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Synthesis and Antimicrobial Activity of Novel Hydrazone Derivatives of 4-(4-chlorophenyl)cyclohexanecarboxylic acid

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ABSTRACT

Hydrazones are of considerable importance because of their diverse biological applications such as antihelmintic, antidiabetic, and trypanocidal activities etc. The present paper describes the synthesis and antibacterial activity of novel hydrazone derivatives ***4a-4k*** *derived from commercially available 4-(4-chlorophenyl)cyclohexanecarboxylic acid* ***1.*** *All the newly synthesized 4-(4-chlorophenyl) cyclo hexane carbohydrazide derivatives* ***4a-4k*** *were evaluated for their in vitro antibacterial activity against Staphylococcus aureus and Bacillus subtilis (Gram-positive bacteria) and Escherichia coli and Pseudomonas aeruginosa (Gram-negative bacteria). On the basis of zone of inhibition, it is observed that compounds* ***4a-4e*** *exhibited good antibacterial activity and compounds* ***4f-4h*** *displayed equipotent activity when compared to the standard drug ciprofloxacin.*

Keywords: Antibacterial Activity, Atovaquone , Gram-positive bacteria, Hydrazones, Synthesis.

INTRODUCTION

Hydrazones possess an azomethine –NHN=CH group which are considered as derivatives of aldehydes and ketones in which the oxygen atom has been replaced by the $=NNH_2$ group. Hydrazones are of considerable importance because of their diverse biological applications such as antimycobacterial, antidepressant, anti-inflammatory, anticonvulsant, analgesic, antiplatelet, antimalarial, antimicrobial, anticancer, vasodilator, antiviral, anti-HIV, anthelmintic, antidiabetic, and trypanocidal activities [1–9].

The appearance of multidrug resistant Gram-positive bacteria, in particular, Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococci (VRE) is causing a serious menace. For the treatment of these intractable infections, a new antiinfectious agent is needed. Therefore, there is an urgent need for development of new antibacterial agents with exclusive structure and with a mechanism of action possibly different from that of existing antimicrobial agents [10, 11].

The present paper describes the synthesis and antibacterial activity of novel hydrazone derivatives **4a-4k** derived from commercially available 4-(4-chlorophenyl)cyclohexanecarboxylic acid **1.** The starting material 4-(4-chlorophenyl)cyclohexanecarboxylic acid is utilized as one of the key raw material in the preparation of Atovaquone [12].

MATERIALS AND METHODS

Chemical and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on EMerck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellet with a Perkin-Elmer spectrum gx FTIR instrument and only diagnostic and/or intense peaks are reported. ^{1}H NMR spectra were recorded in DMSO- d_6 with a Varian Mercury plus 400 MHz instrument. Signals due to the solvent or residual protonated solvent (^{1}H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. . The ^{1}H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (*J*) corresponds to the order of multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under argon atmosphere. The 4-(4-chlorophenyl)cyclohexanecarboxylic acid **1** and all the benzaldehydes used for the preparation of **4a-4k** were purchased from commercial sources.

Methyl 4-(4-chlorophenyl)cyclohexanecarboxylate 2: To a solution of compound **1** (2 g, 8.4 mmol) in methanol (20 mL) was added sulphuric acid (0.1 mL) and refluxed for 10 h. After completion of the reaction, methanol was evaporated under reduced pressure and the obtained residue was taken in ethylacetate (30 mL,), washed with 10% aq; $NaHCO_3$ solution (2 x 10 mL) followed by water and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford compound **2.** Yellow oily liquid, Yield: 2.0 g, 94%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.32 (d, *J* = 12.0 Hz, 2H), 7.24 (d, *J* = 12.0 Hz, 2H), 3.61 (s, 3H), 2.51-2,42 (m, 1H), 2.39-2.35 (m, 1H), 1.97 (d, *J* = 12 Hz, 2H), 1.82 (d, *J* = 12 Hz, 2H), 1.82 (d, *J* = 12 Hz, 2H), 1.54-1.40 (m, 4H);

4-(4-chlorophenyl)cyclohexanecarbohydrazide 3: To a solution of compound **2** (1.5 g, 5.95 mmol) in ethanol (15.0 mL) was added hydrazine hydrate (24 m mol) and heated to reflux for 3 h. After completion of the reaction, ethanol was concentrated under reduced pressure to obtain crude compound **8**. The crude compound was slurred in n-Hexane, filtered at the high vaccum pump and dried to obtain compound **8.** White solid, Yield: 1.35 g, 86%; m.p: 121 - 122 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.97 (br.s, 1H), 7.32 (d, *J* = 12.0 Hz, 2H), 7.24 (d, *J* = 12.0 Hz, 2H), 4.16 (br.s, 2H), 2.55-2,47 (m, 1H), 2.16-2.08 (m, 1H), 1.82-1.60 (m, 4H), 1.32-1.56 (m, 4H);

General Experimental Procedure for the Synthesis of Hydrazone derivatives (4a-4k): To a stirred solution of compound **3** (100 mg, 0.40 mmol) in ethanol was added corresponding benzaldehydes (1.0 mmol) and refluxed for 1 h. The reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain the pure compounds. Yields of the products varied between 84 and 94%.

