International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(3): May-June 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 04-06-2012; Revised; Accepted: 15-06-2012

DESIGN, SYNTHESIS AND CHARACTERIZATION OF NOVEL TRIAZOLE DERIVATIVES AS POTENT ANTIMICROBIAL AGENTS

Swati C. Gajera*, Jagath Pillai, Nilesh Darji, Dr. Bhagirath Patel

Sat Kaival College of Pharmacy, Sarsa, Anand, Gujarat, India

Keywords:

Triazole derivatives, Azole moiety, Antimicrobial activity

For Correspondence:

Swati C. Gajera

Sat Kaival College of Pharmacy, Sarsa, Anand, Gujarat, India

E-mail:

swatiyaxi@yahoo.co.in

ABSTRACT

Triazole shows activities Antibacterial, Antifungal, Antiviral, and Anti-inflammatory, Anticonvulsant, and Antituberculosis, Antiparasitic activity. The Azole moiety is an important part of antimicrobial drugs; hence it was thought of to carry out synthesis of substituted triazole. As part of ongoing studies in developing new antimicrobials, a novel series of substituted-N-(nitrobenzylidene)-3-p-tolyl-4H 1, 2, 4-triazole-4-amine were synthesized in order to determine their antibacterial and antifungal activity. The structures of these compounds were confirmed by IR, 1H NMR, MASS spectra and also micro-analytical data. The novel synthesized compounds were evaluated for their antimicrobial activity against variety of bacterial and fungal strains. From the antimicrobial activity done, out of all the synthesised compounds, the compounds SKSG101204 & SKSG101205 show maximum antimicrobial activity.

