Open Access Journal of Chemistry
Volume 1, Issue 1, 2017, PP 39-47

Synthesis and Antimicrobial Activities Evaluation of Some New Thiadiazinone and Thiadiazepinone Derivatives Bearing Sulfonamide Moiety

Hany M. M. Dalloul,[a]* Khaled A. El-Nwairy,[b] Ali Z. Shorafa,b Ahmed S. Abu Samaha[c]

[a]*Department of Chemistry, Faculty of Applied Science, Alaqsa University of Gaza, P.O.Box 4051, Gaza 76888, PNA.*
[b] *Ministry of Education & Higher Education, Beirut street, Southern remal, Gaza, PNA.*
[c] *Department of Biology, Faculty of Applied Science, Alaqsa University of Gaza, P.O.Box 4051, Gaza 76888, PNA.*

***Corresponding author:** Hany M. M. Dalloul, Department of Chemistry, Faculty of Applied Science, Alaqsa University of Gaza, P.O.Box 4051, Gaza 76888, PNA.*

ABSTRACT

A new series of novel functionalized 1,3,4-thiadiazin-5-ones and 1,3,4-thiadiazepin-5-ones bearing sulfonamide moieties by 1,3-dipolar cyclocondensation reaction of nitrilimines with α-mercaptoesters and mercaptosuccinic acid respectively. The structures of the newly prepared compounds were elucidated by spectral methods (IR,^{1}H-NMR,^{13}C-NMR and MS spectroscopy) and elemental analysis. The newly synthesized compounds were screened for their in vitro antimicrobial activity. Some of titled compounds exhibited significant antimicrobial activity on several strains of microbes.

Keywords: *Nitrilimines, Sulfa drugs, α-Mercaptoesters, 1,3,4-Thiadiazinone, 1,3,4-Thiadiazepinone.*

INTRODUCTION

Sulfonamides represent an important class of medicinally effective molecules and are known to possess various types of biological activities such as antibacterial [1-3], antiviral [4-7], anti-carbonic an hydrase [8,9], high-ceiling diuretic [10], hypoglycemic [11,12], anti thyroid [11], anti-inflammatory [13], and anti glaucoma [10,11]. It is also known that aromatic or hetero aromatic sulfonamides may act as antitumor agents through perturbation of cell cycle in the G1 phase, distribution of microtubule assembly or angiogenesis inhibition [14-17]. Moreover, numerous sulfonamides were found to act as antitumor agents through carbonic an hydrase (CA) inhibition [17-26].It is well known that the nitrogen and sulfur containing heterocyclic compounds play important role in medicinal chemistry and pharmaceutical communities as these molecules have potent biological activities [27]. Among them, 1,3,4-thiadiazines,a therapeutically important class of heterocyclic compounds. They are known to exhibit various pharmacological and medicinal applications [27-29]. 1,3,4-Thiadiazinone derivatives have attracted a great deal of interest due to a variety of interesting biological activities. They are known as spasmolytic [30] and antibacterial agents [31], important matrix metalloproteinase inhibitors [32,33], and they also display cardio tonic, hypertensive [34,35], and other biological activities [36-38]. Thiadiazepines are reported for their potent antimicrobial activity [39], antifungal activity [40] and inhibition of metalloproteinase [41]. The literature survey revealed that some fused thiadiazepines exhibit antidepressant [42], central nervous depressant [43], bactericidal [44,45], fungicidal [44,45], and anticancer activity [46]. Recently, 1,4,5-dibenzo[b,f] thiadiazepine was reported to show good neuro protective properties against neurodegenerative diseases without anti cholinergic effects [47].

Taking into account all previous commentaries of the biological activities of sulfonamides and in continuation of our study on the synthesis of biologically active hetero cycles[48,49], efforts have been made to synthesize a series of new 1,3,4-thiadiazin-5-one and 1,3,4-thiadiazepin-5-one derivatives incorporating sulfonamide moiety via cyclocondensation reaction of nitrilimines containing moiety of sulfonamide

with α-mercaptoesters and mercaptosuccinic acid in anticipation of expected interesting biological activities.

Experimental Section

Instruments and Reagents

Melting points were determined using an electro thermal melting temperature apparatus and are uncorrected. The IR spectra were measured as KBr pellets using a Satellite 3000 Mid infrared spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM 300 MHz spectrometer at r. t. in DMSO-d_6 solution u sing tetramethylsilane (TMS) as internal reference. Chemical shifts are expressed in δ (ppm) downfield from TMS and coupling constants are in Hertz (Hz). Electron impact (EI) mass spectra were run on a Shimadzu GCMS-QP1000 EX spectrometer at 70 eV. Elemental analysis were carried out at micro analytical laboratory, Cairo University, Cairo, Egypt. Ethyl mercapto acetate, mercaptosuccinic acid, triethylamine (TEA), tetrahydrofuran (THF), di cyclo hexylcarbodiimide (DCC) and 1,4-dioxane were purchased from Avocado Research Chemicals, England, and used without further purification. Hydrazonoyl chlorides **1a-c** employed in this study, were prepared via direct coupling of the appropriate sulfa drug diazonium chloride with α-chloroacetoacetanilide in sodium acetate/ ethanol solution following standard procedures [50].

General Procedure For Synthesis of 3, 5,6-Thiadiaza-4-Hexenoates 3a-c.

To a stirred solution of the appropriate hydrazonoyl halide **1** (10 mmol) and ethyl mercaptoacetate (15 mmol) in tetrahydrofuran (THF) (50 mL), triethylamine (5 mmol) in THF (10 mL) was drop wise added at r. t. Stirring was continued for 72 hr, then the solvent was removed under vacuum, and the residual solid was washed with water (100 mL). The solid products were collected and recrystallized from an appropriate solvent to afford the desired compounds. The following compounds were prepared by this method.

Ethyl 4-phenylaminocarbonyl-6-[4-(thiazol-2-yl-sulfamoyl)phenyl]-3,5,6-thiadiaza-4-hexenoate (3a). M.p. 213-215 ◦C (ethanol). Yield 76 %. IR: ν = 3365, 3346, 3270 (NH), 1718 (ester C=O), 1650 (amide C=O), 1597 (C=N), 1225 (C–S), 1150 (S=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 12.70 (s, 1H, SO_2NH), 10.65 (s, 1H, NH), 9.96 (s, 1H, PhNH), 8.74 (d, 1H,*J* = 9.2 Hz, thiazole), 7.93-7.04 (m, 9H, Ar-H), 6.64 (d, 1H,*J* = 4.5 Hz, thiazole), 4.25-4.12 (q, 2H, OCH_2), 3.95 (s, 2H, SCH_2), 1.28-1.15 (t, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.1 (ester C=O), 159.4 (amide C=O), 141.7 (C=N), 167.9-119.3 (Ar-C and thiazole-C), 61.3 (OCH_2), 33.2 (SCH_2), 13.9 (CH_3) ppm. MS: *m/z* = 519 [M$^{+\cdot}$]. Anal. Calcd. for $C_{21}H_{21}N_5O_5S_3$ (519.62): C, 48.54; H, 4.07; N, 13.48; Found C 48.31; H, 3.98; N, 13.60.

