WALAILAK JOURNAL
http://wjst.wu.ac.th Health Sciences

Design, Synthesis, Antimicrobial, and Antioxidant Activities of Novel-{4, 5-(substituted diphenyl)-4H-1, 2, 4-triazol-3-ylthio} Acetyl Chloride

Mukesh KUMAR, Nelam MALIK, Priyanka DHIMAN, Prabhakar Kumar VERMA* and Anurag KHATAHAR

Department of Pharmaceutical Sciences, Maharashi Dayanand University, Rohtak, Haryana, India

(*Corresponding author's e-mail: vermapk422@rediffmail.com)

Received: 2 February 2016, Revised: 29 September 2017, Accepted: 27 October 2017

Abstract

A series of 20 new biologically active derivatives of 2-{4, 5-(substituted diphenyl)-4H-1,2,4-triazol-3-ylthio}acetyl chloride has been synthesized, with the aim to investigate antimicrobial, free radical scavenging activity. All the synthesized compounds were characterized by spectroscopic data and elemental analysis. The final compounds were tested for antibacterial activity against Gram-positive bacteria: *Staphylococcus aureus* MTCC 3160, *Bacillus subtilis* MTCC 441; Gram-negative bacteria: *Escherichia coli* MTCC 443, and, for antifungal activity, against *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281, taking ciprofloxacin as antibacterial and fluconazole as antifungal standard drugs. Compound **$7a_6$** was found to be the most effective antibacterial (MIC = 3.12 µg/ml), and compounds **$7a_2$** and **$7d_1$** (MIC = 3.12 and 6.25 µg/ml) had the most effective antifungal effects on the selected strains, as compared to the standard drugs. The results of antioxidant studies revealed that compound **$7b_1$** was found to be most active antioxidant, with 40.4±0.687 µg/ml, and compounds **$7b_3$**, **$7d_7$**, and **$7d_4$** also showed promising free radical scavenging activity, as compared with the standard drug ascorbic acid.

Keywords: Antibacteria, antifungus, MIC, antioxidant,structure activity relationship triazole

Introduction

The treatment of bacterial infections remains an important and challenging therapeutic problem, due to factors that include emerging infectious diseases and the increasing number of multi-drug-resistant microbial pathogens. In spite of the large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic-resistant bacterial strains in recent decades constitutes a substantial need for new classes of antibacterial agents [1]. Oxidative stress is implicated in contributing to potentially harmful free radicals, which play a pivotal role in the pathogenesis of various diseases, including rheumatoid arthritis, immunosuppression, age-related degeneration, ischemic heart disease, inflammation, atherosclerosis, diabetes, hair loss, cancer initiation, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. The purpose of antioxidants is to prevent ROS concentrations from reacting up to harmful intracellular levels and instigating degenerative processes by various mechanisms, including scavenging of free radicals [2]. The nitrogen containing heterocyclic moiety has served as the foundation for many medicinal compounds. Triazole ring containing derivatives have attracted researcher attention in the past several years. A variety of derivatives were synthesized by various scientists, who reported various triazole compounds which were active against several diseases [3,4]. These triazoles are 5-membered rings, with 2 carbon and 3 nitrogen atoms. Triazoles exist in isomeric forms, according to the position of nitrogen atoms, and are

characterized by the position of nascent hydrogen. Therefore, 1,2,4-triazoles exist in 2 forms, i.e., 1*H* and 4*H* [5,6].

Triazoles have also been reported for their pharmacological activity, and are found in applications for the treatment of many disorders. Triazoles are associated with a wide range of biological activities, such as cytotoxic [7], antiviral [8], analgesic [9], antimalarial [10], antitubercular [11,12], antidiabetic [13], antifungal [14], antiurease [15], antihelmentic [16], anticonvulsant [17], antioxidant [18,19], and antiinflammatory [20] activities. In view of these facts, and as part of our efforts to discover potentially active new agents, we have synthesized some new triazoloe derivatives, and evaluation them for their antimicrobial, free radical scavenging activity.

Materials and methods

All reactions were carried out by standard techniques for the exclusion of moisture. Melting points were determined in open glass capillaries on a Sonar melting point apparatus, and were uncorrected. Reaction progress was monitored by thin layer chromatography on silica gel sheets (Merck silica gel-G) in the solvent system chloroform, benzene and glacial acetic acid (3:1:1), and the spots were located in iodine and a UV chamber. Infra-red (IR) analysis was performed on a SHIMAZDU FT-IR instrument using KBr pellets, and was recorded in cm^{-1}. ^{1}H NMR spectra were recorded on a Bruker DRX-300 FTNMR spectrometer using DMSO-d_6 solvent, and was expressed in parts per million (d, ppm) downfield from the internal standard TMS. ^{13}C-NMR (Nuclear Magnetic Resonance) spectra were recorded on Bruker (400 MHz) and (100 MHz) spectrometers, using DMSO-d_6 as a solvent and TMS as an internal standard. Elemental analysis of all the synthesized compounds was carried out on a Thermo Scientific Flash 2000 CHN analyzer. Mass spectra were taken on a Bruker Compass Data Analysis 4.0 mass spectrometer.

General procedure for synthesis of ethyl aryl carboxylate 3(a-d)

A mixture of substituted aromatic acid (0.001 mol) and absolute alcohol (50 ml) was heated under reflux for 6 - 8 h in the presence of a few drops of conc. H_2SO_4 in anhydrous condition. TLC was used to monitor the progress of the reaction. After completion of the reaction, the reaction mixture was poured on crushed ice and filtered under suction; the precipitate thus obtained was washed with water and recrystallized using ethanol.

General procedure for synthesis of aryl carbonyl hydrazide 4(a-d)

A mixture of ethyl aryl carboxylate (0.01 mol), hydrazine hydrate (0.2 mol), and absolute alcohol (50 ml) was heated under reflux for 6 - 8 h. A condenser with a calcium guard tube was attached to the flask, and the mixture was refluxed for 6 - 8 h. TLC was used to monitor the progress of the reaction. After completion of the reaction, the reaction mixture was poured on crushed ice. It was kept for 4 - 6 h at room temperature, and the solid mass separated out was filtered, dried, and recrystallized using ethanol.