(E)-N'-(4-fluorobenzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4a): White solid; Yield: 88%; m.p: 92-93 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41 (*11.19, s, 1H), 8.19 (*8.03, s, 1H), 7.76-7.73 (m, 2H), 7.36-7.26 (m, 6H), 3.28-3.21 (*2.32-2.28, m, 1H), 2.62-2.50 (m, 1H), 1.92-1.84 (m, 4H), 1.61-1.40 (m, 4H); ESI-MS: m/z, 359.2.

(E)-N'-(3-(trifluoromethyl)benzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4b): White solid; Yield: 92%; m.p: 93-94 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.61 (* 11.38, s, 1H), 8.28 (* 8.08,

s, 1H), 8.03-7.97 (m, 2H), 7.79-7.66 (m, 2H), 7.41-7.27 (m, 4H), 3.30-3.10 (* 2.58-2.40, m, 1H), 2.78-2.66 (m, 1H), 1.98-1.96 (m, 4H), 1.60-1.40 (m, 4H); ESI-MS: m/z, 409.2.

(E)-N'-(4-(trifluoromethyl)benzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4c): White solid; Yield: 90%; m.p: 98-99 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.61 (* 11.39, s, 1H), 8.27 (* 8.07, s, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.37-7.27 (m, 4H), 3.40-3.10 (* 2.40-2.20, m, 1H), 2.68-2.50 (m, 1H), 1.98-1.96 (m, 4H), 1.95-1.46 (m, 4H); ESI-MS: m/z, 409.6.

(E)-N'-(4-(trifluoromethoxy)benzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4d):White solid; Yield: 90%; m.p: 107-108 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50 (* 11.30, s, 1H), 8.22 (* 8.02, s, 1H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.36-7.27 (m, 4H), 3.20-3.10 (* 2.40-2.20, m, 1H), 2.58-2.50 (m, 1H), 1.88 (br.t, *J* = 16.0 Hz, 4H), 1.57 (br.t, *J* = 16 Hz, 4H).

(E)-N'-(4-(difluoromethoxy)benzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4e): White solid; Yield: 92%; m.p: 111-112 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.42 (*11.21, s, 1H), 8.19 (*7.99, s, 1H), 7.72 (d, *J* = 12.8 Hz, 1H), 7.36-7.23 (m, 6H), 3.28-3.21 (*2.32-2.28, m, 1H), 2.62-2.50 (m, 1H), 1.92-1.84 (m, 4H), 1.61-1.40 (m, 4H); ESI-MS: m/z, 407.0.

(E)-N'-(2,4-difluorobenzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4f): White solid; Yield: 90%; m.p: 118-119 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (* 11.31, s, 1H), 8.38 (* 8.15, s, 1H), 7.98-7.87 (m, 1H), 7.41-7.17 (m, 6H), 3.42-3.20 (* 2.31-2.23, m, 1H), 2.56-2.31 (m, 1H), 1.92-1.84 (m, 4H), 1.66-1.40 (m, 4H); ESI-MS: m/z, 377.3.

(E)-N'-(2,6-difluorobenzylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide (4g): White solid; Yield: 88%; m.p: 90-92 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.55 (*11.35, s, 1H), 8.36 (*8.12, s, 1H), 7.53-7.43 (m, 1H), 7.36-7.16 (m, 6H), 3.14-3.07 (*2.32-2.28, m, 1H), 2.62-2.50 (m, 1H), 1.92-1.84 (m, 4H), 1.61-1.40 (m, 4H); ESI-MS: m/z,377.3.

(E)-N'-(3,4-difluorobenzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4h): White solid; Yield: 94%; m.p: 121-122 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (*11.30, s, 1H), 8.18 (*7.97, s, 1H), 7.78-7.69 (m, 1H), 7.56-7.47 (m, 2H), 7.37-7.27 (m, 4H), 3.32-3.14 (* 2.40-2.42, m, 1H), 2.72-2.48 (m, 1H), 1.98-1.80 (m, 4H), 1.60-1.42 (m, 4H).