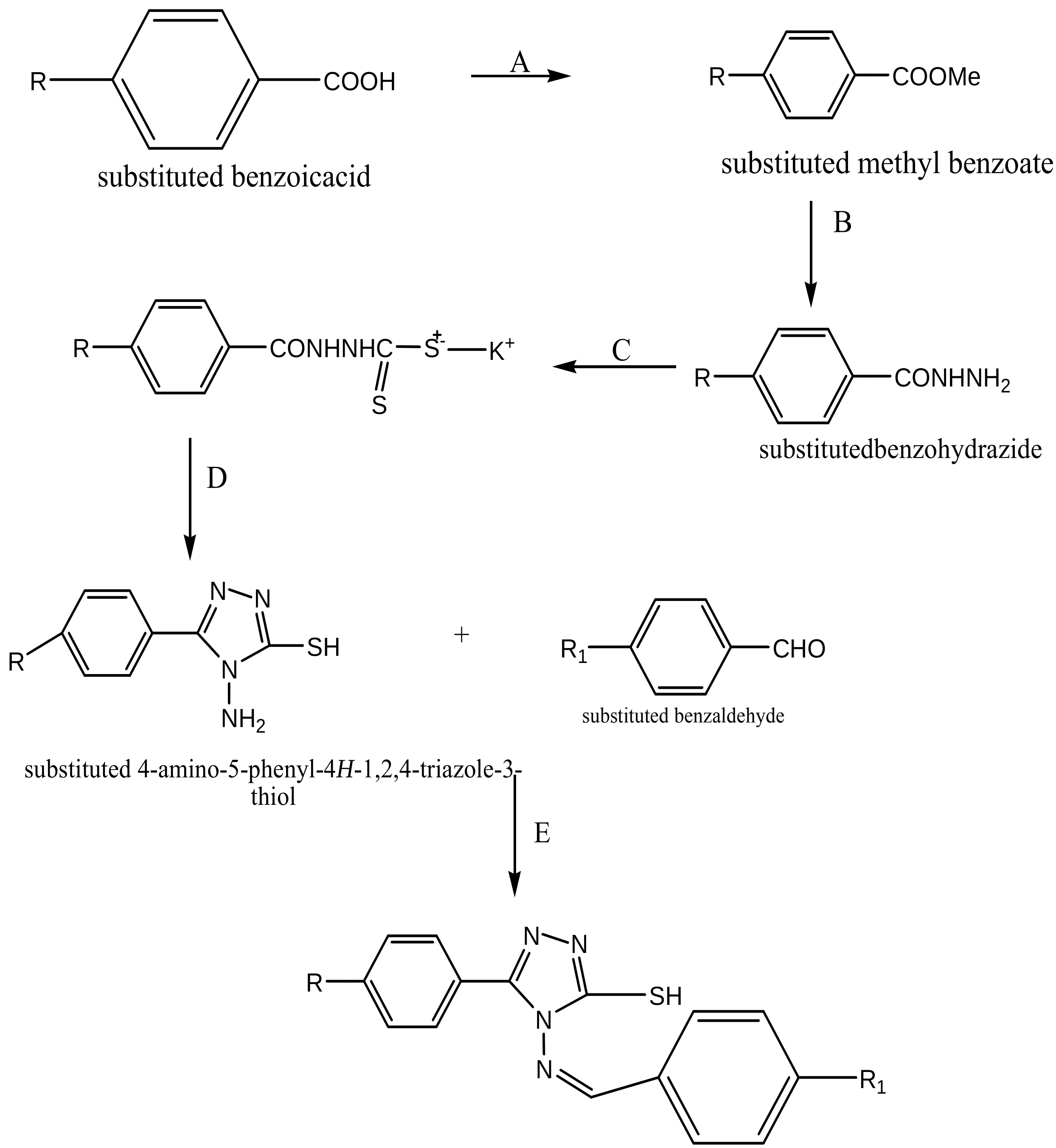
INTRODUCTION

The major drawback of current treatment of infectious diseases are challenging due to resistance to antimicrobial agents and their side effects. Numbers of new infectious diseases have been discovered. So, there is an urgent need to develop novel drugs molecule, with fewer side effects, broad spectrum activity, better stability and drugs with shorter lengths for the treatment of infectious disease. Microbial infection indirectly invites another disease due to low immunity power during infectious conditions. In the global market there are many antimicrobial agents but they all have some or more drawbacks, so to remove these drawbacks it is necessary to develop new antimicrobial agents. Rationale behind this work is to develop a molecule having good anti-microbial activity. With the reference of the various literatures, it revealed that the Triazole show a diverse range of biological activities. They possess activities antibacterial[2], antifungal[3], antiviral[4], and anti-inflammatory[6], antituberculosis[5], anti parasitic activity[5], antilmalerial[8], anticonvulsant[9]. The Azole moiety is an important and frequent insecticide, agrochemical structure feature of many biologically active compounds such as cytochrome P450 enzyme inhibitors and peptide analogue inhibitor.[7] Various 1, 2, 4-triazole and 4, 5-dihydro-1H-1, 2, 4-triazole-4-ones have been found to be associated with diverse pharmalogical activites. For e.g. Antifungal for the treatment of superficial and systematic infections, diuretic, anti-inflammatory, and plan growth regulators.[10] The synthesized compounds will be tested for their in vitro antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus, Bacillus subtilus*, the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa.*

MATERIAL AND METHODS

All Chemicals,Solvents were obtained from E-Merck, India (AR grade) and were used further purification. The melting point of the synthesized compounds were determined in open capillary using VEEGO MELTING POINT APPARATUS model VMP-D and recorded in Celsius without correction. Purity of the compound was verified by precoated TLC plates (E- Merck Kieselgel 60 F_{254}). The Infrared spectra for the synthesized compounds were recorded using SHIMADZU-FTIR 8400S spectrometer using KBr as a back ground. ^{1}H NMR spectra of the synthesized compounds were taken using BRUKER Advance-II 400 NMR spectrometer using Tetramethyl silane as an internal standard. ^{1}H NMR spectra were recorded with DMSO as a solvent & the chemical shift data were expressed as delta values related to TMS. Mass spectra of the synthesized compounds were taken using 2010EV LCMS SHIMADZU instrument at 70 eV.