General procedure for synthesis of 5-substituted phenyl-1, 3, 4-oxadiazole-2-thiols 5(a-d)

A mixture of substituted aryl carbonyl hydrazides (0.01 mol) in ethanol, potassium hydroxide (0.015 mol) in ethanol, and carbon disulphide (0.015 mol) was refluxed for 7 - 8 h. After completion of the reaction, the reaction mixture was detected by thin layer chromatography, acidified with dil. HCl, and the resulting solid collected, washed with distilled water, and dried in vacuum and recrystallized using ethanol.

General procedure for synthesis of substituted 4, 5-diphenyl 4H-1, 2, 4-triazole-3-thiols 6(a_{1-8})(b_{1-3})(c_{1-2})(d_{1-7})

A mixture of substituted 1,3,4-oxadiazoles (0.01 mol) in ethanol and 0.03 mol of different substituted anilines was refluxed for 8 - 10 h. TLC was used to monitor the progress of the reaction. After completion of the reaction, the reaction mixture was poured on crushed ice. The resulting product was filtered, dried in vacuum, and recrystallized using ethanol.

General procedure for synthesis of 2-{4, 5-substituted diphenyl)-4H-1, 2, 4-triazol-3-ylthio} acetyl chloride 7(a_{1-8})(b_{1-3})(c_{1-2})(d_{1-7})

A mixture of substituted 4, 5-diphenyl 4H-1, 2, 4-triazole-3-thiols (0.01 mol) in ethanol, and 0.02 mol of chloro acetylchloride was refluxed for 4 - 7 h. The reaction progress was monitored by thin layer chromatography on silica gel-coated glass plate, and the purity of the compound was ascertained by single spot on TLC sheet. The reaction mixture was poured on crushed ice. The solid product formed was filtered, dried, and recrystallized using ethanol as a solvent (**Table 1**).

Table 1 Arrangement of substituents used for the target compound.

Compd.	R	X	Compd.	R	X
$7a_1$	4-NO_2	2,3-Cl	**$7b_3$**	2-OH	2-NO_2
$7a_2$	4-NO_2	2,4-CH_3	**$7c_1$**	3,5-NO_2	2,4-CH_3
$7a_3$	4-NO_2	4-Cl	**$7c_2$**	3,5-NO_2	2-NO_2
$7a_4$	4-NO_2	4-OCH_3	**$7d_1$**	4-NH_2	2,4-CH_3
$7a_5$	4-NO_2	2-NO_2	**$7d_2$**	4-NH_2	4-Cl
$7a_6$	4-NO_2	2,4-Cl	**$7d_3$**	4-NH_2	2-NO_2
$7a_7$	4-NO_2	4-NO_2	**$7d_4$**	4-NH_2	2,3-Cl
$7a_8$	4-NO_2	H	**$7d_5$**	4-NH_2	4-NO_2
z	2-OH	2,3-Cl	**$7d_6$**	4-NH_2	H
$7b_2$	2-OH	2,4-CH_3	**$7d_7$**	4-NH_2	2,4-Cl

Biological evaluation of final target compounds

***In vitro* antimicrobial activity**

The test antimicrobial activity was performed against Gram-positive bacteria: *Staphylococcus aureus* MTCC 3160, *Bacillus subtilis* MTCC 441; Gram negative bacteria: *Escherichia coli* MTCC 443, and fungal strains: *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 by the tube dilution method, using ciprofloxacin and fluconazole as standard drugs for antibacterial and antifungal activity, respectively. The standard and test samples were dissolved in DMSO to give concentrations of 100 µg/ml. Dilutions of test and standard compounds [ciprofloxacin (antibacterial) and fluconazole (antifungal)] were prepared in double strength nutrient broth-I.P (bacteria) and Sabouraud dextrose broth I.P. (fungi) [21]. The samples were incubated at 37 °C for 24 h (bacteria), at 25 °C for 7 days (*A. niger*), and at 37 °C for 48 h (*C. albicans*). After the mentioned incubated periods, the results were recorded in terms of minimum inhibitory concentration (MIC) (the lowest concentration of test substance which inhibited the growth of microorganisms) [22] (**Table 2**).

Free-radical scavenging activity

Free radical scavenging activity of synthesized compounds against stable free radical 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was determined spectrophotometrically. Electron-releasing ability of the corresponding compounds was measured from bleaching of purple-colored methanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [23]. Fifty microliters of various concentrations (25, 50, 75, and 100 µg/ml) of the compounds dissolved in methanol was added to 5 ml of a 0.004 % methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at

517 nm. Compound concentrations providing 50 % inhibition IC_{50} were calculated by a method based on Bondet *et al*. [24]. Tests were carried out in triplicate, and ascorbic acid was used as a positive control. Scavenging of DPPH free radicals was calculated as: DPPH scavenging activity (%) = $[(A_c-A_t) / A_c]\times100$, where A_c is the absorbance of the control reaction, and A_t is the absorbance of the test compound (**Table 3**).

Table 2 Minimum Inhibitory Concentration (MIC) of the target compound.

Compound	Bacterial species (MBC)		Fungal species (MFC)		
	S. aureus	***B. subtilis***	***S.aureus***	***C. albicans***	***A.niger***
$7a_1$	0.76	1.51	6.25	25	12.5
$7a_2$	1.09	2.19	25	3.12	6.25
$7a_3$	1.06	4.27	25	>50	25
$7a_4$	1.46	1.46	25	>50	25
$7a_5$	1.06	1.06	06.25	12.5	12.5
$7a_6$	2.02	1.01	3.12	12.5	12.5
$7a_7$	0.70	1.40	6.25	6.25	12.5
$7a_8$	0.51	2.06	>50	>50	12.5
$7b_1$	1.91	1.91	6.25	25	25
$7b_2$	0.70	2.79	25	6.25	6.25
$7b_3$	0.26	0.51	>50	12.5	>50
$7c_1$	1.91	1.91	>50	>50	>50
$7c_2$	1.40	0.70	>50	25	12.5
$7d_1$	1.03	1.03	25	3.12	6.25
$7d_2$	0.48	0.95	6.25	6.25	12.5
$7d_3$	1.27	0.63	6.25	12.5	12.5
$7d_4$	0.48	0.96	6.25	25	12.5
$7d_5$	0.84	1.68	25	12.5	25
$7d_6$	0.58	1.16	25	25	>50
$7d_7$	0.90	0.45	6.25	6.25	6.25
Ciprofloxacin	0.74	1.49	3.12	-	-
Fluconazole	1.36	2.73	-	6.25	3.12

Table 3 Free radical scavenging activity of the target compound.