Ethyl 4-phenylaminocarbonyl-6-[4-(pyrimidin-2-yl-sulfamoyl)phenyl]-3,5,6-thiadiaza-4-hexenoate (3b). M.p. 236-238 ◦C (ethanol). Yield 75%. IR: ν =3358, 3348, 3270 (NH), 1715 (ester C=O), 1655 (amide C=O), 1595 (C=N), 1230 (C–S), 1139 (S=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 12.65 (s, 1H, SO_2NH), 10.64 (s, 1H, N-NH), 9.93 (s, 1H, PhNH), 8.84 (d, 2H, *J* = 8.8 Hz, pyrimidine ring), 8.02-7.07 (m, 9H, Ar-H), 6.86 (t, 1H, *J* = 7.5 Hz, pyrimidine ring), 4.23-4.12 (q, 2H, OCH_2), 3.96 (s, 2H, SCH_2), 1.27-1.16 (t, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.8 (ester C=O), 159.9 (amide C=O), 141.2 (C=N), 168.2-113.8 (Ar-C and pyrimidine-C), 61.8 (OCH_2), 34.2 (SCH_2), 14.1 (CH_3) ppm. MS: *m/z* = 514 [M$^{+\cdot}$]. Anal. Calcd. for $C_{22}H_{22}N_6O_5S_2$ (514.59): C, 51.35; H, 4.31; N, 16.33; Found C, 51.58; H, 4.45; N, 16.22.

Ethyl 4 - phenylaminocarbonyl - 6- [4- (5-methyloxazol – 3 – yl - sulfamoyl) phenyl]-3,5,6-thiadi-aza-4-hexenoate (3c.) M.p. 218-220 ◦C (ethanol). Yield 73 %. IR: ν = 3372, 3343, 3270 (N–H), 1712 (ester C=O), 1650 (amide C=O), 1598 (C=N), 1226 (C–S), 1138 (S=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 12.62 (s, 1H, SO_2NH), 10.46 (s, 1H, N-NH), 9.95 (s, 1H, PhNH), 7.88-7.06 (m, 9H, Ar-H), 6.24 (s, 1H, oxazole ring), 4.13-4.01 (q, 2H, OCH_2), 3.89 (s, 2H, SCH_2), 2.38 (s, 3H, CH_3 on oxazole ring), 1.25-1.13 (t, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 170.4 (ester C=O), 159.4 (amide C=O), 141.5 (C=N), 167.4-115.4 (Ar-C and oxazole-C), 61.3 (OCH_2), 33.5 (SCH_2), 13.8 (CH_3), 12.40 (CH_3 oxazole) ppm. MS: *m/z* = 517 [M$^{+\cdot}$]. Anal. Calcd. for $C_{22}H_{23}N_5O_6S_2$ (517.59): C, 51.05; H, 4.48; N, 13.53; Found C, 50.82; H, 4.60; N, 13.65.

Cyclization of Compounds 3a-C To 1, 3,4-Thiadiazin-5-Ones 4a-c

*Method A:*Compounds **3a-c** (5 mmol) were added to a methanolic solution of sodium methoxide -prepared from sodium metal (0.12 g, 5 mmol) and methanol (20 mL)- under stirring at r. t. The resulting solution was refluxed for 2-

3 h. After cooling the solvent was removed under reduced pressure, and the residue was washed with water. The solid was collected and recrystallized from ethanol to give the desired 1,3,4-thiadi-azinones **4a-c**.

Method B: To a stirred solution of compounds **3a-c** (5 mmol) in dry THF (30 mL) was carefully added lithium hydride (0.08 g, 10 mmol) at r. t. The resulting reaction mixture was refluxed for 30 min. After cooling excess lithium hydride was destroyed with some drops of glacial acetic acid. The solvent was evaporated under reduced pressure and the product extracted three times with chloroform. The combined organic extracts were dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The resulting solid product was collected and recrystallized from ethanol to give compounds **4a-c** that were identical with the ones prepared by method A.

2-phenylaminocarbonyl-4-[4-(thizol-2-yl-sulfamoyl)phenyl]-6H-1,3,4-thiadiazin-5-one (4a). M. p. 251-253 ◦C (ethanol). Yield 73 %. IR: ν = 3365, 3273 (NH), 1678 (lactam C=O), 1650 (amide C=O), 1610 (C=N), 1149 (S=O), 684 (C-S) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.61 (s, 1H, SO_2NH), 10.12 (s, 1H, PhN–H), 8.76 (d, 1H, *J* = 9.1 Hz, thiazole), 7.87-7.03 (m, 9H, Ar-H), 6.63 (d, 1H *J* = 4.5 Hz, thiazole), 3.90 (s, 2H, CH_2) ppm. ^{13}C NMR (DMSO-d_6): δ = 161.5 (lactam C=O), 158.5 (amide C=O), 143.3 (C=N), 166.7-119.2 (Ar-C and thiazole-C), 26.2 (CH_2) ppm. MS: *m/z* = 473 $[M]^{+\cdot}$ Anal. Calcd. for $C_{19}H_{15}N_5O_4S_3$ (473.55): C, 48.19; H, 3.19; N, 14.79; Found C, 48.41; H, 3.30; N, 14.67.

2-phenylaminocarbonyl-4-[4-(pyrimidin-2-yl-sulfamoyl)phenyl]-6H-1,3,4-thiadiazin-5-one (4b). M. p. 241-243 ◦C (ethanol). Yield 72 %. IR: ν = 3375, 3273 (NH), 1680 (lactam C=O), 1640 (amide C=O), 1615 (C=N), 1170 (S=O), 683 (C-S) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.60 (s, 1H, SO_2NH), 10.14 (s, 1H, PhNH), 8.86 (d, 2H, *J* = 8.8 Hz, pyrimidine), 7.97-7.11 (m, 9H, Ar-H), 6.89 (t, 1H, *J* = 7.5 Hz, pyrimidine), 3.91 (s, 2H, CH_2) ppm. ^{13}C NMR (DMSO-d_6): δ = 162.2 (lactam C=O), 159.5 (amide C=O), 143.6 (C=N), 167.9-110.3 (Ar-C and pyrimidine-C), 26.6 (CH_2) ppm. MS: *m/z* = 468 $[M]^{+\cdot}$ Anal. Calcd. for $C_{20}H_{16}N_6O_4S_2$ (468.52): C, 51.27; H, 3.44; N, 17.94; Found C, 51.46; H, 3.35; N, 18.05.