EXPERIMENTAL PROCEDURE



substituted-N-(nitrobenzylidene)-3-p-tolyl-4H 1,2,4-triazole-4-amine

A= CH_3OH, H_2SO_4 , 12 hr reflux
B= NH_2NH_2
C=KOH, CS_2, 5hr stirring
D=NH_2NH_2, 8hr reflux
E = CH_3OH, 5hr reflux

Figure 1: ROUTE OF SYNTHESIS

A methanolic solution of 4- substituted benzoic acid is refluxed for 12 h in presence of H_2SO_4 to yield ester. The resulting product is treated with hydrazine hydrate to get acid hydrazide. The hydrazide (C) (0.02 mole) is treated with alcoholic solution of KOH (0.03 mole) and carbon disulphide for 5 h at room temperature with constant stirring. The potassium salt, also known as dithiocarbazate is filtered and washed with ether. In dithiocarbazate (0.02 mole), hydrazine hydrate (0.04 mole) is added drop wise and the mixture is refluxed for 8 hours in oil bath. It is poured in water; acidified and resulting solid is filtered, washed with water and purified by KOH treatment to give target molecule. Equimolar of target molecule and different aldehydes are taken in methanol.The mixture is heated in water bath for about 10 h. The solution is poured in ice cold water and the resulting solid is filtered, washed and recrystallized from ethanol. Melting points are determined on an electro thermal capillary melting point apparatus and are uncorrected. All the compounds are checked for their homogeneity by TLC using silica as stationary phase checked. IR spectra wares recorded by KBr (cm^{-1}) pellet method, ^1H NMR spectra are recorded by taking TMS as internal standard (chemical shift in σ ppm).

(Z)-4-(4-nitrobenzylideneamino)-5-phenyl-4H-1, 2, 4-triazole-3-thiol

Yield (%): 76; M.P. (^{O}C): 208-212; R_f: 0.53; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-$C\underline{H}_3$), 1.20-1.33 [(m, 12H, -NH-CH_2-$(C\underline{H}_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-$C\underline{H}_3$], 1.59-1.67 [(t, 2H, -NH-$C\underline{H}_2$-CH_2-$(CH_2)_5$- CH_3], 4.42-4.47 (q, 2H, $COOC\underline{H}_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-$\underline{H}$), 7.87-7.89 [(t, 1H, -N$\underline{H}$-CH_2-$(CH_2)_6$-CH_3], 8.25 (s, 1H, heteroaromatic-$\underline{H}$); Mass spectra (m/z): $326(M+1)^+$.

(Z)-4-(4-nitrobenzylideneamino)-5-(4-aminophenyl)-4H-1, 2, 4-triazole-3-thiol

Yield (%): 84; M.P. (^{O}C): 208-212; R_f: 0.53; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-$C\underline{H}_3$), 1.20-1.33 [(m, 12H, -NH-CH_2-$(C\underline{H}_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-$C\underline{H}_3$], 1.59-1.67 [(t, 2H, -NH-$C\underline{H}_2$-CH_2-$(CH_2)_5$- CH_3], 4.42-4.47 (q, 2H, $COOC\underline{H}_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-$\underline{H}$), 7.87-7.89 [(t, 1H, -N$\underline{H}$-CH_2-$(CH_2)_6$-CH_3], □ 8.25 (s, 1H, heteroaromatic-$\underline{H}$); Mass spectra (m/z): $341(M+1)^+$.

(Z)-4-(4-nitrobenzylideneamino)-5-(4-nitrophenyl)-4H-1, 2, 4-triazole-3-thiol

Yield (%): 88; M.P. (^{O}C): 174-176; R_f: 0.69; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-$C\underline{H}_3$), 1.20-1.33 [(m, 12H, -NH-CH_2-$(C\underline{H}_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-$C\underline{H}_3$], 1.59-1.67 [(t, 2H, -NH-$C\underline{H}_2$-CH_2-$(CH_2)_5$- CH_3], 4.42-4.47 (q, 2H, $COOC\underline{H}_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-H), 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3], 8.25 (s, 1H, heteroaromatic-H); Mass spectra (m/z): $371(M+1)^+$.