Compound	$IC_{50\ (Mean \pm S.D.)}$ (µg/ml)	Compound	$IC_{50\ (Mean \pm S.D.)}$ (µg/ml)
$7a_1$	48.9±0.089	**$7b_3$**	41.3±0.172
$7a_2$	63.2±0.118	**$7c_1$**	69.4±0.473
$7a_3$	50.7±0.162	**$7c_2$**	50.2±0.164
$7a_4$	64.8±0.210	**$7d_1$**	47.4±0.473
$7a_5$	46.9±0.139	**$7d_2$**	55.6±0.125
$7a_6$	44.9±0.089	**$7d_3$**	43.8±0.789
$7a_7$	45.6±0.067	**$7d_4$**	42.6±0.210
$7a_8$	52.6±0.067	**$7d_5$**	45.8±0.210
$7b_1$	40.4±0.687	**$7d_6$**	63.2±0.118
$7b_2$	45.5±0.586	**$7d_7$**	41.8±0.148
Ascorbic acid	41.9±0.089		

Results and discussion

Chemistry

The synthetic work to synthesize the target compound has been sketched in **Figure 1**. The substituted benzoic acid was refluxed with conc. sulphuric acid and ethanol for 6-8 h to form ethyl aryl carboxylate. Further into treatment with hydrazine hydrate, ethanol aryl carbonyl hydrazides were formed, which yielded 5-substituted phenyl-1, 3, 4-oxadiazole-2-thiols when refluxed with potassium hydroxide, carbon disulphide, and ethanol for 4 - 6 h, followed by acidification by dil. HCl. Further, the substituted 1,3,4-oxadiazole-2-thiols heated with substituted aniline derivatives yielded 4, 5-diphenyl 4H-1, 2, 4-triazole-3-thiols. Finally, the chloracetylchloride was refluxed with 4,5-diphenyl 4H-1, 2, 4-triazole-3-thiols, which synthesized the desired products. All the synthesized derivatives were purified and recrystallized using ethanol and were characterized using elemental analysis and spectroscopic techniques. The target compounds were in full agreement with their structures. The characteristic strong peaks at 3364, 1678, and 1813 cm^{-1} corresponded to the aromatic ring, and the presence of carbonyl groups confirmed the structure of the final compound.

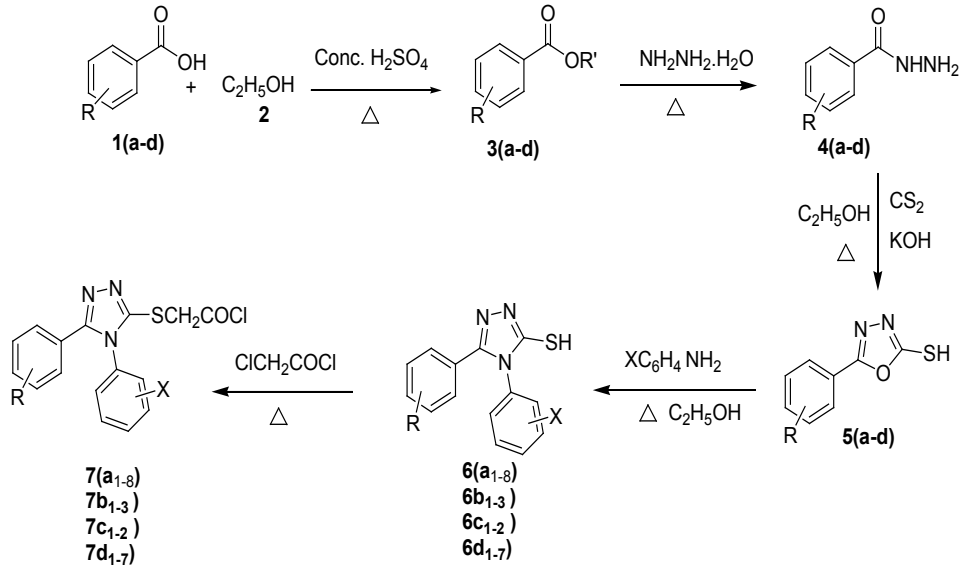

Figure 1 Synthetic route followed for the synthesis of novel 2-{4, 5-(substituted diphenyl)-4H-1,2,4-triazol-3-ylthio}acetyl chloride derivatives.

2-(4-(2,6-dichlorophenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride (7a_1)
Yield: 71.46 %, M.P.(°C): 112-114, Solid white, R_f value: 0.76, IR (cm^{-1}): 3042 (C-H str., aromatic), 1709 (C=O str., carbonyl), 1549 (N-O sym. str., nitro), 1321 (C-N str., aromatic), 1691 (C=C str., aromatic ring), 1436 (C-C str., aromatic in-ring), 1436 (C-H bend., CH_2), 768 (C-Cl str., aryl halide); MS ES + (ToF): *m/z* 639[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.74, 8.25, 8.25, 7.74, 7.2, 7.1, 7.2. Elem. Anal. Calcd. $C_{24}H_{19}N_3O_2S$: C, 68.51; H, 4.61; N, 10.11; Found: C, 67.51; H, 4.33; N, 10.15.