2-phenylaminocarbonyl-4-[4-(5-methyloxazol-3-yl-sulfamoyl)phenyl]-6H-1,3,4-thiadi-azin-5-one (4c). M. p. 232-234 ◦C (ethanol). Yield 71 %. IR: *ν* =3382, 3272 (NH), 1675 (lactam C=O), 1645 (amide C=O), 1612 (C=N), 1153 (S=O), 682 (C-S) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.58 (s, 1H, SO_2NH), 10.13 (s, 1H, PhNH), 7.78-7.10 (m, 9H, Ar-H), 6.34 (s, 1H, oxazole ring), 3.92 (s, 2H, CH_2), 2.35 (s, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = 160.8 (lactam C=O), 158.6 (amide C=O), 143.4 (C=N), 166.9-115.6 (Ar-C and oxazole-C), 26.3 (CH_2), 12.4 (CH_3). ppm. MS: *m/z* = 471 $[M]^+$. Anal. Calcd. for $C_{20}H_{17}N_5O_5S_2$ (471.52): C, 50.95; H, 3.63; N, 14.85; Found C, 51.15; H, 3.52; N, 14.97.

Synthesis of Compounds 5a-C (General Procedure)

Reaction of nitrilimines with mercaptosuccinic acid: To a mixture of the appropriate hydrazonoyl halide **1a-c** (0.01 mol) and mercaptosuccinic acid (7.50 g, 0.05 mol) in dry tetrahydrofuran or 1,4-dioxane (100 mL), triethylamine (5 mL, 0.05 mol) was added at room temperature and the reaction mixture was controlled by TLC. The stirring continued until the starting substrates were completely consumed (4-6 days). The triethylammonium chloride salt was filtered off, the solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were extracted with saturated $NaHCO_3$ solution, washed twice with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was treated with ethanol, whereby **5a-c** could be isolated by slow evaporation, or immediately cyclized to **6,7**.

The Following Compounds Were Prepared Using This Method:

2-{2-Anilino-2-oxoethanehydrazonoyl-N-[4-(thiazol-2-yl-sulfamoyl)phenyl] }thiosuccinic acid (5a). White solid, yield 73%, mp 216-218°C, ^1H NMR (DMSO-d_6) δ: 3.66 (d, 2H, *J* = 6.7 Hz, CH_2), 3.76 (t, 1H, *J* = 6.7 Hz, CH), 6.64 (d, 1H,*J* = 4.5 Hz, thiazole), 7.24-8.22 (m, 9H, Ar-CH), 8.78 (d, 1H, *J* = 9.2 Hz, thiazole), 9.86 (NH anilino), 10.52 (s, 1H, ArNH),12.70 (s, 1H, SO_2NH).^{13}C NMR (DMSO-d_6) δ: 39.7 (CH_2), 42.3 (CH), 125.2-141.6 (Ar-C), 145.9 (C=N), 159.6 (C=O amide), 171.8, 172.5 (COOH). IR (KBr) *ν*/cm^{-1}: 1237 (C-S), 1621 (C=N), 1654 (C=O amide), 1723, 1734 (C=O), 2539, 3240 (OH), 3265, 3347 (NH). MS, (*m/z*): 549 $[M]^+$. Analysis (% Calculated / found) for $C_{21}H_{19}N_5O_7S_3$ (Mw 549.61) C: 45.89/46.15, H: 3.48/3.62, N: 12.74/12.63.

*2-{2-Anilino-2-oxoethanehydrazonoyl-N-[4-(pyrimidin-2-yl-sulfamoyl)phenyl]}thio-succinic acid (5b).*White solid, yield 72%, mp 220-222 °C, ^{1}H NMR (DMSO-d_6) δ: 3.67 (d, 2H, *J* = 6.7 Hz, CH_2), 3.74 (t, 1H, *J* = 6.7 Hz, CH), 6.86 (t, 1H, *J* = 7.5 Hz,pyrimidine), 7.06-7.98 (m, 9H, Ar-CH), 8.82 (d, 2H, *J* = 8.8 Hz, pyrimidine), 9.88 (NH anilino), 10.51 (s, 1H, ArNH), 12.65 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 39.7 (CH_2), 42.3 (CH), 125.2-141.6 (Ar-C), 145.9 (C=N), 159.5 (C=O amide), 171.8-172.5 (COOH). IR (KBr) *v*/cm^{-1}: 1236 (C-S), 1623 (C=N), 1656 (C=O amide), 1723, 1734 (C=O), 2533-3240 (OH), 3248-3341 (NH). MS, (*m/z*): 544 [M]$^+$. Analysis (% Calculated/found) for $C_{22}H_{20}N_6O_7S_2$ (Mw 544.57) C: 48.52/48.75, H: 3.70/3.57, N: 15.43/15.55.

*2-{2-Anilino-N-[5-(methyloxazol-3-yl-sulfamoyl) phenyl]-2-oxoethanehydrazonoyl}thio-succinic acid (5c).*White solid, yield 70%, mp 230-232 °C, ^{1}H NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3 of oxazole), 3.68 (d, 2H, *J* = 6.7 Hz, CH_2), 3.76 (t, 1H, *J* = 6.7 Hz, CH), 6.21 (s, 1H, oxazole proton),7.08-7.78 (m, 9H, Ar-CH), 9.86 (NH anilino), 10.54 (s, 1H, ArNH), 12.62 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 21.6 (CH_3), 39.7 (CH_2), 42.3 (CH), 125.2-141.6 (Ar-C), 145.9 (C=N), 159.7 (C=O amide), 171.8, 172.5 (COOH). IR (KBr) *v*/cm^{-1}: 1238 (C-S), 1625 (C=N), 1665 (C=O amide), 1723, 1734 (C=O), 2534-3227 (OH), 3241, 3334 (NH). MS, (*m/z*): 547 [M]$^+$. Analysis (% Calculated/found) for $C_{22}H_{21}N_5O_8S_2$ (Mw 547.57) C: 48.26/48.05, H: 3.87/4.02, N: 12.79/12.65.