(E)-N'-(2-chloro-3-(trifluoromethyl)benzylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide (4i): White solid; Yield: 85%; m.p: 102-103 ^{o}C;^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (*11.52, s, 1H), 8.70 (*8.40, s, 1H), 8.20 (t, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.64 (t, *J* = 8.2 Hz, 1H), 7.36-7.28 (m, 4H), 3.32-3.28 (* 2,30, m, 1H), 2.56-2.51 (m, 1H), 1.94-1.85 (m, 4H), 1.58-1.41 (m, 4H); ESI-MS: m/z, 443.1.

(E)-N'-(5-bromo-2-fluorobenzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4j): White solid; Yield: 84%; m.p: 87-88 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.65 (*11.21, s, 1H), 8.36 (*8.14, s, 1H), 7.95 (d, *J* = 3.8 Hz, 1H), 7.42-7.22 (m, 5H), 3.28-3.21 (*2.32-2.28, m, 1H), 2.62-2.50 (m, 1H), 1.92-1.84 (m, 4H), 1.61-1.40 (m, 4H); ESI-MS: m/z, 438.8.

(E)-N'-(4-fluoro-2-methylbenzylidene)-4-(4-chlorophenyl) cyclohexanecarbohydrazide (4k): White solid; Yield: 90%; m.p: 114-115 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (*11.11, s, 1H), 8.42 (*8.23, s, 1H), 7.83-7.74 (m, 1H), 7.36-7.28 (m, 4H), 7.15-7.08 (m, 2H), 3.26-3.10 (* 2.26-2.20, m, 1H), 2.72-2.68 (m, 1H), 2.40 (s, 3H), 1.98-1.72 (m, 4H), 1.62-1.46 (m, 4H); ESI-MS: m/z, 373.3.

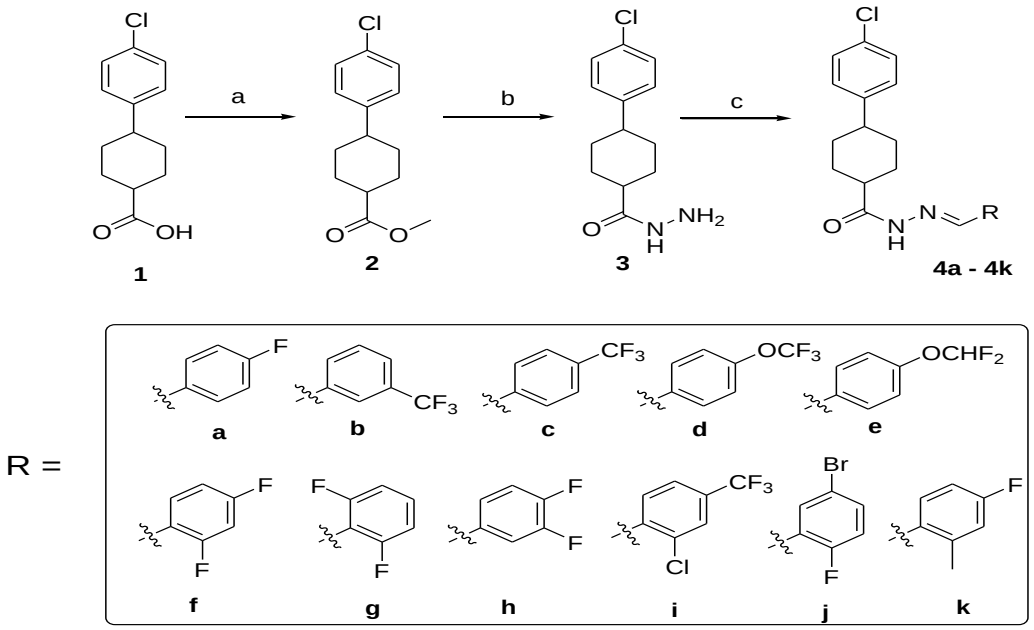
Biological Assay

In Vitro Antibacterial Assay: All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 x 10^8 cfu mL^{-1} [13]. The antibacterial activity of newly synthesized 4-(4-chlorophenyl)cyclohexanecarbohydrazide derivatives was evaluated by agar well diffusion method [14]. Into the each Petri plate, 20 mL of Mueller Hinton agar medium was poured and the agar plates were swabbed with 100 µL inoculant of each test bacterium and kept for 15-20 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into seeded agar plates and these were loaded with a 100 µL volume with concentration of 4.0 mg mL^{-1} of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity of the newly synthesized 4-(4-chlorophenyl)cyclohexanecarbohydrazide derivatives was evaluated by measuring the zone of growth inhibition against the test bacteria. DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates. The antibacterial activity of the compounds was compared with ciprofloxacin as standard. Minimum Inhibitory Concentration (MIC) of newly synthesized compounds against tested bacteria was determined using macro dilution tube method as recommended by NCCLS [14, 15]. MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 h.