2-(4-(2,4-dimethylphenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride (7a_2)
Yield: 73.26 %, M.P.(°C): 122 - 124, Solid pale yellow, R_f value: 0.66 IR (cm^{-1}): 3014 (C-H str., aromatic), 1654 (C=O str., carbonyl), 1504 (N-O sym. str., nitro), 1340 (C-N str., aromatic), 1686 (C=C str., aromatic ring), 1434 (C-C str., aromatic in-ring), 1448 (C-H bend., CH_2), 738 (C-Cl str., alkyl halide); MS ES + (ToF): *m/z* 439[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.74, 8.25, 8.25, 7.74, 6.9, 6.9, 7.0, 2.35, 2.35. Elem. Anal. Calcd. $C_{24}H_{17}N_3O_2S$: C, 38.36; H, 3.00; N, 6.45; Found: C, 49.42; H, 3.37; N, 7.24.

2-(4-(4-chlorophenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7a_3$)
Yield: 61.36 %, M.P.(°C): 140 - 142, Solid white, R_f value: 0.69 IR (cm^{-1}): 3080 (C-H str., aromatic), 1671 (C=C str., aromatic ring), 1345 (C-N str., aromatic), 1614 (C=O str., carbonyl), 742 (C-Cl str., aryl halide), 1437 (C-H bend., CH_2), 1491 (N-O sym. str., nitro), 1437 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 558 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.74, 8.25, 8.25, 7.74, 7.2, 7.3, 7.3, 7.2. Elem. Anal. Calcd. $C_{12}H_{11}N_3O_4S$: C, 47.24; H, 3.67; N, 13.32; Found: C, 48.17; H, 3.61; N, 14.17.

2-(4-(4-methoxyphenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7a_4$)
Yield: 67.93 %, M.P.(°C): 118 - 120, Solid white, R_f value: 0.71, IR (cm^{-1}): 3098 (C-H str., aromatic), 1638 (C=C str., aromatic ring), 1324 (C-N str., aromatic), 1678 (C=O str., carbonyl), 736 (C-Cl str., alkyl halide), 1437 (C-H bend., CH_2), 1530 (N-O sym. str., nitro), 1400 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 459 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.74, 8.25, 8.25, 7.74, 7.2, 6.8, 6.8, 7.2, 3.73. Elem. Anal. Calcd. $C_{24}H_{19}N_3O_3S$: C, 57.12; H, 4.34; N, 8.67; Found: C, 59.15; H, 4.41; N, 8.89.

2-(4-(2-nitrophenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7a_5$)
Yield: 62.23 %, M.P.(°C): 160 - 162, Solid yellow, R_f value: 0.65, IR (cm^{-1}): 3014 (C-H str., aromatic), 1624 (C=C str., aromatic ring), 1405 (C-N str., aromatic), 1686 (C=O str., carbonyl), 844 (C-Cl str., alkyl halide), 1434 (C-H bend., CH_2), 1504 (N-O sym. str., nitro), 1340 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 673 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.74, 8.25, 8.25, 7.74, 8.2, 7.5, 7.7, 7.5. Elem. Anal. Calcd. $C_{23}H_{16}N_3O_3S$: C, 48.89; H, 2.82 N, 7.10; Found: C, 48.25; H, 2.88; N, 7.12.

2-(4-(2,4-dichlorophenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7a_6$)
Yield: 67.35 %, M.P.(°C): 146 - 148, Solid pale white, R_f value: 0.59, IR (cm^{-1}): 3035 (C-H str., aromatic), 1638 (C=C str., aromatic ring), 1324 (C-N str., aromatic), 167 8 (C=O str., carbonyl), 736 (C-Cl str., aryl halide), 1437 (C-H bend., CH_2), 1530 (N-O sym. str., nitro), 1400 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 509 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)** 4.48, 7.74, 8.25, 8.25, 7.74, 7.3, 7.2, 7.1. Elem. Anal. Calcd. $C_{13}H_{12}N_3O_5S$: C, 47.10; H, 3.38; N, 13.39; Found: C, 45.25; H, 3.31; N, 12.43.

2-(4,5-bis(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7a_7$)
Yield: 72.09 %, M.P.(°C): 165 - 167, Solid yellow, R_f value: 0.86, IR (cm^{-1}): 3068 (C-H str., aromatic), 1645 (C=C str., aromatic ring), 1388 (C-N str., aromatic), 1720 (C=O str., carbonyl), 774 (C-Cl str., alkyl halide), 1403 (C-H bend., CH_2), 1530 (N-O sym. str., nitro), 1403 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 508 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.74, 8.25, 8.25, 7.74, 7.5, 8.2, 8.2, 7.5. Elem. Anal. Calcd. $C_{22}H_{17}N_3O_2S$: C, 62.35; H, 4.01; N, 9.18; Found: C, 59.39; H, 4.07; N, 8.41.

2-(5-(4-nitrophenyl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7a_8$)
Yield: 63.46 %, M.P.(°C): 198 - 200, Solid yellow, R_f value: 0.73, IR (cm^{-1}) 3067 (C-H str., aromatic), 1625 (C=C str., aromatic ring), 1410 (C-N str., aromatic), 1716 (C=O str., carbonyl), 853 (C-Cl str., alkyl halide), 1440 (C-H bend., CH_2), 1507 (N-O sym. str., nitro), 1345 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 659 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.3, 7.3, 7.3, 7.3, 7.3, 7.74, 8.25, 8.25, 7.74. Elem. Anal. Calcd. $C_{23}H_{14}N_2O_3S$: C, 41.79; H, 2.46; N, 6.74; Found: C, 43.42; H, 2.24; N, 7.11.

2-(4-(2,6-dichlorophenyl)-5-(2-hydroxyphenyl)-4H-1,2,4-triazol-3-ylthio)acetylchloride ($7b_1$)
Yield: 55.36 %, M.P.(°C): 145 - 147, Solid pale white, R_f value: 0.76, IR (cm^{-1}): 3014 (C-H str., aromatic), 1643 (C=C str., aromatic ring), 1431 (C-N str., aromatic), 1728 (C=O str., carbonyl), 1481 (C-H bend., CH_2), 846 (C-Cl str., alkyl halide), 3629 (O-H str.), 1341 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 645 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 6.79, 7.05, 6.88, 7.31, 5.0, 7.2, 7.1, 7.2. Elem. Anal. Calcd. $C_{11}H_9ClN_3O_2S$: C, 42.98; H, 3.07; N, 11.72; Found: C, 41.76; H, 3.21; N, 11.59.

