1.1 mmol), 20 ml of ethanol and copper chloride (0.05 mmol) were transferred into a 250 ml round bottomed flask. The reaction mixture was refluxed for 1.5 hrs, then cooled and poured into ice cold water. The product obtained was separated by filtration and dried. It was recrystallized from absolute ethanol [20]. Yield: 82%; m.p: 161-163°C.

Similarly compounds **4b – 4o** were prepared by adopting similar procedure using appropriate aldehydes**.**

Synthesis of 5-benzylidene-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl) acetyl) thiazolidine-2,4-dione(5a)

2-methyl-quinazolin-4(3H)-one (0.01 mol) dissolved in 50 ml of 10% sodium hydroxide solution was transferred into a conical flask. To this added 5-benzylidene-3-(2-chloroacetyl) thiazolidine-2,4-dione (0.01 mol) dropwise with stirring. The reaction mixture was stirred for 3hrs and refluxed for 1 hr to complete the reaction. The product obtained was washed with water, separated by filtration and dried. It was recrystallized from absolute ethanol [19].

Similarly compounds **5b – 5o** were prepared by adopting similar procedure using appropriate 5-(substituted benzylidene) thiazolidine-2,4-diones.

5-benzylidene-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione(5a): Yield: 74%, m.p: 230 -232°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3095 (Ar C-H), 1681 (C=O), 1345 (C-N), 728 (C-S) .^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.28 (s, 3H, CH_3) , 4.17 (s, 2H, CH_2) , 7.38–7.68 (m, 9H, Ar-H), 8.59 (s, 1H, C_6H_5CH=C). EI-MS m/z: 406 (M+1). Anal.Calcd. For $C_{21}H_{15}N_3O_4S$: C, 62.12; H, 3.73; N, 10.36; Found: C, 62.16; H, 3.74; N, 10.34.

5-(4-chlorobenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione (5b): Yield: 69%, m.p: 243-245°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3018 (Ar C-H), 1679 (C=O), 1348 (C-N), 651 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.34 (s, 3H, CH_3), 4.29 (s, 2H, CH_2) , 7.54–8.05 (m, 8H, Ar-H), 8.28 (s, 1H, C_6H_5CH=C). EI-MS m/z: 440 (M+1). Anal.Calcd. For $C_{21}H_{14}N_3O_4SCl$: C, 57.34; H, 3.21; N, 9.55; Found: C, 57.30; H, 3.18; N, 9.58.

5-(4-nitrobenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione

(5c): Yield: 78%, m.p: 261-263 °C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3082 (Ar C-H), 1716 (C=O), 1343 (C-N), 629 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.16 (s, 3H, CH_3) ,4.29 (s, 2H, CH_2) , 7.52–7.74 (m, 8H, Ar-H), 8.21(s, 1H, C_6H_5CH=C). EI-MS m/z: 451 (M+1). Anal.Calcd. For $C_{21}H_{14}N_4O_6S$: C, 56; H, 3.13; N, 12.44; Found: C, 44; H, 3.16; N, 12.42.

5-(4-dimetylaminobenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl) acetyl) thiazolidine-2,4-dione (5d): Yield: 75%, m.p: 225-227°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3092 (Ar C-H), 1676 (C=O), 1335 (C-N), 692 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.08 (s, 3H, CH_3), 2.38 (s, 6H, $N(CH_3)_2$, 4.28 (s, 2H, CH_2), 7.26–7.40 (m, 8H, Ar-H), 8.23 (s, 1H, C_6H_5CH=C). EI-MS m/z: 449 (M+1). Anal.Calcd. For $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49; Found: C, 61.56; H, 4.18; N, 12.47.

5-(3,4,5-trimethoxybenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl) acetyl) thiazolidine-2,4-dione (5e): Yield: 80%, m.p: 253-255°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3100 (Ar C-H), 1705 (C=O), 1347 (C-N), 677 (C-S) .^{1}H NMR (400 MHz, DMSO – d_6) δ: 1.71 (s, 3H, CH3),3.51-3.58 (s, 9H, (OCH3)3), 4.36 (s, 2H, CH2) , 7.16–8.06 (m, 6H, Ar-H), 8.47 (s, 1H, C6H5CH=C).EI-MS m/z: 436 (M+1). Anal.Calcd. For $C_{24}H_{21}N_3O_7S$: C, 58.17; H, 4.27; N, 8.48; Found: C, 58.14; H, 4.25; N, 8.46.

