Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2023, 12 (2): 85-91

(International Peer Reviewed Journal)

Synthesis and evaluation of antibacterial potential of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against Gram positive and Gram negative bacteria

Nisar Ahmad Lone[1] and Ali Mohd Lone[2]*

1. Department of Botany, Govt. Degree College for Women Baramulla, Jammu & Kashmir-193101, **INDIA**
2. Department of Chemistry, Govt. Degree College for Women Baramulla, Jammu & Kashmir-193101, **INDIA**

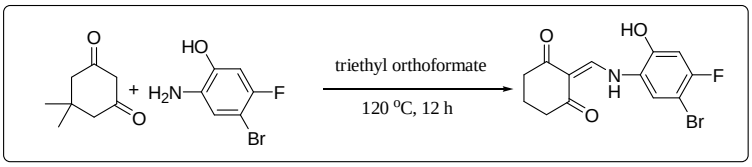
Email: loneali33@gmail.com

Accepted on 11th February, 2023

ABSTRACT

In the present study 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione was synthesized and evaluated for antibacterial activity. Disc diffusion method revealed that the zone of inhibition of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against Acetobacter aceti, Staphylococcus aureus, Klebsiella pneumonia and Enterobacter aerogenes bacterial strains was 18.5, 25, 19 and 28 mm, respectively at 5 µM concentration. The compound also exhibited good antibacterial activity against Acetobacter aceti, Klebsiella pneumonia and Entrobacter aerogenes bacterial strains at 2.5 µM concentration. The zone of inhibition of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against Entrobacter aerogenes, Klebsiella pneumonia and Acetobacter aceti was 17, 19 and 22 mm, respectively. In summary, the present study demonstrates that 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione has potential as effective anti-bacterial agent.

Graphical Abstract:



Synthesis of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione.

Keywords: Condensation, Antibacterial activity, Zone of inhibition, Chemotherapy, Disc diffusion method.

INTRODUCTION

Presently the world is confronted with a serious challenge because of the evolution of bacterial and fungal infections having resistance to the available drugs. Development of drug-resistant bacterial and fungal strainsis related with the extensive use of available antimicrobial agents. Antimicrobial

resistance is harmful to mankind, because most of the infectious microorganisms understand the mechanism of drug action and develop tolerance to it [**1-4**]. Therefore discovery of new generation drugs against these infections either from synthetic or natural sources are highly desired. In concern to drawbacks of conventional medicine, the use of natural products as an alternate to the conventional treatment in healing and treatment of various diseases has been rise in the last few decades [**5, 6**]. Grams positive as well as Gram negative bacterial infections are the cause of various diseases/disorders in human beings. Nosocomial infections are the result of Staphylococcus aureus infection whileas food poisoning is caused by the Bacillus subtilis [**7, 8**]. Pseudomonas aeruginosa leads to urinary tract infections [**9**] and Escherichia coli causes diarrhea, food poisoning and sepsis [**10-13**].

1,3-cyclohexandione commonly known as dimedone has been used efficiently as pharmacophoric building block for the synthesis of various antimicrobial agents including, xanthenes [**14, 15**], substituted chromenes [**16**], macrocyclic metal complexes [**17**], quinazoline derivatives [**18**], tetrahydro quinolone diones [**19**] and acridine based compounds [**20**]. Dimedone acts as a versatile building block and because of its amicable functionality the molecules is extensively used by the synthetic organic chemists to synthesize libraries of the compounds for screening against various diseases. While working on the synthesis and modification of bioactive molecules for the development of drug candidates [**21-31**] we thought ofsynthesis and investigation of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5, 5-dimethyl-cyclohexane-1, 3-dionefor antibacterial activity (Figure 1).

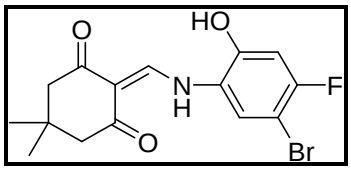


Figure 1. Structure of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione.