Synthesis of Compounds 6 And 7 (General Procedure)

Cyclization of compounds 5: To a stirred solution of compounds **5-a-c** in THF (30 mL) was added 1 equivalent DCC in THF (10 mL) at room temperature. The stirring continued until the starting substrates were completely consumed (2-3 h). The precipitate (dicyclohexyl urea) was filtered off, and the filtrate was evaporated under reduced pressure. The residue (viscous or crude solid) was dissolved in hot ethanol, and by slow cooling and evaporation of the solvent the desired cyclic compounds were obtained as a mixture which chromatographed on preparative TLC plates, using Merck silica gel 60 HF_{254} as the adsorbent, and $CHCl_3$/ EtOAc (5:1). The characteristic data of the title compounds **6a-c** and**7a-c** were listed bellow:

5-Oxo-{2-Phenylaminocarbonyl-4-[4-(thiazol-2-yl-sulfamoyl)phenyl]-5,6-dihydro-4H-1,3,4-thiadiazin-6-yl}-acetic acid (6a). yellow solid, yield 64%, mp 196-198 °C, ^{1}H NMR (DMSO-d_6) δ: 2.51 (s, 3H, CH_3), 3.61 (d, 2H, *J* = 7.1 Hz, CH_2), 4.59 (t, 1H, *J* = 7.1 Hz, CH), 6.62 (d, 1H, *J* = 4.5 Hz, thiazole), 7.16-7.98 (m, 9H, Ar-CH), 8.77 (d, 1H, *J* = 9.1 Hz, thiazole), 9.95 (PhNH), 12.45 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 24.7 (CH_3), 32.5 (CH_2), 34.3 (CH), 126.3-139.2 (Ar-C), 144.6 (C=N), 157.8 (C=O amide), 159.8 (C=O lactam), 171.4 (COOH). IR (KBr) *v*/cm^{-1}: 1248 (C-S), 1626 (C=N), 1660 (C=O amide), 1723 (C=O), 2550-3200 (OH). MS, (*m/z*): 531 [M]$^+$. Analysis (% Calculated/found) for $C_{21}H_{17}N_5O_6S_3$ (Mw 531.59) C: 47.45/47.63, H: 3.22/3.35,N:13.17/13.30.*5-Oxo-{2 Phenylaminocarbonyl-4-[4-(pyrimidin-2-yl-sulfamoyl)phenyl]-5,6-dihydro-4H-1,3,4-thiadiazin-6-yl}-acetic acid (6b).*Pale yellow solid, yield 61%, mp 183-185 °C, ^{1}H NMR (DMSO-d_6) δ: 3.68 (d, 2H, *J* = 7.1 Hz, CH_2), 4.55 (t, 1H, *J* = 7.1 Hz, CH), 6.87 (t, 1H, *J* = 7.5 Hz, pyrimidine), 7.11-7.89 (m, 9H, Ar-CH),8.84 (d, 2H, *J* = 8.8 Hz, pyrimidine), 9.93 (PhNH), 12.65 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 32.7 (CH_2), 34.5 (CH), 126.6-139.7 (Ar-C), 143.7 (C=N), 157.9 (C=O amide), 159.5 (C=O lactam), 171.6 (COOH). IR (KBr) *v*/cm^{-1}: 1247 (C-S), 1624 (C=N), 1655 (C=O amide), 1723 (C=O), 2535-3230 (OH). MS, (*m/z*): 526 [M]$^+$. Analysis (% Calculated/found) for $C_{22}H_{18}N_6O_6S_2$ (Mw 526.55) C: 50.18/50.35, H: 3.45/3.33, N: 15.96/16.12.

*4-{[5-(Methyloxazol-3-yl-sulfamoyl)phenyl]-5-oxo-2-phenylaminocarbonyl-5,6-dihydro-4H-1,3,4-thiadiazin-6-yl}acetic acid (6c).*White off solid, yield 63%, mp 246-248 °C, ^{1}H NMR (DMSO-d_6) δ: 2.36 (s, 3H, CH_3 of oxazole), 3.61 (d, 2H, *J* = 7.1 Hz, CH_2), 4.59 (t, 1H, *J* = 7.1 Hz, CH), 6.21 (s, 1H, oxazole proton), 7.06-7.84 (m, 7H, Ar-CH), 9.93 (PhNH), 12.70 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 32.2 (CH_2), 33.7 (CH), 126.6-139.7 (Ar-C), 143.8 (C=N), 157.8 (C=O amide), 159.8 (C=O lactam), 171.9 (COOH). IR (KBr) *v*/cm^{-1}: 1224 (C-S), 1626 (C=N), 1660 (C=O amide), 1721 (C=O), 2540-3235 (OH). MS, (*m/z*): 529 [M]$^+$. Analysis (% Calculated/ found) for $C_{22}H_{19}N_5O_7S_2$ (Mw 529.55) C: 49.90/50.15, H: 3.62/3.50, N: 13.22/13.11.

*5-Oxo-2-phenylaminocarbonyl-4-[4-(thiazol-2-yl-sulfamoyl)phenyl]-4,5,6,7-tetrahydro-1,3,4-thiadiazepine-7-carboxylic acid(7a).*Yellow solid, yield 57%, mp 232-234 °C, ^{1}H NMR (DMSO-d_6) δ: 3.64 (d, 2H, *J* = 6.9 Hz, CH_2), 4.89 (t, 1H, *J* = 6.9 Hz, CH), 6.62 (d, 1H, *J* = 4.5 Hz, thiazole), 7.16-7.91 (m, 9H, Ar-CH), 8.76 (d, 1H, *J* = 9.2 Hz, thiazole), 9.89 (PhNH), 11.75

(s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 24.7 (CH_3), 31.9 (CH_2) 36.8 (CH), 126.6-139.7 (Ar-C), 144.3 (C=N), 160.5 (C=O ring), 171.4 (COOH), 193.6 (CH_3C=O),. IR (KBr) ν/cm^{-1}: 1208 (C-S), 1624 (C=N), 1692 (RC=O), 1723 (C=O), 2560-3210 (OH). MS, (*m/z*): 531 $[M]^+$. Analysis (% Calculated/found) for $C_{21}H_{17}N_5O_6S_3$ (Mw 531.59) C: 47.45/47.65, H: 3.22/3.35, N: 13.17/13.30.

*5-Oxo-2-phenylaminocarbonyl-4-[4-(pyrimidin-2-yl-sulfamoyl)phenyl]-4,5,6,7-tetra-hydro-1,3,4-thiadiazepine-7-carboxylic acid(7b).*Yellow solid, yield 56%, mp 279-281 °C, ^1H NMR (DMSO-d_6) δ: 3.46 (d, 2H, *J* = 6.9 Hz, CH_2), 4.66 (t, 1H, *J* = 6.9 Hz, CH), 6.85 (d, 1H, *J* = 7.5 Hz, pyrimidine), 7.14-7.98 (m, 9H, Ar-CH), 8.86 (d, 2H, *J* = 8.8 Hz, pyrimidine), 9.87 (PhNH), 11.78 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 31.7 (CH_2), 36.6 (CH), 126.6-139.7 (Ar-C), 144.7 (C=N), 160.8 (C=O ring), 171.4 (COOH), 187.6 (CH_3C=O). IR (KBr) ν/cm^{-1}: 1208 (C-S), 1624 (C=N), 1665 (RC=O), 1723 (C=O), 2520-3230 (OH),. MS, (*m/z*): 526 $[M]^+$. Analysis (% Calculated/ found) for $C_{22}H_{18}N_6O_6S_2$ (Mw 526.55) C: 50.18/49.90, H: 3.45/3.55, N: 15.96/16.11.