5-(3-nitrobenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl) acetyl) thiazolidine-2,4-dione (5f): Yield: 75%, m.p: 237-239°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3095 (Ar C-H), 1681 (C=O),1345 (C-N), 728 (C-S).^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.06 (s, 1H, CH_3), 4.18 (s, 2H, CH_2) , 7.24–7.90 (m, 8H, Ar-H), 8.13(s, 1H, C_6H_5CH=C). EI-MS m/z: 451 (M+1). Anal.Calcd. For $C_{21}H_{14}N_4O_6S$: C, 56; H, 3.13; N, 12.44; Found: C, 56.13; H, 3.16; N, 12.42.

5-(4-methoxybenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione (5g): Yield: 68%, m.p: 229-231°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3017.43 (Ar C-H), 1656 (C=O), 1383 (C-N), 647 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.07 (s, 1H, CH3),3.34 (s, 3H, OCH3), 4.36 (s, 2H, CH2) , 7.14–7.94 (m, 8H, Ar), 8.47 (s, 1H, C6H5CH=C). EI-MS m/z: 436 (M+1). Anal.Calcd. For $C_{22}H_{17}N_3O_5S$: C, 60.98; H, 3.93; N, 9.65; Found: C, 60.94; H, 3.96; N, 9.68.

5-(3,4-dimethoxybenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione (5h): Yield: 73%, m.p: 217-219°C. IR (KBr, $\nu_{max, Cm^{-1}}$) : 3098 (Ar C-H), 1605 (C=O), 1343 (C-N), 633 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 1.98 (s, 1H, CH3), 3.17-3.19 (s, 6H, (OCH3)2), 4.75 (s, 2H, CH2). 7.14–8.47 (m, 7H, Ar-H), 9.78 (s, 1H, C6H5CH=C). EI-MS m/z: 466 (M+1). Anal.Calcd. For $C_{23}H_{19}N_3O_6S$: C, 59.35; H, 4.11; N, 9.03; Found: C, 59.34; H, 4.13; N, 9.08.

5-(4-bromobenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione (5i): Yield: 80%, m.p: 225-227°C..IR (KBr, $\nu_{max, Cm^{-1}}$): 3071 (Ar C-H), 1607 (C=O), 1309 (C-N), 641 (C-

S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.19 (s, 1H, CH3),4.07 (s, 2H, CH2) , 7.63–8.13 (m, 8H, Ar-H), 8.62 (s, 1H, C6H5CH=C).EI-MS m/z: 484 (M+1). Anal.Calcd. For $C_{21}H_{17}N_2O_4SBr$: C, 53.29; H, 3.62; N, 5.92; Found: C, 53.24; H, 3.60.; N, 5.94.

5-(4-hydroxybenzylidene)-3-(2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione (5j): Yield: 74%, m.p: 207-209°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3098 (Ar C-H), 1732 (C=O), 1383 (C-N), 646 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 2.09 (s, 1H, CH_3), 4.14 (s, 2H, CH_2) , 7.28–7.94 (m, 8H, Ar-H), 8.16(s,1 H, C_6H_5CH=C) 10.34 (s, 1H, -OH). EI-MS m/z: 422 (M+1). Anal.Calcd. For $C_{21}H_{15}N_3O_5S$: C, 59.35; H, 3.54; N, 9.87; Found: C, 59.32; H, 3.56; N, 9.84.

5-benzylidene-3-(2-(4-oxoquinazolin-3(4H)-yl) acetyl) thiazolidine-2,4-dione (5k): Yield: 78%, m.p: 227-229°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3020 (Ar C-H), 1728 (C=O), 1374 (C-N), 676.89 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ:4.16 (s, 2H, CH_2) , 7.14–7.82 (m, 10H, Ar-H), 8.16 (s, 1H, C_6H_5CH=C). EI-MS m/z: 392 (M+1). Anal.Calcd. For $C_{20}H_{13}N_3O_4S$: C, 61.37; H, 3.35; N, 10.74; Found: C, 61.36; H, 3.34; N, 10.71.