2-(4-(2,4-dimethylphenyl)-5-(2-hydroxyphenyl)-4H-1,2,4-triazol-3-ylthio)acetylchloride ($7b_2$)
Yield: 54.60 %, M.P.(°C): 167 - 169, Solid pale white, R_f value: 0.68, IR (cm^{-1}) 3045 (C-H str., aromatic), 1588 (C=C str., aromatic ring), 1328 (C-N str., aromatic), 1666 (C=O str., carbonyl), 819 (C-Cl str., alkyl

halide), 1445 (C-H bend., CH_2), 1402 (C-C str., aromatic in-ring), 3639 (O-H str.); MS ES + (ToF): *m/z* 648 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 6.79, 7.05, 6.88, 7.31, 5.0, 6.9, 6.9, 7.0, 2.35, 2.35. Elem. Anal. Calcd. $C_{22}H_{17}N_2O_2S$: C, 60.35; H, 4.04; N, 9.27; Found: C, 64.18 H, 4.12; N, 9.11.

2-(5-(2-hydroxyphenyl)-4-(2-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetylchloride (7b$_3$)
Yield: 61.30 %, M.P.(°C): 148 - 150, Solid yellow, R_f value: 0.70, IR (cm^{-1}): 3059 (C-H str., aromatic), 1570 (C=C str., aromatic ring), 1343 (C-N str., aromatic), 1624 (C=O str., carbonyl), 1438 (C-H bend., CH_2), 741 (C-Cl str., alkyl halide), 1540 (N-O sym. str., nitro), 1395 (C-C str., aromatic in-ring), 3479 (O-H str.); MS ES + (ToF): *m/z* 623 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 8.2, 7.5, 7.7, 7.5, 6.79, 7.05, 6.88, 7.31, 5.0. Elem. Anal. Calcd. $C_{24}H_{16}lN_3O_2S$: C, 45.49; H, 2.65; N, 6.94; Found: C, 47.52; H, 2.42; N, 6.47.

2-(4-(2,3-dimethylphenyl)-5-(3,5-dinitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetylchloride (7c$_1$)
Yield: 52.79 %, M.P.(°C): 172 - 174, Solid yellow, R_f value: 0.54, IR (cm^{-1}): 3015 (C-H str., aromatic), 1620 (C=C str., aromatic ring), 1344 (C-N str., aromatic), 1654 (C=O str., carbonyl), 1440 (C-H bend., CH_2), 819 (C-Cl str., alkyl halide), 1507 (N-O sym. str., nitro), 1375 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 429 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 8.80, 9.08, 8.80, 6.9, 6.9, 6.9, 2.35, 2.35. Elem. Anal. Calcd. $C_{23}H_{14}N_2O_2S$: C, 47.19; H, 2.26; N, 6.41; Found: C, 47.25; H, 2.41; N, 6.57.

2-(5-(3,5-dinitrophenyl)-4-(2-nitrophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride (7c$_2$)
Yield: 71.23 %, M.P.(°C): 145 - 147, Solid yellow, R_f value: 0.56, IR (cm^{-1}): 3015 (C-H str., aromatic), 1624 (C=C str., aromatic ring), 1435 (C-N str., aromatic), 1707 (C=O str., carbonyl), 1445 (C-H bend., CH_2), 843 (C-Cl str., alkyl halide), 1506 (N-O sym. str., nitro), 1343 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 369 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 8.2, 7.5, 7.7, 7.5, 8.80, 9.08, 8.80. Elem. Anal. Calcd. $C_{12}H_{10}N_3O_4S$: C, 43.68; H, 3.07; N, 11.82; Found: C, 42.84; H, 3.12; N, 11.98.

2-(5-(4-aminophenyl)-4-(2,4-dimethylphenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride (7d$_1$)
Yield: 70.36 %, M.P.(°C): 122 - 124, Solid pale white, R_f value: 0.63, IR (cm^{-1}): 3043 (C-H str., aromatic), 1667 (C=C str., aromatic ring), 1487 (C-N str., aromatic), 1725 (C=O str., carbonyl), 1413 (C-H bend., CH_2), 817 (C-Cl str., alkyl halide), 1337 (C-C str., aromatic in-ring), 3464 (O-H str.), 3362 (N-H str., amino); MS ES + (ToF): *m/z* 693 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 6.9, 6.9, 7.0, 2.35, 2.35, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{24}H_{18}N_3O_2S$: C, 62.35; H, 4.02; N, 9.18; Found: C, 62.39; H, 4.15; N, 9.21.

2-(5-(4-aminophenyl)-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride (7d$_2$)
Yield: 68.46 %, M.P.(°C): 180 - 182, Solid white, R_f value: 0.78, IR (cm^{-1}): 3053 (C-H str., aromatic), 1674 (C=C str., aromatic ring), 1368 (C-N str., aromatic), 1708 (C=O str., carbonyl), 1403 (C-H bend., CH_2), 1343 (C-C str., aromatic in-ring), 3358 (N-H str., amino), 767 (C-Cl str., aryl halide); MS ES + (ToF): *m/z* 483[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.2, 7.3, 7.3, 7.2, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{22}H_{15}Cl_2N_3O_2S$: C, 41.59; H, 2.26; N, 6.34; Found: C, 41.63; H, 2.18; N, 6.49.

2-(5-(4-aminophenyl)-4-(2-nitrophenyl)-4H-1,2,4-triazol-3-ylthio) acetyl chloride (7d$_3$)
Yield: 63.20 %, M.P.(°C): 174 - 176, Solid yellow, R_f value: 0.73, IR (cm^{-1}): 3040 (C-H str., aromatic), 1673 (C=C str., aromatic ring), 1374 (C-N str., aromatic), 1720 (C=O str., carbonyl), 1438 (C-H bend., CH_2), 742 (C-Cl str., alkyl halide), 1344 (C-C str., aromatic in-ring), 3375 (N-H str., amino), 1509 (N-O str., nitro); MS ES + (ToF): *m/z* 453[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 8.2, 7.5, 7.7, 7.5, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{11}H_{10}N_2O_4S$: C, 41.98; H, 3.08; N, 11.82; Found: C, 41.92; H, 3.11 N, 12.69.