MATERIALS AND METHODS

General: ^{1}H and ^{13}C NMR were recorded on 400 MHz Bruker Daltonics spectrometer. The chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as internal standard and coupling constants were measured in Hz. Mass spectra were recorded on Bruker Daltonics electro spray ionization apparatus. Column chromatography was carried out on silica gel (Qualigens, 60-120 mesh) and pre-coated silica gel thin layer chromatographic (TLC) plates were viewed with ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots. Ceric sulfate was used as visualizing agents. Retinoic acid was purchased from Sigma-Aldrich (Sigma, St. Louis, MO, USA).

General procedure for the synthesis of compound: A clean and dry 50 mL round bottom flask fitted with reflux condenser was charged with dimedone (500 mg, 4.46 mmol), 5-bromo-4-fluoro-2-hydroxy-aniline (487 mg, 4.46 mmol) and triethylorthoformate (1.1 mL, 6.69 mmol). The reaction mixture was stirred for 2 h at 120^{o}C. The crude solid material formed was then purified by silica gel column chromatography to obtain the desired compound in 15% ethyl acetate: hexane.

Preparation of Inoculum: A single glycerol stock of the bacterial and fungal strains were thawed and added into 250 mL conical flasks containing 50 mL of respective broth media. The bacterial cultures were incubated at 37°C and grown to mid log growth phase. The exponentially grown bacterial cultures were appropriately diluted based on optical density measured at 600 nm and used for inoculation.

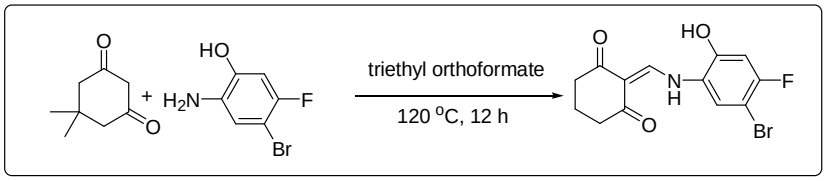
Test organism used: The antibacterial activity of *Pithecellobiumdulce* root extract was evaluated against four strains. Among four tested strains one was Gram positive (*Staphylococcus aureus* (MTCC–3160) and three Gram negative (*Acetobacteraceti*, MTCC–2623; *Acetobacteraceti* MTCC–2623; *Klebsiella pneumoniae* (MTCC–3384). These bacterial strains were obtained from BouraSagar Institute Jhansi, Uttar Pradesh-India. The cultural medium for the bacteria contained yeast extract (5 g L^{-1}), peptone (5 g L^{-1}) and mannitol (25 g L^{-1}).

Antibacterial Assay: The antimicrobial activity of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dionewas analyzed using Disc Diffusion Method [32-34]. In brief, the Mueller Hinton Agar (Himedia, Mumbai, India) plates were prepared by pouring into sterile petriplates 15 mL of the molten media. After 5 min, the dried plates were swabbed uniformly with 0.1 % of inoculum suspension and subsequently allowed to dry. The sterile individual discs loaded with 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5, 5-dimethyl-cyclohexane-1,3-dione(at 0.75, 1.25, 2.5 and 5 µM concentrations) were put onto the medium surface (pH 6.8-7.2). After diffusion of the extracts into the medium plates were incubated for 24 h at 37°C. For negative control, the discs were loaded with dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) alone whereas streptomycin (10 µg/disc; Sigma-Aldrich, St. Louis, MO, USA) was used as positive control. After incubation the zone of inhibition around the disc was measured in millimeter using a transparent ruler. The measurements were performed in triplicates to determine the mean of inhibition zone.

RESULTS AND DISCUSSION

At present development of resistance to antibiotic at almost geometric scale [**35, 36**] is associated with the confrontation of serious challenges by medical practitioners in the treatment of infectious diseases [**37**]. Therefore proper attention needs to be given to bioactive compounds and synthesis of organic compounds to reap the antimicrobial benefits inherited to these compounds. After screening of the bioactive compounds with therapeutic potential, the molecules should be subjected to structural modification to develop the compounds with more promising activity. Also its tolerable levels in the human body as well as any toxic effects have to be investigated.

In the present study, 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dionewas synthesized by the condensation reaction between dimedone and 5-bromo-4-fluoro-2-hydroxy-aniline (Scheme 1). The reaction was performed for 12 h under reflux at 120^{o}C in triethylorthformate to obtain 5-bromo-4-fluoro-2-hydroxy-aniline. The crude product was purified by column chromatography on silica gel using 15% ethyl acetate and petroleum ether solvent mixture. The product was characterized using the ^{1}H NMR, ^{13}C NMR and MS spectral techniques.