5-(4-(dimethylamino)benzylidene)-3-(2-(4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione (5l): Yield: 75%, m.p: 233-235°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3019 (Ar C-H), 1728 (C=O),1335 (C-N), 664 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ:2.36 (s, 6H, $N(CH_3)_2$), 4.17 (s, 2H, CH_2) , 7.14–7.89 (m, 9H, Ar-H), 8.24 (s, 1H, C_6H_5CH=C). EI-MS m/z: 435 (M+1). Anal.Calcd. For $C_{21}H_{18}N_4O_4S$: C, 60.82; H, 4.18; N, 12.9; Found: C, 60.80; H, 4.16; N, 12.6.

5-(4-nitrobenzylidene)-3-(2-(4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione(5m): Yield:83%, m.p: 250-252°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3014 (Ar C-H),1682 (C=O), 1343 (C-N), 684 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 4.28 (s,2 H, CH_2) , 7.28–7.94 (m, 9H, Ar-H), 8.11 (s,1H, C_6H_5CH=C). EI-MS m/z: 437 (M+1). Anal.Calcd. For $C_{20}H_{12}N_4O_6S$: C, 55.04; H, 2.77; N, 12.84; Found: C, 55.02; H, 2.76; N, 12.86.

5-(4-chlorobenzylidene)-3-(2-(4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione(5n): Yield: 78%, m.p: 261-263°C. IR (KBr, $\nu_{max, Cm^{-1}}$): 3022 (Ar C-H), 1732(C=O), 1367 (C-N), 644 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 4.16 (s, 2H, CH_2) ,7.14–7.89 (m, 9H, Ar-H), 8.23 (s, 1H, C_6H_5CH=C). EI-MS m/z: 426 (M+1). Anal.Calcd. For $C_{20}H_{12}N_3O_4SCl$: C, 56.41; H, 2.84; N, 9.87; Found: C, 56.39; H, 2.86; N, 9.88.

5-(3,4-dimethoxybenzylidene)-3-(2-(4-oxoquinazolin-3(4H)-yl)acetyl)thiazolidine-2,4-dione(5o): Yield: 68%, m.p: 201-203°C. IR (KBr, $\nu_{max, Cm^{-1}}$) :3032 (Ar C-H), 1746 (C=O), 1338 (C-N), 644 (C-S). ^{1}H NMR (400 MHz, DMSO – d_6) δ: 3.82-3.94 (s, 6H, $(OCH_3)_2$), 4.16 (s, 2H, CH_2) , 7.22–7.93 (m, 8H, Ar-H), 8.14 (s, 1H, C_6H_5CH=C). EI-MS m/z: 452 (M+1). Anal.Calcd. For $C_{22}H_{17}N_3O_6S$: C, 58.53; H, 3.8; N, 9.31; Found: C, 58.50; H, 3.6; N, 9.28.

***Invitro* Screening for Antimicrobial and Antioxidant Activities**

Antimicrobial activity

Cup plate method was used for determination of antimicrobial activity by measuring zone of inhibition. All the compounds were screened for antimicrobial activity against gram negative *Salmonella paratyphi, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis,* gram positive *Bacillus subtilis, Streptococcus pyogene bacteria* and fungi *Aspergillus niger*, *Penicillium notatum* and *Colletotrichum coffeanum* at a concentration of 50 µg/ml. Streptomycin and Griseofulvin were used as standard drugs at a concentration of 50 µg/ml. Muller Hinton agar media for bacterial strain and Sabouraud dextrose agar (SDA) for fungal strain was used as culture medium & dimethylsulfoxide (DMSO) was used as solvent control [21, 22].

Bores were made on the medium using sterile borer. A volume of 0.1 ml of test solution was added to respective bores. Streptomycin at a concentration of 50 µg/ml was taken as standard. A control having only DMSO in the cup was maintained in each plate. The petriplates were kept in the refrigerator at 4°C for 15 min for diffusion to take place. Afterwards they were incubated at 37°C for 24 hrs and zone of inhibition was measured. Similarly, the antifungal activity of synthesized compounds was determined by cup plate method using Sabouraud dextrose broth and the petriplates were incubated at 37°C for 48 hrs and the zone of inhibition was measured [23, 24, 25]. The results of antimicrobial activity of synthesized compounds are presented in the Table. No.2.