2-(5-(4-aminophenyl)-4-(2,6-dichlorophenyl)-4H-1,2,4-triazol-3-ylthio) acetylchloride ($7d_4$)
Yield: 71.35 %, M.P.(°C): 125 - 127, Solid white, R_f value: 0.76, IR (cm^{-1}): 3036 (C-H str., aromatic), 1624 (C=C str., aromatic ring), 1410 (C-N str., aromatic), 1717 (C=O str., carbonyl), 1436 (C-H bend., CH_2), 3362 (N-H str., amino), 1344 (C-C str., aromatic in-ring), 743 (C-Cl str., aryl halide); MS ES + (ToF): *m/z* 473[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.2, 7.1, 7.2, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{22}H_{17}Cl_2N_3O_2S$ C, 57.54; H, 3.48; N, 8.43; Found: C, 38.50; H, 3.54; N, 8.68.

2-(5-(4-aminophenyl)-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio) acetyl chloride ($7d_5$)
Yield: 57.26 %, M.P.(°C): 175 - 177, Solid yellow, R_f value: 0.66, IR (cm^{-1}): 3072 (C-H str., aromatic), 1627 (C=C str., aromatic ring), 1406 (C-N str., aromatic), 1716 (C=O str., carbonyl), 848 (C-Cl str., alkyl halide), 1437 (C-H bend., CH_2), 3375 (N-H str., amino), 1343 (C-C str., aromatic in-ring), 1308 (N-O str., nitro) ; MS ES + (ToF): *m/z* 659[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.5, 8.2, 8.2, 7.5, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{22}H_{14}N_3O_2S$: C, 43.33; H, 2.28; N, 6.36; Found: C, 41.29; H, 2.26; N, 6.32.

2-(5-(4-aminophenyl)-4-phenyl-4H-1, 2, 4-triazol-3-ylthio) acetyl chloride ($7d_6$)
Yield: 62.70 %, M.P.(°C): 163 - 165, Solid pale white, R_f value: 0.71, IR (cm^{-1}): 3034 (C-H str., aromatic), 1674 (C=C str., aromatic ring), 1399 (C-N str., aromatic), 1717 (C=O str., carbonyl), 1443 (C-H bend., CH_2), 764 (C-Cl str., alkyl halide), 3366 (N-H str., amino), 1345 (C-C str., aromatic in-ring); MS ES + (ToF): *m/z* 383[M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.3, 7.3, 7.3, 7.3, 7.3, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{10}H_{11}N_3O_4S$: C, 38.72; H, 2.81; N, 11.39; Found: C, 38.48; H, 2.67; N, 10.95.

2-(5-(4-aminophenyl)-4-(2,3-dichlorophenyl)-4H-1,2,4-triazol-3-ylthio)acetyl chloride ($7d_7$)
Yield: 62.70 %, M.P.(°C): 189 - 191, Solid white, R_f value: 0.68, IR (cm^{-1}): 3034 (C-H str., aromatic), 1678 (C=C str., aromatic ring), 1407 (C-N str., aromatic), 1813 (C=O str., carbonyl), 1453 (C-H bend., CH_2), 3350 (N-H str., amino), 1345 (C-C str., aromatic in-ring), 766 (C-Cl str., aryl halide); MS ES + (ToF): *m/z* 541 [M^++1]; **^{1}H NMR, δppm (DMSO-d6)**4.48, 7.2, 7.1, 7.1, 7.23, 6.52, 6.52, 7.23, 4.0. Elem. Anal. Calcd. $C_{24}H_1Cl_2N_3O_2S$: C, 51.44; H, 3.16; N, 7.29; Found: C, 51.40; H, 3.22; N, 7.30.

***In vitro* antimicrobial activity**

All the newly synthesized 2-{4, 5-(substituted diphenyl)-4H-1, 2, 4-triazol-3-ylthio} acetyl chloride derivatives were evaluated for their antibacterial and antifungal activity by the tube dilution method. (**Figure 1**).

From the observed MIC values, it was concluded that the compound **$7a_6$** was found to be most active antibacterial against the selected strains; this may be due to the presence of strong electron-withdrawing dichloro groups on the benzene ring attached to the triazole ring (**Figure 2**). Further, the compounds **$7a_1$ = $7d_4$ > $7b_1$ > $7d_1$** were found to have significant antibacterial activity. The high antibacterial activity of compound **$7a_6$**, **$7b_1$**, and **$7d_4$** due to presence of electron withdrawing groups is supported by the reports given by Hussain *et al*. [25]. The compounds **$7a_2$** and **$7d_1$** were found to have better antifungal activity against the selected strains when compared with the standard drug Fluconazole. Increases in antifungal activity may be contributed to by electron-donating dimethyl groups on the benzene ring attached to the triazole ring, while the compounds **$7d_7$ = $7b_2$ > $7a_7$ > $7a_5$ = $7a_6$** were found to have moderate activity against the selected fungal strains. The role of electron-donating groups to increase antifungal activity is also supported by the study of Naika and Chikhalia [26]. Hence, from the synthesized triazole derivatives, a good antimicrobial activity was established.