(Z)-4-(4-chlorobenzylideneamino)-5-(4-chlorophenyl)-4H-1, 2, 4-triazole-3-thiol

Yield (%): 45; M.P. (^OC): 138-140; R_f: 0.43; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-CH_3), 1.20-1.33 [(m, 12H, -NH-CH_2-$(CH_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-CH_3], 1.59-1.67 [(t, 2H, -NH-CH_2-CH_2-$(CH_2)_5$- CH_3], 8.25 (s, 1H, heteroaromatic-H), 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3], 7.26-7.41 (m, 3H, Aromatic-H), 4.42-4.47 (q, 2H, $COOCH_2$-CH_3); Mass spectra (m/z): $350(M+1)^+$.

(Z)-4-((3-(4-chlorophenyl)-5-mercapto-4H-1, 2, 4-triazol-4-ylimino)methyl)phenol

Yield (%): 75; M.P. (^OC): 188-190; R_f: 0.58; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-CH_3), 1.20-1.33 [(m, 12H, -NH-CH_2-$(CH_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-CH_3], 1.59-1.67 [(t, 2H, -NH-CH_2-CH_2-$(CH_2)_5$- CH_3], 4.42-4.47 (q, 2H, $COOCH_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-H), 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3], 8.25 (s, 1H, heteroaromatic-H); Mass spectra (m/z): $331(M+1)^+$.

(Z)-4-(4-nitrobenzylideneamino)-5-p-tolyl-4H-1, 2, 4-triazole-3-thiol

Yield (%): 67; M.P. (^OC): 212-214; R_f: 0.45; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-CH_3), 1.20-1.33 [(m, 12H, -NH-CH_2-$(CH_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-CH_3], 8.25 (s, 1H, heteroaromatic-H), 4.42-4.47 (q, 2H, $COOCH_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-H), 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3], 1.59-1.67 [(t, 2H, -NH-CH_2-CH_2-$(CH_2)_5$- CH_3]; Mass spectra (m/z): $340(M+1)^+$.

(Z)-2-((3-mercapto-5-(4-nitrophenyl)-4H-1, 2, 4-triazol-4-ylimino) methyl) phenol

Yield (%): 67; M.P. (^OC): 240-242; R_f: 0.45; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-CH_3),1.20-1.33 [(m, 12H, -NH-CH_2-$(CH_2)_6$- CH_3], 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-CH_3],1.59-1.67 [(t, 2H, -NH-CH_2-CH_2-$(CH_2)_5$- CH_3], 4.42-4.47 (q, 2H, $COOCH_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-H), 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3], 8.25 (s, 1H, heteroaromatic-H); Mass spectra (m/z): $342(M+1)^+$.

(Z)-4-(2-amino-3, 4-dimethylbenzylideneamino)-5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol

Yield (%): 69; M.P. (^OC): 236-238; R_f: 0.55; ^1H- NMR (DMSO, ppm): 0.86-0.89 (t, 3H, $COOCH_2$-CH_3), 1.20-1.33 [(m, 12H, -NH-CH_2-$(CH_2)_6$- CH_3], 1.40-1.49 [(3H, -NH-$(CH_2)_7$-CH_3], 1.59-1.67 [(t, 2H, -NH-CH_2-CH_2-$(CH_2)_5$- CH_3], 4.42-4.47 (q, 2H, $COOCH_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-H), 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3], 8.25 (s, 1H, heteroaromatic-H); Mass spectra (m/z): $369(M+1)^+$.

(Z)-4-(3-nitrobenzylideneamino)-5-(2-aminophenyl)-4H-1, 2, 4-triazole-3-thiol

Yield (%): 78; M.P. (^OC): 220-222; R_f: 0.65; ^1H- NMR (DMSO, ppm): 8.25 (s, 1H, heteroaromatic-H), 0.86-0.89 (t, 3H, $COOCH_2$-CH_3),1.20-1.33 [(m, 12H, -NH-CH_2-$(CH_2)_6$-CH_3], 7.87-7.89 [(t, 1H, -NH-CH_2-$(CH_2)_6$-CH_3],1.59-1.67 [(t, 2H, -NH-CH_2-CH_2-$(CH_2)_5$-CH_3], 4.42-4.47 (q, 2H, $COOCH_2$-CH_3), 7.26-7.41 (m, 3H, Aromatic-H), 1.40-1.49 [(t, 3H, -NH-$(CH_2)_7$-CH_3]; Mass spectra (m/z): $341(M+1)^+$.