Table 2 – Antibacterial activity of compounds 5a-5o against different bacteria and fungi at 50 µg/ml

S.NO	Compounds	ZONE OF INHIBITION (mm)								
		Gram negative				Gram positive		Fungal		
		SP	PA	EC	PM	BS	S.pyogenes	AN	PN	CC
1	5a	17	15	14	10	11	10	9	18	11
2	5b	11	16	17	11	16	9	20	23	12
3	5c	18	9	15	NA	12	9	9	NA	10
4	5d	24	22	26	21	23	10	18	14	13
5	5e	13	10	14	NA	9	NA	10	15	NA
6	5f	11	NA	10	11	10	NA	NA	17	NA
7	5g	19	16	17	12	15	12	9	16	13
8	5h	18	12	15	NA	13	10	NA	11	10
9	5i	21	19	20	19	18	13	21	22	16
10	5j	11	17	10	NA	9	NA	17	9	10
11	5k	17	18	15	19	19	10	NA	15	12
12	5l	26	23	24	20	22	11	19	20	14
13	5m	10	14	16	10	12	NA	11	17	15
14	5n	10	NA	9	NA	11	NA	NA	11	10
15	5o	23	21	22	20	21	12	20	21	15
16	Streptomycin	30	32	32	33	28	30	NT	NT	NT
17	Griseofulvin	NT	NT	NT	NT	NT	NT	25	30	35

SP: *Salmonella paraytphi*; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*;PM: Proteus mirabilis; BS: *Bacillus subtilis*; S.pyogenes: *Streptococcus pyogenes* ; AN: *Aspergillus niger*; PN: *Penicillium notatum* ; CC: *Colletotrichum coffeanum*.

ANTIOXIDANT ACTIVITY

The antioxidant activity of all the synthesized compounds were evaluated by *in-vitro* free radical scavenging activity using DPPH (2, 2-diphenyl-1-picryl hydrazyl), hydrogen peroxide and nitric oxide assay methods [26,27, 28].

Table 3 – Antioxidant activity data of compounds 5a-5o

Compounds	% Inhibition at 100µM		
	DPPH	H_2O_2	NO
5a	57±0.5	41±3.5	56±0.6
5b	34±2.2	17±2.4	36±1.4
5c	40±2.8	25±2.8	44±2.6
5d	61±1.5	44±2.5	70±0.5
5e	33±0.5	19±1.5	32±0.8
5f	46±3.5	30±0.5	49±1.6
5g	54±2.7	49±2.5	58±1.8
5h	43±1.5	28±1.6	46±0.9
5i	50±2.5	39±0.8	54±2.7
5j	53±0.5	56±1.8	64±0.6
5k	52±1.5	34±2.2	59±1.8
5l	63±2.1	54±2.5	72±2.4
5m	38±2.5	22±3.5	42±2.8
5n	49±3.8	32±3.2	52±2.8
5o	56±1.5	47±1.5	68±3.2
Ascorbic acid	66±0.5	58±1.2	74±1.4

DPPH: Diphenyl picryl hydrazine; H_2O_2: Hydrogen peroxide; NO: Nitric oxide methods; Results are presented as Mean±SEM in Triplicate.

DPPH method

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical with purple colour. Antioxidant reduces DPPH to 2, 2-diphenyl-1-picrylhydrazine, a colourless compound which is measured at an absorbance of 517 nm using ethanol as blank. 2 ml of standard drug, ascorbic acid and title compounds (100 µM) were added to 2 ml of 100 µM alcoholic DPPH solution. The tubes were kept at an ambient temperature for 20-30 min protected from light by covering with aluminum foil. All the tests were carried out in triplicate.

The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of test}}{\text{absorbance of control}} \times 100 \rightarrow (1)$$

Hydrogen peroxide method: A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (PBS) (pH 7.4). The test compounds (100µM) in 3.4 ml phosphate buffer were added to H_2O_2 solution (0.6 mL, 40 mM). After 10 min, the absorbance was measured at 230 nm. The percentage inhibition was calculated by using equation (1).