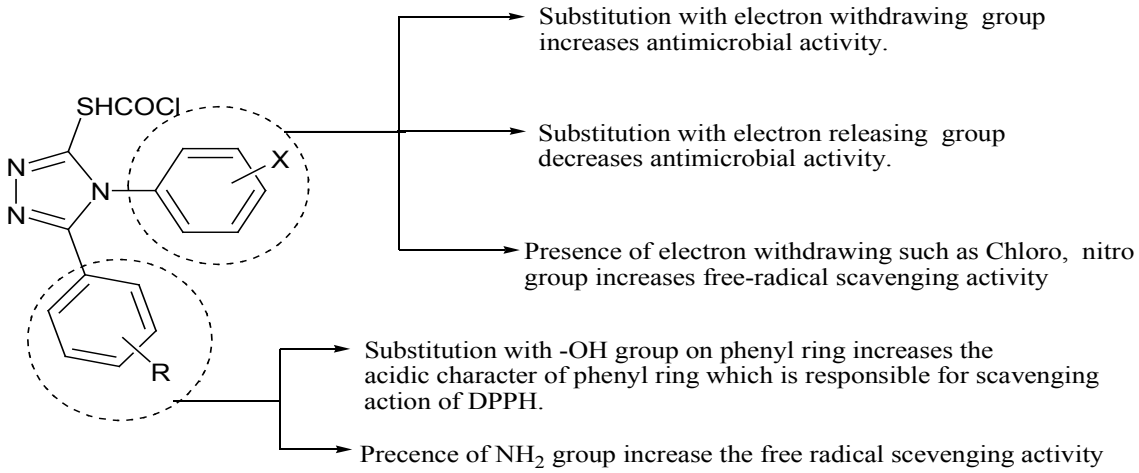

Figure 2 Structural activity relationship of the synthesized compounds in relation to their antimicrobial activity.

Free-radical scavenging activity

The results of antioxidant activity showed that few synthesized compounds exhibited significant antioxidant activity. Chloro (**7b$_1$, 7d$_7$, 7d$_4$**) and the substituted aromatic ring attached to the main triazole moiety showed a significant increase in antioxidant activity (among which **7b$_1$** was most active antioxidant), which is also favoured by Paulrasu *et al.* [27]. The presence of the -OH group **7b$_1$** and **7b$_3$** on the phenyl ring increases the acidic character of phenyl ring, which leads to an easy release of H^{+}, which is responsible for the scavenging action of DPPH. This is also supported by Mondal *et al.* [28], who explained the role of OH in increasing antioxidant activity. Substitution of the nitro group also increased the radical scavenging activity of compound **7b$_3$**, which is supported by Trujillo *et al.* [29]. In general, the presence of the electron-withdrawing group on the phenyl ring causes increases in antioxidant activity. The order of the antioxidant efficacy of the active compounds in the series is: **7b$_1$ > 7b$_3$ > 7d$_7$ > 7d$_4$**.

Structural activity relationship

From the results of the antimicrobial activity of the synthesized substituted 2-{4, 5-(substituted diphenyl)-4H-1,2,4-triazol-3-ylthio}acetyl chloride derivatives, the following structure activity relationships can be derived;

1) The presence of electron-withdrawing groups on the phenyl ring (**7a$_6$**, **7b$_1$**, **7d$_4$**) improved the antibacterial activity of the synthesized compound.

2) The presence of electron-releasing groups (-CH_3, -OCH_3, compound **7a$_2$**, **7d$_1$** and **7c$_1$**) increased the antifungal activity of the synthesized 1, 2, 4 triazole derivatives.

3) The antioxidant activity results indicated that the presence of -OH group **7b$_1$** and **7b$_3$** on the phenyl ring improved the acidic character of the phenyl ring, which is responsible for the scavenging action of DPPH.

4) The presence of the electron-withdrawing Chloro (**7b$_1$, 7d$_7$, 7d$_4$**) group on the phenyl ring attached to the triazole ring increases free-radical scavenging activity.

5) Substitution of nitro group **7b$_3$** on the phenyl ring also increases free-radical scavenging activity.

Conclusions

In the present study, the synthesis of novel 2-{4, 5-(substituted diphenyl)-4H-1,2,4-triazol-3-ylthio}acetyl chloride derivatives has been reported. All the synthesized derivatives were evaluated for their *in vitro* antimicrobial activity and free-radical scavenging activity. Among the evaluated derivatives, compound **7a$_6$** was found to be the most effective antibacterial, and compounds **7a$_2$** and **7d$_1$** had the most effective antifungal effect on the selected strains, as compared to the standard drugs. The results of the antioxidant activity showed that compound **7b$_1$** was found to be most active antioxidant, with 40.4±0.687 µg/ml. The results of the antioxidant assays proved that compounds **7b$_3$**, **7d$_7$**, and **7d$_4$** had the most promising free radical scavenging activity. From these observations, it may be suggested that substitution of the electron-withdrawing groups enhanced the antibacterial effect, and electron-donating groups enhanced the antifungal effect with substitution on the triazoles ring. Hence, the study may become useful for further research to identify new antimicrobial compounds to combat the problems of infection.

Acknowledgements

The authors acknowledge the Department of Pharmaceutical Sciences, M.D. University, Rohtak, for their full support during the work.

References

[1] NM Khalifa, AH Adel, SI Abd-Elmoez, OA Fathalla and AAA El-Gwaad. Antimicrobial activity of newly synthesized imidazolones, their oxadiazolyl and acyclic C-nucleosides. *Res. Chem. Intermediat.* 2015; **41** 1-11.

[2] Z Chen, R Bertin and G Froldi. Evaluation of anogeissus acuminata (Roxb. ex DC.) in diabetes mellitus and its complications *Food. Chem*. 2013; **138**, 414-20.

[3] M Aufort, J Herscovici, P Bouhours, N Moreau and C Girard. Synthesis and antibiotic activity of a small molecules library of 1,2,3-triazole derivatives. *Bioorg. Med. Chem. Lett.* 2008; **18**, 1195-8.

[4] SMI Badr and RM Barwa. Synthesis of some new [1,2,4]triazolo[3,4-b][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles starting from 5-nitro-2-furoic acid and evaluation of their antimicrobial activity. *Bioorg. Med. Chem. Lett.* 2013; **19**, 4506-12.

[5] GL Almajan, SF Barbuceanu, ER Almajan, C Draghici and G Saramet. Synthesis, characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties. *Eur. J. Med. Chem.* 2009; **44**, 3083-9.

[6] H Yuksek, Z Ocak, M Alkan, S Behceci and M Ozdemir. Synthesis and determination of pKa values of some new 3,4-disubstituted-4,5-dihydro-1H-1,2,4-triazol-5-one derivatives in non-aqueous solvents. *Molecules* 2004; **9**, 232-40.