BIOLOGICAL EVALUATION

Determination of Antimicrobial Activity

Modified Kirby-Bauer method was used for the evaluation of antimicrobial activity of the synthesized compounds. Circular paper disks of 6 mm diameter were impregnated with the specific amount of the test sample and were placed on a Mueller Hinton agar medium in the petri plates, which was inoculated on its surface with one of the test organisms After incubation, the plates were observed for the growth inhibition zones around the paper disks. The diameter of the zone of inhibition is proportional to the antimicrobial activity of the substance. The diameters of the zones of inhibition were compared with that produced by the standard antibiotics.

All the test compounds were tested at 50µg level. To obtain this, solutions containing 10mg/ml of the test compounds were prepared in sterile dimethylformamide (DMF) and 5µl each of the solutions was added into each disk using a micropipette. Ampicillin(10µg) and griseofulvin(2.5g) were used as standard antibiotics. All the solutions were prepared using aseptic precautions.

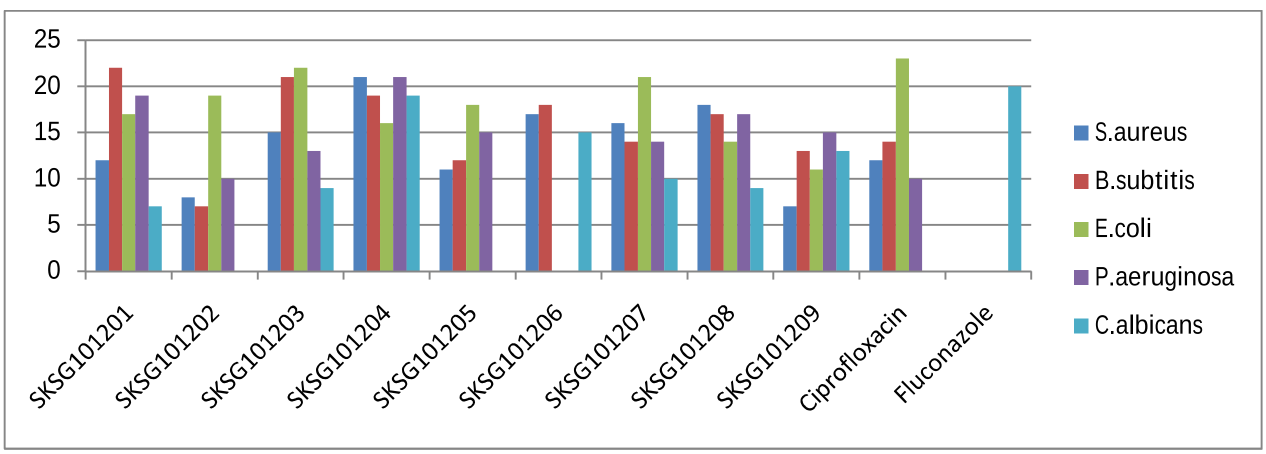


Figure 2: Biological activity (Zone of inhibition) of synthesized compounds

RESULTS AND DISCUSSION

All synthesized compounds were evaluated for antibacterial activity against *E. coli, P. aeruginosa, B. subtilis, S. aureus* and antifungal activity against *C. albicans*.

Table 1: Zone of Inhibition of Synthesized target compounds for antibacterial and antifungal activity

Sr. No	Compound Number	Diameter of zone of inhibition (mm)				
		S.aureus	***B.subtitis***	***E.coli***	***P.aeruginosa***	***C.albicans***
1	SKSG101201	12	22	17	19	7
2	SKSG101202	8	7	19	10	-
3	SKSG101203	15	21	22	13	9
4	SKSG101204	21	19	16	21	19
5	SKSG101205	11	12	18	15	-
6	SKSG101206	17	18	-	-	15
7	SKSG101207	16	14	21	14	10
8	SKSG101208	18	17	14	17	9
9	SKSG101209	7	13	11	15	13
11	Ciprofloxacin	12	14	23	10	-
12	Fluconazole	-	-	-	-	20