Nitric oxide method

1 ml of sodium nitroprusside (10 mM) dissolved in 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to the test compounds at 100 µM concentration and incubated for 150 min at 25˚C and 1 ml of the reaction mixture was treated with 1 ml of Griess reagent. The absorbance was measured at 546 nm. The percentage inhibition was calculated by using equation (1).

RESULTS & DISCUSSION

Chemistry

2,4-TZD (**1**) was synthesized with chloroacetic acid and thiourea in hot water. 5-substituted-thiazolidine-2,4-dione (**2**) was obtained with Knoevenagel condensation of 2,4-TZD (**1**) and aromatic aldehyde in Toluene in presence of piperidine. 5-(substituted benzylidene)-3-(2-chloroacetyl) thiazolidine-2,4-dione **(3)** was prepared by acetylation of **(2)** with chloroacetyl chloride in sodium hydroxide solution. Quinazolinones **(4)** were obtained by reaction of anthranilamide, aliphatic aldehyde and copper chloride in ethanol. A series of 5-(substituted benzylidene)-3-(2-(2-substituted-4-oxoquinazolin-3(4H)-yl) acetyl) thiazolidine-2,4-dione **(5a-5o)** were synthesized by condensation of 5-(substituted benzylidene)-3-(2-chloroacetyl) thiazolidine-2,4-dione **(3)** and Quinazolinone **(4)** in sodium hydroxide solution.

The structure of the title compounds was elucidated by ^{1}H NMR, Mass and IR spectroscopy. All spectral data were in accordance with the assumed structures. IR spectra of the compounds **(5a-5o)** showed C-S stretching at 728-629 cm−1, C-N stretching at 1383-1309 cm−1 and C = O stretching bonds at 1746-1605 cm−1 respectively. ^{1}H NMR of the compounds showed methylene protons at 4.16-4.38 ppm, aromatic protons at 7.14-7.96 ppm, benzylidene protons at 7.84-8.32 ppm. The molecular ion peaks (M+1) was observed at 406, 439, 451, 448 and 436 for the compounds **5a**, **5b**, **5c**, **5d** and **5e** respectively.

Antimicrobial activity

The Results of antimicrobial evaluation (Table.No:2) revealed that, the compounds **5d,5l** and **5o** with dimethyl amino and dimethoxy derivatives showed good antibacterial activity against gram positive *Bacillus subtilis* gram negative *Salmonella paratyphi, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis* bacteria with zone of inhibition 20-26 mm. The compounds **5a-5o** also screened for antifungal activity against different fungal strains. The compounds **5b, 5i** and **5o** with 4-chloro, 4-bromo and 3,4-dimethoxy derivatives showed good antifungal activity against fungi *Aspergillus. niger* and *Penicillium. notatum* with zone of inhibition ranging from 20-23 mm.

Antioxidant activity

Free radical scavenging activity by DPPH assay method

All the compounds **(5a-5o)** were screened by DPPH scavenging assy method. The compounds **5d (61%)**, **5l (63%)** showed good scavenging effect, indicating that electron donating groups enhanced the antioxidant activity.

Hydrogen peroxide scavenging assay method

All the compounds **(5a-5o)** were screened by hydrogen peroxide scavenging assay method. The compounds **5d (70%)** and **5l (72%)** showed good scavenging effect.

Nitric oxide scavenging assay method

All the compounds **(5a-5o)** were screened by nitric oxide scavenging assay method. The compounds **5j (56%)** and **5l (54%)** showed good antioxidant activity. These results revealed that, the compounds with electron donating groups exhibited good activity when compared with respective electron withdrawing substituted compounds. On the basis of

structure activity relationship, the presence of electron donating group on benzylidene ring essential for antioxidant activity.

CONCLUSION

The thiazolidine-2,4-dione based quinazolinone derivatives **(5a–5o)** were synthsised by using conventional procedures. The compounds **5d, 5l** and **5o** showed good activity against gram positive *Bacillus subtilis* and gram-negative *Salmonella paratyphi, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis* bacteria and fungi *A. niger* and *P. notatum*. The compounds **5d** exhibited good antioxidant activity by DPPH, hydrogen peroxide and nitric oxide scavenging methods. These compounds could be selected as lead molecules for further synthetic and biological evaluation.

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