[7] V Padmavathi, RG Sudhakar, A Padmaja, P Kondaiah and A Shazia. Synthesis and antimicrobial studies of novel methylene bridged benzisoxazolyl imidazo[2,1-b][1,3,4]thiadiazole derivatives. *Eur. J. Med. Chem.* 2012; **44**, 2106-12.

[8] P Selvam, M Chandramohan, ED Clercq and M Witvrouw. Synthesis and anti-HIV activity of 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N (4,6-dimethyl-2-pyrimidinyl)-benzenesulphonamide and its derivatives. *Eur. J. Pharm. Sci.* 2001; **14**, 313-6.

[9] SP Aytac, B Tozkoparan, F Betu, B Kaynak, G Aktay, O Goktas and S Unuvar. Synthesis and pharmacological evaluation of 1,3,4-oxadiazole bearing bis(heterocycle) derivatives as anti-inflammatory and analgesic agents. *Eur. J. Med. Chem.* 2009; **44**, 4528-38.

[10] M Dhooghe, S Vandekerckhove, K Mollet, K Vervisch, S Dekeukeleire and L Lehoucq. Synthesis of 2-amino-3-arylpropan-1-ols and 1-(2,3-diaminopropyl)-1,2,3-triazoles and evaluation of their antimalarial activity. *Beil. J. Org. Chem.* 2011; **7**, 1745-52.

[11] MS Costa, N Boechat, EA Rangel, FD Silva, AM Souza and CR Rodrigues Synthesis, tuberculosis inhibitory activity, and SAR study of N-substituted-phenyl-1,2,3-triazole derivatives. *Bioorg. Med. Chem. Lett.* 2006; **14**, 8644-53.

[12] P Shanmugavelan, S Nagarajan, MS Kumar, A Ponnuswamy, P Yogeeswari and D Sriram. Efficient synthesis and in vitro antitubercular activity of 1,2,3-triazoles as inhibitors of Mycobacterium tuberculosis.*Bioorg. Med. Chem. Lett.* 2011; **21**, 7273-6.
[13] HM Faidallah, AK Khalid and MA Abdullah. Synthesis and biological evaluation of new N,N′-bis(1-substituted-ethylidene)-ethane1,2-diamine and their acetyl and trifluoroacetyl derivatives as cytotoxic and antimicrobial agents. *J. Fluorine. Chem.* 2011; **132**, 870-77.
[14] S Oida, T Yawara, K Toshiyuki, N Yoshie, S Atsushi and T Teruo. Synthesis and antifungal activities of R-102557 and related dioxane-triazole derivatives. *Chem. Pharm. Bull.* 2000; **48**, 694-707.
[15] M Serwar, T Akhtar, S Hameed and KM Khan. Synthesis, urease inhibition and antimicrobial activities of some chiral 5-aryl-4-(1-phenylpropyl)-2H-1,2,4-triazole-3(4H)-thiones. *Arch. Org. Chem.* 2009; **7**, 210-21.
[16] RK Sharma, K Shrivastava, V Daniel, MS Panwar and S Goyal. Critical analysis of cell block versus smear examination in effusions*Int. J. Pharm. Sci.* 2010; **2**, 502-7.
[17] N Siddiqui and W Ahsan. Triazole incorporated thiazoles as a new class of anticonvulsants: Design, synthesis and in vivo screening. *Eur. J. Med. Chem.* 2010; **45**, 1536-43.
[18] I Khan, S Ali, S Hameed, NH Rama and MT Hussain. Synthesis, antioxidant activities and urease inhibition of some new 1,2,4-triazole and 1,3,4-thiadiazole derivatives. *Eur. J. Med. Chem.* 2010; **45**, 5200-7.
[19] K Sancak, Y Unver, D Unluer, E Dugdu, G Kor, F Celik and E Birinci. Synthesis of 2-acylamino, 2-aroylamino and ethoxycarbonyl imino-1, 3, 4-thiadiazoles as antitumor agents. *Turk. J. Chem.* 2010; **36**, 457-66.
[20] B Tozkoparan, G Aktay and E Yesilada. Synthesis of some 1,2,4-triazolo[3,2-b]-1,3-thiazine-7-ones with potential analgesic and antiinflammatory activities. *Farmaco* 2002; **57**, 145-52.
[21] Pharmacopoeia of India Ministry of Health Department. Vol II. Ministry of Health and Family Welfare. Nirman Bhavan, Government of India, New Delhi, 1996, p. A88.
[22] JG Cappucino and N Sherman. *Microbiology: A Laboratory Manual*. Addison Wesley, California 1999, p. 263.
[23] MS Blois. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; **181**, 1199-200.
[24] V Bondet, W Brand-Williams and C Berset. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *Food. Sci. Tech.* 1997; **30**, 609-15.
[25] S Hussain, J Sharma and M Amir. Synthesis and antimicrobial activities of 1,2,4-triazole and 1,3,4-thiadiazole derivatives of 5-amino-2-hydroxybenzoic acid. *Eur. J. Med. Chem.* 2008; **5**, 963-8.
[26] TA Naik and KH Chikhalia. Studies on synthesis of pyrimidine derivatives and their pharmacological evaluation. *J. Chem*. 2007; **4**, 60-6.
[27] K Palrasu, A Duraikannu and B Thirunavukkarasu. Synthesis of 4-methyl-N'-(3-alkyl-2r,6c-diarylpiperidin-4-ylidene)-1,2,3-thiadiazole-5-carbohy drazides with antioxidant, antitumor and antimicrobial activities. *Org. Biomol. Chem*. 2014; **12**, 5911-21.
[28] P Mondal, S Jana and LK Kanthal. Synthesis of some new isoxazoline derivatives of chalconised indoline 2-one as a potential analgesic, antibacterial and anthelmimtic agents. *T. Ph. Res.* 2010; **3**, 17-26.
[29] M Trujillo, E Gallardo, A Madrona, L Bravo, B Sarria, JA Gonzaalez-Correa and JL Espartero. Synthesis and antioxidant activity of nitrohydroxytyrosol and its acyl derivatives *J. Agr. Food Chem.* 2014; 62, 10297-303.