*4-[5-(Methyloxazol-3-yl-sulfamoyl)phenyl]-5-oxo-2-phenylaminocarbonyl-4,5,6,7-tetra-hydro-1,3,4-thiadiaze-pine-7-carboxylic acid(7c).*White solid, yield 53%, mp 254-256 °C, ^1H NMR (DMSO-d_6) δ: 2.35 (s, 3H, CH_3 of oxazole), 3.48 (d, 2H, *J* = 6.9 Hz, CH_2),4.61 (t, 1H, *J* = 6.9 Hz, CH), 6.21 (s, 1H, oxazole proton), 7.07-7.84 (m, 9H, Ar-CH), 9.88 (PhNH), 11.90 (s, 1H, SO_2NH). ^{13}C NMR (DMSO-d_6) δ: 31.4 (CH_2), 36.7 (CH), 126.6-139.7 (Ar-C), 144.4 (C=N), 160.8 (C=O ring), 171.3 (COOH), 176.2 (RC=O). IR (KBr) ν/cm^{-1}: 1208 (C-S), 1624 (C=N), 1660 (RC=O), 1723 (C=O), 2530-3235 (OH). MS, (*m/z*): 529 $[M]^+$. Analysis (% Calculated/found) for $C_{22}H_{19}N_5O_7S_2$ (Mw 529.55) C: 49.90/50.15, H: 3.62/3.55, N: 13.22/13.35.

Antimicrobial Activity Screening

Antimicrobial activity screening of the synthesized compounds was determined by the agar dilution technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [51]. The tested compounds were dissolved in dimethyl sulfoxide (DMSO). An inoculum of about 1.5 x 10^8 colony forming unit per spot was applied to the surfaces of Mueller–Hinton agar plates containing graded concentrations of the respective compound; plates were incubated at 37 °C for 18 h. All organisms used in this study were standard strains were obtained from the Microbiology laboratory (Al-Aqsa University) and included bacterial strain such as *Enterococci, Escherichia coli, Staphylococcus aureus, Klebsiella spp, Proteus spp,* and fungi strain such as *Aspergillus niger, Candida albicans.* The MIC of Tetracycline and fluconazole was determined concurrently as reference for antibacterial and antifungal activities, respectively (Table 1). Control DMSO was carried out with each experiment.

Table1. *Antimicrobial screening results of the tested compounds**

Comp.No.	Antibacterial activity					Antifungal activity	
	En.	*E. coli*	*S. aureus*	*K. spp*	*P. spp*	*C. alb.*	*A. niger*
4a	18	16	17	15	14	18	16
4b	19	19	15	17	14	19	18
4c	16	16	17	16	15	16	19
6a	18	19	19	18	13	19	17
6b	17	18	18	19	16	19	16
6c	17	15	18	19	18	18	17
7a	18	18	16	16	19	15	13
7b	16	16	19	18	16	16	17
7c	15	17	18	15	13	17	19
DMSO	--	--	--	--	--	--	--

**Calculated as average of three values.*

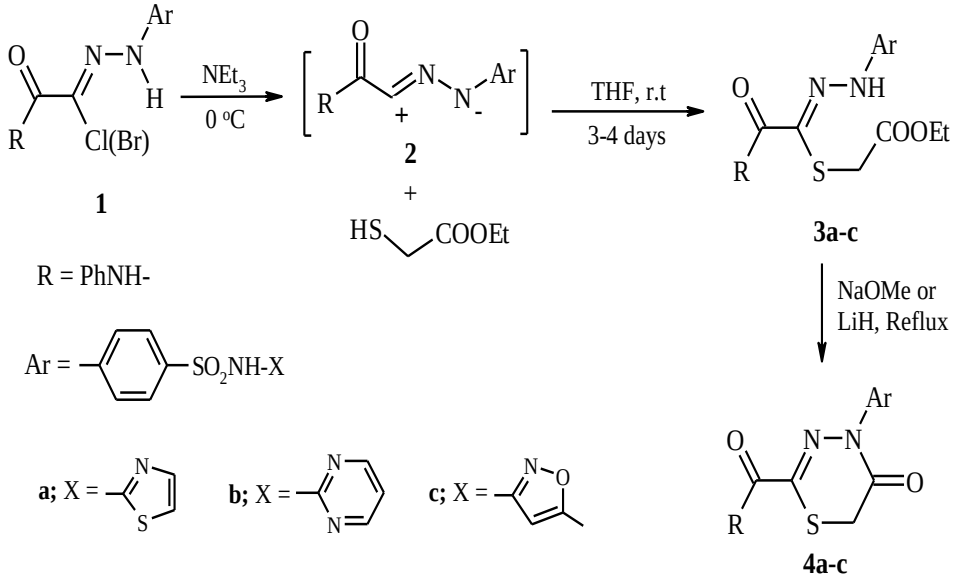
Ent=Enterococci,E. coli =Escherichia coli,S aureus = Staphylococcus aureus, K.spp =Klebsiella spp, P. spp = Proteus spp,C. alb. = Candida albicans, A. niger =Aspergillus niger.

Results And Discussion

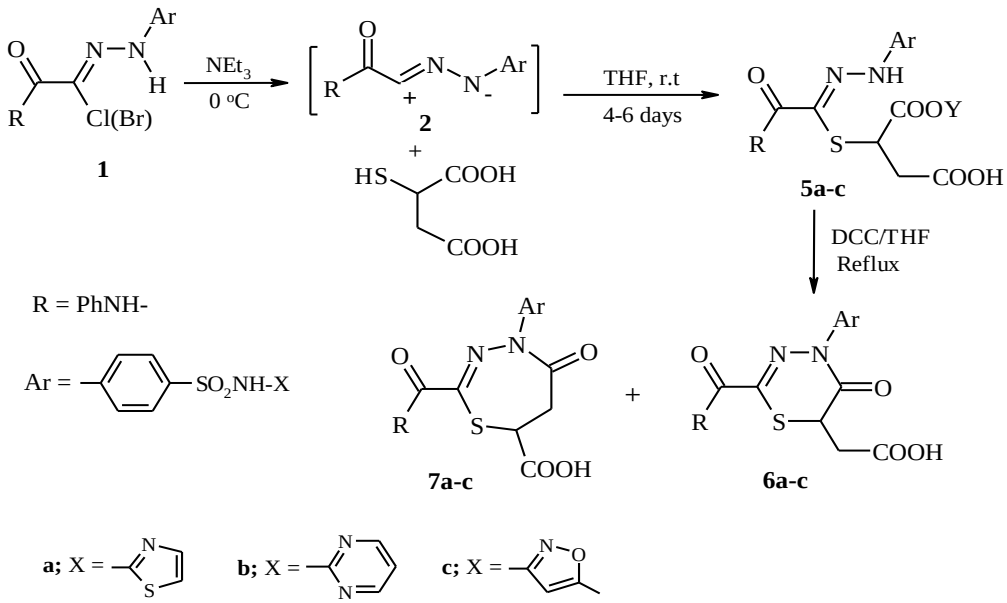
Chemistry

Hydrazonoyl halides (nitrilimines precursors) have been widely used for the synthesis of heterocyclic compounds. In recent years, cyclo condensations using nitrilimines have received considerable attention because they have been shown to be an efficient synthetic tool for the