Scheme 1. Synthesis of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione.

The synthesized compound was screened for antibacterial activity against the Gram positive and Gram negative bacterial strains. The data showed that 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dioneexhibits variable degrees of antimicrobial activity against the tested bacterial strains.

Disc diffusion method revealed that the compound showed good activity against *Acetobacteraceti, Staphylococcus aureus*, *Klebsiella pneumonia* and *Enterobacter aerogenes* bacterial strains (Figure 2). The zone of inhibition of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-

dimethyl-cyclohexane-1,3-dione against *Acetobacteraceti, Staphylococcus aureus, Klebsiella pneumonia* and *Enterobacter aerogenes* bacterial strains was 18.5, 25, 19 and 28 mm, respectively at 5µM concentration.

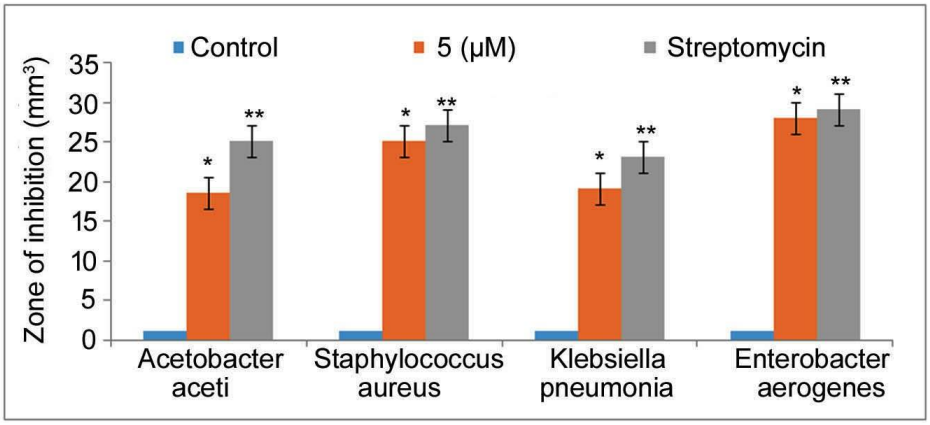


Figure 2. Antibacterial activity of 5µM concentrationof 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1, 3-dioneagainst the indicated strains- Zone of inhibition in diameter (mm).

The 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione also exhibited good antibacterial activity against *Acetobacteraceti, Klebsiella pneumonia* and *Entrobacter aerogenes* bacterial strains at 2.5µM concentration. The zone of inhibition of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against *Acetobacteraceti, Klebsiella pneumonia* and *Entrobacteraerogenes* was 17, 19 and 22 mm, respectively. The antibacterial activity of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against *Staphylococcus aureus* was found to be negligible at 2.5 µM concentration (Figure 3).

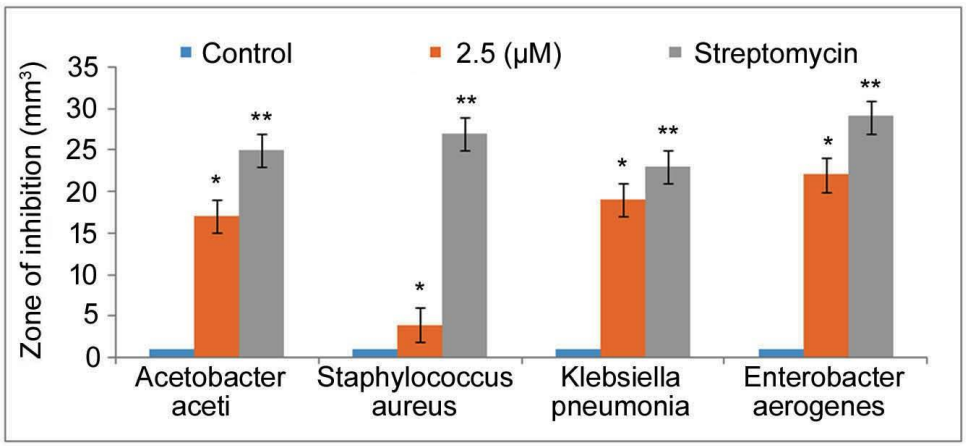


Figure 3. Antibacterial activity of 2.5µM concentrationof 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against the indicated strains- Zone of inhibition in diameter (mm).