Triazole moiety possess activities antibacterial, antifungal, antiviral, and anti-inflammatory, anticonvulsant, and antituberculosis,antiparasitic activity. The Azole moiety is an important and frequent insecticide, agrochemical structure feature of many biologically active compounds such as cytochrome P450 enzyme inhibitors and peptide analogue inhibitor & hence was found to be good antimicrobial. Synthesis of 1, 2, 4- triazoles & its derivatives was carried out using substituted benzoic acids & substituted aromatic aldehydes. From the antimicrobial activity done, out of all the synthesised compounds, the compounds SKSG101204 & SKSG101205 showed more & better antimicrobial activity than the other synthesized compounds due to the presence of halogen element. The antimicrobial activity of the synthesised compounds was taken using ciprofloxacin as standard.

CONCLUSION

Triazole moiety possess activities antibacterial, antifungal, antiviral, and anti-inflammatory, anticonvulsant, and antituberculosis,antiparasitic activity. Synthesis of 1,2,4- triazoles & its derivatives was carried out using substituted benzoic acids & substituted aromatic aldehydes. The antimicrobial activity of the synthesised compounds was taken using ciprofloxacin as standard. From the antimicrobial activity done, out of all the synthesised compounds, the compounds SKSG101204 & SKSG101205 showed more & better antimicrobial activity than the other synthesized compounds due to the presence of halogen element.

ACKNOWLEDGEMENT

The authors are thankful to SAIF, Panjab University, Chandigarh and Oxygen healthcare pvt ltd. for providing the spectral and analytical data of the compounds. Authors are also thankful to the Head, Department of Chemistry for providing the laboratory facility. We are grateful to the AICTE, New Delhi, India, for financial assistance.

BIBLIOGRAPHY

1. Bauman, R. "Bacterial Cell Wall Structure", *Microbiology*, 2005.
2. ROY, R (2005), "Synthesis and Antimicrobial Activity of 1, 2, 4-Triazoles." *E-Journal of Chemistry*, Vol. 2(6), 1-5.
3. Freddy, H (2011), "Synthesized and antifungal activity of a series of compound 3-[4-(substituted phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl-methoxy)-phenyl]-2-phenyl-3*H*-quinazoline-4-one", *J. Chem. Pharma. Res.*, Vol. 2(2):120-133.
4. Sztanke, K; Tuzimski, T; Szerszen, MK (2008), *Eur. J. Med. Chem.*, Vol. 43, 404-41.
5. Krzysztof, S (2011), "Synthesized series 3-(un)substituted-7-aryl-5H-6,7-dihydroimidazo[2,1-c][1,2,4]triazoles compounds and its derivatives which is screened for antimicrobial and antifungal activity," *J. Chem. Pharma. Res.*, Vol. 3(2):126-133.
6. Prasad (2011), "Synthesized a series of derivatives of [4-Amino-3-Aryloxy alkyl, 5-Mercapto-1,2,4-Triazole] and evaluated them for anti-inflammatory activity", *J. Chem. Pharma. Res.*, Vol. 3(2):150-159.
7. Kumar, H (2001), "Synthesized and anti-inflammatory activity of a series of compound 5-[(Biphenyl-4-yloxy)methyl]-4-n- substituents-3-marcapto-(4H)-1,2,4-triazole", *Bioorg. Med. Chem.*, Vol. 9, 2149-2153.
8. Freddy, H; Havaldar (2011), "Synthesized and anti malarial activity of triazole derivatives", *Bioorg. Med. Chem.*, 15, 5888-5904.
9. Mohammad, SA; Yoshihisa, O; Dong-Ung Lee (2011), "Structure-Anticonvulsant Activity Relationship of 4- or 5-Substituted 1-(2, 6-Dichloro-4-trifluoromethylphenyl)-1*H-1, 2, 3-triazole Analogues" J. Korean Soc. Appl. Biol. Chem.* Vol. 54(1), 149-153.
10. Singhal, N (2011) "Recent advancement of triazole derivatives and their biollogical significance", *Asian J. Pharm. Hea. Sci.*, 1,158-162.