preparation of various thia-aza hetero cycles. The reactive nitrilimines are found to react with 2-sulfanyl alkanoic acids or ethyl sulfanylacetate yielding acyclic adducts (4-arylhydrazono-5-oxo-3-thiahexanoic acid or ethyl6-aryl-4-aroyl-3,5,6-thiadiaza-4-hexenoate) which underwent cyclization to 1,3,4-thiadiazinone ringsin the presence of dicyclohexylcarbodiimide (DCC) or lithium hydride, or methanolic sodium methoxide [52]. In the present study, the nitrilimines **2a-c** having sulfonamide moieties were generated *in situ* from the respective hydrazonoyl chlorides **1a-c**, are found to react readily with ethyl mercaptoacetate for 3-4 days at room temperature gave acyclic electrophilic addition products (3,5,6-thiadiaza-4-hexenoates) **3a-c** (Scheme 1). Cyclization to the corresponding 1,3,4-thiadiazin-5-ones **4a-c** did not observed. The 3,5,6-thiadiaza-4-hexenoates **3a-c** were cyclized intramolecularly to the corresponding 2,4-disubstituted 1,3,4-thiadiazin-5-ones **4a-c** by heating them with methanolic sodium methoxide (NaOMe) or lithium hydride (LiH) (Scheme 1).



Scheme1. *Synthetic pathway for the preparation of compounds **3a-c** and **4a-c**.*



Scheme2. *Synthetic pathway for the preparation of compounds **5-7a-c**.*

Similarly, the mercaptosuccinic acid reacts with reactive nitrilimines **2a-c** for 4-6 days at room temperature yielding acyclic electrophilic addition products **5a-c** (Scheme 2)**.** Acyclic adducts **5a-c** underwent cyclization upon loosing water molecule, to the corresponding (4-

Heterylsulfamoylphenyl-2 phenylaminocarbonyl-5-oxo-5,6-dihydro-4H-1,3,4-thiadiazin-6-yl)-acetic acid **6a-c** and 1,3,4-thiadiazepine-5-ones **7a-c**, in the presence of dicyclohexylcarbodiimide (DCC) in refluxing tetrahydrofuran (THF) (Scheme 2). The antimicrobial activities of the synthesized compounds **4a-c, 6a-c** and **7a-c** were investigated.

Spectroscopic Data for Compounds 3-7a-c

The assignment of structures **3-7a-c** is based on their analytical and spectroscopic data. Physical properties, molecular ion peaks and elemental analysis are presented in the Experimental Section. The characteristic data of compounds **3-7a-c** are given in detail in the Experimental Section. All compounds gave satisfactory combustion analysis for the proposed structures which were confirmed on the basis of their spectroscopic data. For compounds **3a-c**, the electron impact (EI) mass spectra displayed the correct molecular ions ($M^{+\cdot}$) in accordance with the suggested structures. Their IR spectra showed three NH absorption bands at the region 3360-3200 cm^{-1}. The carbonyl absorption of the ester and amide groups appeared in the regions 1720-1710 cm^{-1} and 1655-1650 cm^{-1}, respectively. The C–S stretching band appeared in the region 1230-1220 cm^{-1} and SO_2 of sulfonamide group bands appeared around 1150 and 1060 cm^{-1}. The ^1H NMR spectra of compounds **3a-c** showed signals of the ethyl protons at δ = 1.3–1.1 ppm (t, 3H, CH_3) and 4.2-4.1 ppm (q, 2H, OCH_2), indicating clearly that the ethyl group of the ester was not lost, and that the compounds have acyclic structure. Also the N–NH proton appeared as a singlet at δ = 10.6-10.4 ppm. The ^{13}C NMR spectra illustrate that compounds **3a-c** have the assigned acyclic structures. The carbonyl carbon of the ester group appeared at about δ = 170 ppm, and the signals of the CH_2 and CH_3 carbon atoms of the ethoxy group appeared at about δ = 61and 14 ppm, respectively. The methylene carbon of S–CH_2 appeared at about δ = 34-33 ppm, and the signal at δ ≈ 141 ppm is attributed to the C=N carbon atom. Structure elucidation of the obtained thiadiazin ones **4a-c** was achieved as follows: their mass spectra displayed the correct molecular ion peaks [M^+] in accordance with the suggested structures and showed the loss of an ethoxy group from the acyclic adducts **3a**-c viaethanol elimination. Their IR spectra support the formation of the thiadiazin one ring by the absence of N-NH (around 3340 cm^{-1}) and C=O (around 1710 cm^{-1}) vibration bands of the ester, and the appearance of a new absorption band for a lactam (C=O of the thiadiazinone ring) in the region 1680-1670 cm^{-1}. The ^1H NMR spectra of compounds **5a-c** showed all the signals of the proposed structures, indicating the disappearance of ethyl (CH_2CH_3) and N-NH protons. Finally, the ^{13}C NMR data illustrated that compounds **4a-c** have the assigned cyclic structure by the absence of signals for ester group carbons (170, 61, 14 ppm) and the presence of the signal at δ ≈ 161 ppm which is typical for a lactam group. Furthermore, the signal of the methylene carbon (δ ≈ 34 ppm) of the thioester moiety in the acyclic adducts **3a-c** is shifted up field to δ ≈ 26 ppm in compounds **4a-c**, whereas the signal of the C=N carbon is recorded at δ ≈ 143 ppm. For compounds **5a-c**, their IR spectra are characterized by the 3NH bands in the region 3370-3220 cm^{-1}, a broad hydroxyl bands in the region 3200-2520 cm^{-1} indicating the carboxyl group, a strong and broad carbonyl of the carboxyl groups band in the region 1730-1720 cm^{-1}, a C=N band at 1630-1610 cm^{-1}, and a C-S stretching band appeared in the region 1240-1220 cm^{-1}. The ^1H NMR spectra of compounds **5a-c** showed characteristic signals of the aliphatic and aromatic protons, especially the triplet at 4.4-4.2 ppm for the proton at C-3, and a doublet at 3.9-3.7 ppm for the protons at C-2. Also the N-NH proton appeared as singlet at 10.4-10.6 ppm. Structure elucidation of the obtained 1,3,4-thiadiazin-5-ones **6a-c** and 1,3,4-thiadiazepin-5-ones **7a-c** were achieved by their analytical and spectral data summarized in the experimental section. Their mass spectra displayed the correct molecular ion peaks [M^+] in accordance with the suggested structures. The IR spectra of those compounds **6a-c** and **7a-c** support the formation of the cyclic structures by the absence of NH band and the appearance of a new absorption band for a lactam (C=O of the ring) in the region 1670-1680 cm^{-1}. Their ^1H NMR spectra showed all the signals of the proposed structures, indicating the disappearance of the signal of the proton of NNH. Finally, also the ^{13}C NMR data illustrated that compounds **6a-c** and **7a-c** have the assigned cyclic structure by the presence of the signal at 159-160 ppm which is typical for a lactam group. Whereas the signal of the C=N carbon is recorded at 143-144 ppm.