However, 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dioneat 0.75 and 1.25 µM concentrations did not show any significant antibacterial activity against *Staphylococcus aureus, Klebsiella pneumonia* and *Enterobacter aerogenes* bacterial strains but showed some activity against *Acetobacteraceti.* The zone of inhibition of 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against *Acetobacteraceti* was 11and 9mm, respectively on treatment with1.25 and 0.75 µM concentrations (Figure 4, 5).

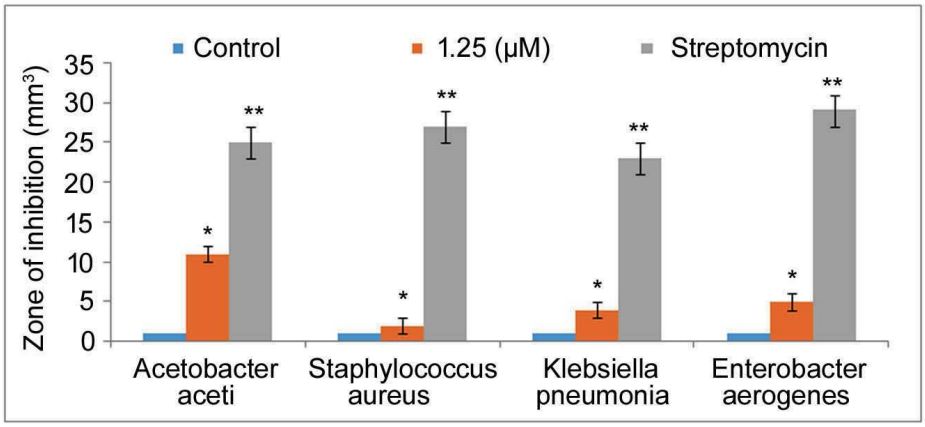


Figure 3. Antibacterial activity of 1.25µM concentrationof 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against the indicated strains- Zone of inhibition in diameter (mm).

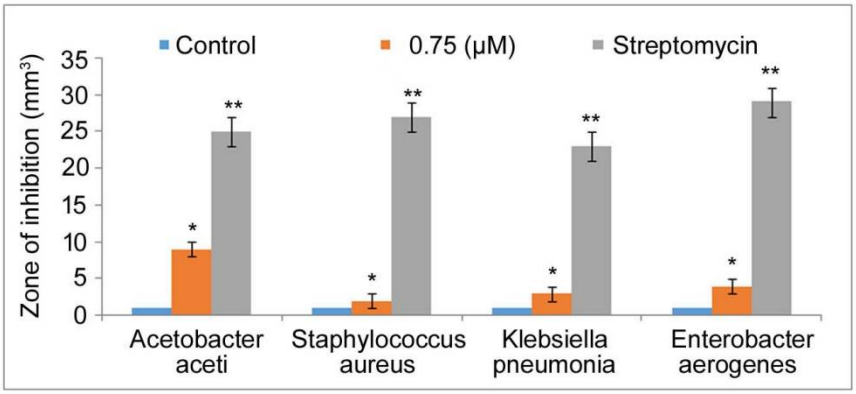


Figure 4. Antibacterial activity of 0.75µM concentrationof 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione against the indicated strains- Zone of inhibition in diameter (mm).

APPLICATION

The study identified 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5, 5-dimethyl-cyclohexane-1, 3-dione as an effective antibacterial agent. More studies are required to investigate the structure-activity-relationship of the synthesized compounds for development of novel and effective antibacterial agents.

CONCLUSION

In summary, 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dione was found to possess antibacterial activity against Gram positive and Gram negative bacterial strains. Therefore, 2-[(5-Bromo-4-fluoro-2-hydroxy-phenylamino)-methylene]-5,5-dimethyl-cyclohexane-1,3-dionecan be investigated further as antibacterial agent.

REFERENCES

[1]. F. Barchiesi, A. M. Tortorano, F. L. Falconi Di, M. Cogliati, G. Scalise, M. A. Viviani, In-vitro activity of five antifungal agents against uncommon clinical isolates of *Candida spp, J. Antimicrob. Chemother.*, **1999**, 43, 295–299.