Antimicrobial Activity

Various sulfonamide moieties substituents were placed on the thiadiazinone and thiadiazepinone rings in order to study their effects on an antimicrobial activity in vitro. Most of the

synthesized compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains such as E*nterococci, Escherichia coli, Staphylococcus aureus, Klebsiella spp, Proteus spp,* and fungi such as *Aspergillus niger, Candida albicans*, employing the nutrient agar disc diffusion method [53] at 1-100 mg/ mL concentration in dimethyl sulfoxide (DMSO) which used as solvent control, by measuring the average diameter of the inhibition zone in mm. All the experiments were carried out in triplicate. The results showed that all the tested compounds exhibited good degree of activity against different strains of bacteria and fungi compared with well-known antibacterial and antifungal substances such as tetracycline and fluconazole respectively. The results are given in Table 1. According to NCCLS (2004) [54], zones of inhibition for tetracycline and fluconazole < 14 mm were considered resistant, between 15 and 18 mm were considered weakly sensitive and > 19 mm were considered sensitive. From the screening results, it found to possess various antimicrobial activities towards all the microorganisms tested. The results confirm that, the antimicrobial activity is strongly dependent on the nature of the substituents on triazinone and thiadiazinone nucleus. The presence of sulfonamide moieties showed a better spectrum of activity than the reference drug (Table 1).

CONCLUSIONS

New series of novel functionalized 1,3,4-thiadiazinones **5a - c, 6a- c**and1, 3, 4 thiadiazepinones **7a - c**containing benzene sulfonamide moiety were synthesized using hydrazonoyl halides as a precursor of nitrilimines and evaluated for their *in vitro* antibacterial, and antifungal activities. From the screening results, it found to possess various antimicrobial activities towards all the microorganisms tested. The results confirm that, the antimicrobial activity is strongly dependent on the nature of the substituents on triazinone and thiadiazinone nucleus. The pyrimidinyl, thiazolylmethoxzolylderivatives generally led to dramatic improvements in activity against both bacteria and fungi.In short, the present study can lead medicinal chemists to design and synthesize similar compounds with enhanced biological potency in future.

ACKNOWLEDGEMENTS

The authors are great thankful to the Qatar Charity for the financial support of this research through Ibhath grant (GCC-07-06).

REFERENCES

[1] A.K. Gadad, C.S. Mahajanshetti, S. Nimbalkar, A. Raichurkar, Eur. J. Med. Chem.**2000**, 35(9), 853-857.

[2] V.S. Misra, V.K. Saxena, R.J. Srivastava, Ind. Chem. Soc.**1982**, 59, 781.

[3] F. Zani, P. Vicini, Arch. Pharm.**1998**, 331, 219-223.

[4] A. Scozzafava, T. Owa, A. Mastrolorenzo, and C. T. Supuran, "Anticancer and antiviral sulfonamides," Curr. Med. Chem., **2003**, 10(11), 925–953, 2003.

[5] B. N. Stranix, J. G. Lavallee, G. Sevigny, "Lysine sulfonamides as novel HIV-protease inhibitors: N-Acyl aromatic α- amino acids," Bioorg. Med. Chem. Lett., **2006**,16(13), 3459–3462.

[6] J. M. Beale, Jr., Anti Infective Agents, in Wilson and Gisvold's Text Book of Organic Medicinal and Pharmaceutical Chemistry (Eds. J. H. Block and J. M. Beale, Jr.), 11th ed., Lippincott William andWilkins, Philadelphia **2004**, pp. 217–281.

[7] Dogruer, D.; Urlu, S.; Onkol, T.; Ozcellik, B.; Sahin, M. F. Synthesis of some pyridazine derivatives carrying urea, thiourea, and sulfonamide moieties and their antimicrobial activity. Turk. J. Chem., **2010**, 34(1), 57-65.

[8] Z. Brzozowski, J. S1awi_nski, F. Sa¸czewski, A. Innocenti, C.T. Supuran, Eur. J. Med.Chem.2010, 45, 2396-2404.

[9] G. Renzi, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett.**2000**, 10, 673-676.

[10] T.H. Maren, Physiol. Rev.**1967**, 47, 595-781.

[11] T.H. Maren, Ann. Rev. Pharmacol. Toxicol.**1976**, 16, 309-327.

[12] J. Drew, Science**2000**, 287, 1960-1964.

[13] J.J. Li, D. Anderson, E. Burton, J. Cogburn, J. Collins, D. Garland, S. Gregory, H. Huang, P. Isakson, C. Koboldt, E. Logusch, M. Norton, W. Perkins, E. Reinhard, K. Seibert, A. Veenhuizem, Y. Zang, D.B. Reitz, Med. Chem.**1995**, 38 4570-4578.

[14] F. Sa¸czewski, A. Innocenti, J. S1awinski, A. Kornicka, Z. Brzozowski, El . Pomarnacka, A. Scozzafava, C. Temperini, C.T. Supuran, Bioorg. Med. Chem.**2008**, 16, 3933-40.

[15] V. Garaj, L. Puccetti, G. Fasolis, J.Y. Winum, J.L. Montero, A. Scozzafava, D. Vullo, A. Innocenti, C.T. Supuran, Bioorg. Med. Chem. Lett.**2005**, 15, 3102-3108.

[16] F. Sa¸czewski, J. S1awi_nski, A. Kornicka, Z. Brzozowski, E. Pomarnacka, A. Innocenti, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett.**2006**, 16, 4846e-4851.

[17] C.T. Supuran, A. Scozzafava, A. Casini, Med. Res. Rev.**2003**,23, 146-189.

[18] C.T. Supuran, Carbonic anhydrases: catalytic and inhibition mechanisms, distribution and physiological roles, in: Carbonic Anhydrase. Its Inhibitors and Activators. CRC Press, Boca Raton, **2004**, pp. 1-23.