[2]. R. E. Hancock, A. Nijnik, D. J. Philpott, Modulating immunity as a therapy for bacterial infections, *Nat. Rev. Microbiol.*, **2012**, 10, 243-254.

[3]. A. P. Mukne, V. Viswanathan, A. G. Phadatare, Structure pre-requisites for isoflavones as effective antibacterial agents, *Pharmacogn. Rev.*, **2011**, 9, 13-8.

[4]. H. M. Dionisi, M. Lozada, N. L. Olivera, Bioprospection of marine microorganisms: biotechnological applications and methods, *Rev. Argent. Microbiol*., **2012**, 44, 49-60.
[5]. R. A. Mothana, U. Lindequist, Antimicrobial activity of some medicinal plants of the island Soqotra, J. *Ethnopharmacol*., **2005**, 96, 177-81.
[6]. J. A. Duke, K. K. Wain, Medicinal plants of the world. [Computer index with more than 85,000 entries], 3 vols, Longman group UK Limited, **1981**.
[7]. S. Bhakdi, J. Tranum-Jensen, Alpha-toxin of Staphylococcus aureus, *Microbiol. Rev.*, **1991**, 55, 733–751.
[8]. S. C. Parija, Textbook of Microbiology & Immunology, Elsevier Health Sciences, **2014**.
[9]. K. Todar, Todar's Online Textbook of Bacteriology, Kenneth Todar University of Wisconsin-Madison Dept. of Bacterology, **2009**.
[10]. P. B. Eckburg, E.M. Bik, C. N. Bernstein, E. Purdom, L. Dethlefsen, M. Sargent, S. R. Gill, K. E. Nelson, D. A. Relman, Diversity of the human intestinal microbial flora, *Science*, **2005**, 308 , 635–1638.
[11]. P. Feng, S. D. Weagant, M.A. Grant, W. Burkhardt, M. Shellfish, B. Water, BAM: enumeration of escherichia coli and the coliform bacteria, *Bacteriol. Anal. Manual*, **2002**, 1– 13.
[12]. A. Thompson, E. coli thrives in beach sands, *Live Sci*., **2007**, 6.
[13]. S. Ishii, M. J. Sadowsky, Escherichia coli in the environment: implications for water quality and human health, Microbes Environ., **2008**, 23, 101–108.
[14]. M. Kaya, E. Demir, H. Bekci, Synthesis, characterization and antimicrobial activity of novel xanthene sulfonamide and carboxamide derivatives, *J EnzymInhib Med Chem*., **2013**, 28(5), 885–893.
[15]. M. Kaya, E. Basar, F. Colak, Synthesis and antimicrobial activity of some bisoctahydroxanthene-1, 8-dione derivatives, *Med Chem Res*., **2011**, 20(8):1214–1219.
[16]. C. B. Sangani, N. M. Shah, M. P. Patel, R. G. Patel, Microwave assisted synthesis of novel 4H-chromene derivatives bearing phenoxypyrazole and their antimicrobial activity assess, *J Serb ChemSoc.*, **2012**, 77(9), 1165–1174.
[17]. D. P. Singh, R. Kumar, P. Surain, K. R. Aneja (2014) Spectroscopic and antimicrobial studies of macrocyclic metal complexes derived from 1, 8-diaminonaphthalene and dimedone, J Incl Phenom Macro., **2014**, 78(1–4), 363–369.
[18]. D. Wang, F. Gao (2013) Quinazoline derivatives: synthesis and bioactivities. Chem Cent J 7(1):95.
[19]. Shahi M, Foroughifar N, Mobinikhaledi A (2015) Synthesis and antimicrobial activity of some tetrahydro quinolone diones and pyrano [2, 3-d] pyrimidine derivatives, *Iran J Pharma Res.*, **2013**, 14(3), 757.
[20]. M. Kaya, Y. Yıldırır, G. Y. Çelik, Synthesis and antimicrobial activities of novel bisacridine-1, 8-dione derivatives, *Med Chem Res.*, **2011**, 20(3), 293–299.
[21]. A. M. Lone, B. A. Bhat, G. Mehta, A general, flexible, ring closing metathesis (RCM) based strategy for accessing the fused furo[3,2-b]furanone moiety present in diverse bioactive natural products, *Tetrahedron Lett.*, **2013**, 54, 5619–5623.
[22]. A. M. Lone, B. A. Bhat, Metal free stereoselective synthesis of functionalized enamides, *Org. Biomol. Chem*., **2014**, 12, 242–246.
[23]. A. M. Lone, B. A. Bhat, W. A. Shah, G. Mehta, A concise synthesis of Hagen's gland lactones, *Tetrahedron Lett*., **2014**, 55, 3610-3612.
[24]. A. M. Lone, N. J. Dar, A. Hamid, W. A. Shah, M. Ahmad, B. A. Bhat, Promise of retinoic acid-triazolyl derivatives in promoting differentiation of neuroblastoma cells, *ACS Chem. Neurosci*., **2016**, 7, 82–89.
[25]. B.A. Dar, A.M. Lone, W.A. Shah, M.A. Qurishi, Synthesis and screening of ursolic acid-benzylidine derivatives as potential anti-cancer agents, *Eur. J. Med. Chem*., **2016**, 111, 26-32.
[26]. A.M. Lone, W.A. Shah, Synthesis and biological evaluation of new triazolyl analogues derived from 1-oxaspiro[4.4]nona-3,6-dien-6-ylmethanol, *J.Applicable Chem.*, **2014**, 3, 513-520.