[19] A.J. Kivela, J. Kivela, J. Saarnio, S. Parkkila, J. World Gastroenterol.**2005**, 11, 155-163.
[20] C.T. Supuran, A. Scozzafava, Bioorg. Med. Chem.**2007**, 15, 4336-4350.
[21] Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. C.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F. J. Med. Chem.**2000**, 43, 775-777.
[22] Thun, M. J.; Henley, S. J.; Patrono, C. J. Natl. Cancer Inst.**2002**, 94, 252-266.
[23] C.C. Wykoff, J. Beasley, H. Watson, K. Turner, J. Pastorek, A. Sibtain, D. Wilson, H. Turley, K.L. Talks, H.P. Maxwell, W.C. Pugh, J.P. Ratcliffe, L.A. Harris, Cancer Res.**2000**, 60, 7075-7083.
[24] S. Parkkila, H. Rajaniemi, A. Parkkila, J. Kivelä, A. Waheed, S. Pastorekova, J. Pastorek, W. Sly, Proc. Natl. Acad. Sci. U.S.A**2000**, 97, 2220-2224.
[25] E. Svastova, A. Hulikova, M. Rafajova, M. Zatovicova, A. Gibadulinova, A. Casini, A. Cecchi, A. Scozzafava, C.T. Supuran, J. Pastorek, S. Pastorekova, FEBS Lett.**2004**, 577,439-445.
[26] A. Cecchi, A. Hulikova, J. Pastorek, S. Pastorekova, A. Scozzafava, J.Y. Winum, J.L. Montero, C.T. Supuran, J. Med. Chem.**2005**,48 4834-4841.
[27] Argyropoulou, I.; Geronikaki, A.; Vicini, P.; Zani, F. Synthesis and biological evaluation of sulfonamide thiazole and benzothiazole derivatives as antimicrobial agents. Arkivoc, **2009**(vi), 89-102.
[28] N. Demirbas, A. Demirbas, S. Karaoglu, E. Celik,Arkivoc**2005**, (i), 75-91.
[29] Zircle, C.; Kaiser, C. In Medicinal Chemistry, Ed. Burger, A.; Wiley- Interscience, New York **1970**, pp. 1410.
[30] A. K. Shakya, P. Mishra, G. K. Patnaik, R. Shukla, R. C. Srimal, Arch. Pharm. Res.,**1998**,21, 753.
[31] M. J. Pawar, A. B. Burungale, B. K. Karale, ARKIVOC, **2009**, xiii, 97.
[32] J. Schroder, A. Henke, H. Wenzel, H. Brandstetter, H. G. Stammler, A. Stammler, W. D. Pfeiffer, H. Tschesche, J. Med. Chem., **2001**, 44, 3231.
[33] H. Tschesche, J. Schroder, EP Pat. Appl. 1191024.
[34] H. Sugawara, M. Endoh, Jpn. J. Pharmacol., **1999**, 80, 55.
[35] H. M. Himmel, G. J. Amos, E. Wettwer, U. Ravens, J. Card. Pharm.,**1999,**33, 301.
[36] H. N. Dogan, A. Duran, S. Rollas, G. Sener, M. K Uysal, D. Gulen, Bioorg. Med. Chem.,**2002**,10, 2893.
[37] S. A. Carvalho, E. Da-Silva, R. M. Santa-Rita, S. De-Castro, C. A. Fraga, Bioorg. Med. Chem. Lett., **2004**, 14, 5967.
[38] S. N. Swamy, Basappa, B. S. Priya, B. Prabhuswamy, B. H. Doreswamy, J. S. Prasad, K. S. Rangappa, Eur. J. Med. Chem., **2006**, 41, 531.
[39] B. Kalluraya, R. E. Gururaja, R. Ganesha, Indian J. Chem.**2003**, 42, 211.
[40] A. Dandia, R. Singh, S. Khaturia, Bioorg. Med. Chem., **2006**,14, 1303.
[41] j. Duan, L. Chen, R. J. Chemey, C. P. Decicco, M. E. Voss, PCT Int. Appl. WO, **1999**, 41246.
[42] W. L. Albrecht, F. W. Sweet, US Pat (**1976**) 3,954,983; Chem. Abstr. 85 (**1976**) 78172g.
[43] W. L. Albrecht, W. Jones, US Pat, (**1976**) 3,954,981; Chem. Abstr. 85 (**1976**) 177501v.
[44] P. Molina, M. Alajarin, M. J. Perez, M. C. Foces, F. Hernandez Cano, R. M. Claramunt, J. Elguero, J. Chem. Soc. Perkin Trans I, **1987**, 1853.
[45] V. Reddy, K. Reddy, Chem. Pharm. Bull., **2010**, 58(8), 1081; Y. A. Ammar, M. M. Ghorab, A. M. El-Sharief, Sh. I. Mohamed, Heteroatom Chem.,**2002**,13, 199.
[46] B. Kalluraya, Ph D Thesis, Bangalore University, India, **1987**.
[47] G. C. Gonzalez-Munoz, M. P. Arce, B. Lopez, C. Perez, M. Villarroya, M. G. Lopez, A. G. Garcia, S. Conde, M. I. Rodriguez-Franco, Eur. J. Med. Chem.,**2010**,45, 6152.
[48] Dalloul, H. M. Can. Chem. Trans.,**2015**, 3, 108-117; J. Chem.,**2015**, 1-7; Open J. Org.Chem., **2015**, 3,12-17.
[49] Hany M. Dalloul, Khaled A. El-nwairy, Ali Z. Shorafa, Ahmed S. AbuSamaha, Inter. J.Adv. Res. Chem. S., **2016**, 3, 1-9.
[50] Nishiwaki, T.; Siato, T. J. Chem. Soc. (C)**1971**, 2648-2652; Frohberg, P.; Drutkowski, G.; Wagner, C. Eur. J.Org. Chem.**2002**, 1654-1663.
[51] Clinical Laboratory Standards Institute, "Methods for dilution antimicrobial susceptibility tests for bacteria grow aerobically", Approved Standard M7-A4, Clinical and Laboratory Standards Institute, Wayne, Pa, USA, **2005**.
[52] B. A. Thaher, H. H. Otto, Monatsh. Chem.,**2002**,133, 1011; H. M. Dalloul, E. A. R. Mohamed, A. Z. El-Shorafa, Z. Naturforsch., **2009**, 63b, 585.
[53] Irobi, O. N.; Moo-Young, M.; Anderson, W. A. Inter. J. Pharm.,**1996**, 34, 87-90; Grayer, R. J.; Harborne, J. B. Phytochemistry, **1994**, 37, 19-42.
[54] National Committee on Clinical Laboratory Standards (NCCLS), Richard A. Van Enk, Ph.D. Bronson Methodist Hospital Kalamazoo, Michigan, **2004**.