[27]. A. Ibad, M. A. Waseem, F. Ibad, K. Ansari, A.M. Lone, G. Watal, I. R. Siddiqui. A green access to tetrahydro-1H-pyrano[2,3-d]pyrimidines: Visible light triggered and ethylene glycol mediated multicomponent one pot process, *Chemistryselect*, **2017**, 17, 4587.
[28]. M.A. Waseem, A.M. Lone, F. Ibad, A. Ibad, V.B. Yadav, K. Ansari, B.A. Shairjogrey, G. Watal, I.R. Siddiqui, Double hydroamination of alkyne via PTC and microwave-activated diastereoselective synthesis of 2,3-dihydroimidazo[1,2-a]pyridine in an aqueous media, *J. Heterocyc Chem.*, **2017,** DOI 10.1002/jhet.2875.
[29]. M. A. Rather, A. M. Lone, B. T, Z. S. Bhat, P. Singh, M. Maqbool, B.A. Shairgojray, M. J. Dar, S. Amin**,** S. K. Yousuf, B. A. Bhat, Z. Ahmad, The synthesis, biological evaluation and structure–activity relationship of 2-phenylaminomethylenecyclohexane-1,3-diones as specific antituberculosis agents, *Med. Chem. Commun*., **2017**, 8, 2133.
[30]. A. M. Lone, M. A. Rather, M. A. Bhat, Z. S. Bhat, I. Q. Tantry, P. Prakash. Synthesis and in vitro evaluation of 2-(((2-ether)amino)methylene)-dimedone derivatives as potential antimicrobial agents, *MicrobPathog*, **2018,** 114, 431–435.
[31]. B. A. Dar, A. M. Lone, M. A. Qurishi, Cytotoxic activity and GC-MS analysis of the constituents of essential oil of *Nepetagovaniana* (Wall.exBenth) from Jammu and Kashmir, India, *International Journal of Herbal Medicine*, **2014**, 2, 58-60.
[32]. J. Megala, A. Geetha, Antiulcerogenic activity of hydroalcoholic fruit extract of Pithecellobiumdulce in different experimental ulcer models in rats, *J Ethnopharmacol*., **2012**, 142 41.
[33]. J.B. Harborne. Phytochemical methods. Chapman and Hall Int., New York, Third Edition (1998).
[34]. C. K. Kokate, Pharmacognosy. NiraliPrakasham, Mumbai, India, Sixteenth Edition, **2001**.
[35]. V. L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Method Enzymol., **1999**, 299 152–178.
[36]. S. Kumazawa, M. Taniguchi, Y. Suzuki, M. Shimura, M. S. Kwon, T. Nakayama, Antioxidant activity of polyphenols in carob pods, *J Agric Food Chem*., **2002**, 50, 373–377.
[37]. D. C. Quettier, Phenolic compounds and antioxidant activities of buck wheat hulls and flour, *J. Ethnopharmacol.*, **2000**, 72 35-42.