RESULTS AND DISCUSSION

Chemistry : Initially, 4-(4-chlorophenyl)cyclohexanecarbohydrazide **3** was obtained by the reaction of methyl 4-(4-chlorophenyl)cyclohexanecarboxylate **2** with hydrazine hydrate in ethanol at reflux for 3 h. The methylester 2 was in turn prepared from corresponding carboxylic acid **1** in presence of catalytic qty; of conc; H_2SO_4 in methanol at reflux for 10 h. The target compounds (**4a-4k**) were synthesized *via* the nucleophilic addition-elimination reaction of 4-(4-chlorophenyl)cyclohexanecarbohydrazide **3** with various fluorinated benzaldehydes. The reaction scheme associated with these derivatives is depicted in Scheme **1.**

The structures of the synthesized compounds were confirmed by ^{1}H NMR and Mass spectral data. All the synthesized hydrazones derivatives **4a-4k** compounds were found to exist as a mixture of two rotameric forms in solution [16] e.g. antiperiplanar (*ap*) and synperiplanar (*sp*) as indicated by their ^{1} H NMR spectra. Two sets of signals were observed for all groups in the ^{1}H NMR spectra of each compound indicating the possibility of equilibrium and interconversion between rotamers (and/or configurational isomers) in solution [16]. As a representative example, the ^{1} H NMR spectra of the compound **4d** is as follows, the broad singlets at 11.50 (* 11.30 ppm) and 8.22 ppm (* 8.02 ppm) corresponds to the protons representing to -N=C*H*- and -N*H*-N=C- groups respectively. The doublets appearing at 7.80 and 7.43 ppm represents to the protons attached to the phenyl ring bearing OCF_3 substituent and a mutiplet at 7.36-7.27 ppm represents to the protons attached to the phenyl ring (having chloro substituent) All the other aliphatic protons were observed at expected regions. The ^{1}H NMR data for the remaining hydrazone derivatives in the series are in agreement with the assigned structures. The mass spectra of compounds showed (M+1) peaks, and are in agreement with their molecular formula.



Experimental Conditions: a) Conc; H_2SO_4, Methanol, reflux, 10 h; b) $NH_2NH_2.H_2O$, ethanol, reflux, 3 h; c) Benzaldehydes **a-k**, ethanol, reflux, 1 h

Scheme 1. Synthesis of Hydrazone derivatives of 4-(4-chlorophenyl)cyclohexanecarboxylic acid **4a – 4k**

Biological Evaluation

In Vitro Antibacterial Activity : All the newly synthesized 4-(4-clorophenyl)cyclohexanecarbohydrazide derivatives **4a-4k** were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis (*Gram-positive bacteria) and *Escherichia coli* and *Pseudomonas aeruginosa (*Gram-negative bacteria) by agar well diffusion method [14,15] using ciprofloxacin as the reference drug. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth surrounding the well in mm. The minimum inhibitory concentration (MIC) measurements were performed using a macro dilution tube method [14, 15].

The results of the antibacterial activity of the tested compounds **4a-4k** is presented in **Table 1**. On the basis of zone of inhibition against the test bacterial strains, compounds **4a-4e** exhibited good antibacterial activity against *E.coli, P.aeruginosa* (with zone of inhibition: 21-23 mm) and *S.aureus* , *B.subtilis* (with zone of inhibition: 21-25 mm) when compared to the standard drug ciprofloxacin (zone of inhibition: 25 mm). Compounds **4f-4h**, showed equipotent activity (zone of inhibition of the test compounds equals to that of zone of inhibition of standard drug Ciprofloxacin) against all the tested bacterial strains. The remaining compounds in the series *viz.*, compounds **4i-4k** displayed moderate antibacterial activity against all the tested bacterial strains.

On the basis of MIC, none of the compounds was found to have substantial antibacterial activity. However, amongst all the compounds, the MIC ranged between 32 and 256 µg/mL against all the tested bacterial strains (Table **1**). It is noteworthy to mention that, in general, R having difluoro substitutions on the phenyl ring (**4f, 4g and 4h**) showed equipotent antibacterial activity while the phenyl ring having 4-Fluoro, 3-CF_3, 4-CF_3, 4-OCF_3 and 4-$OCHF_2$ (compounds **4a-4e)** displayed good antibacterial activity. Therefore it can be inferred that, further structural activity relationship can be explored by varying the suitable R group in the scaffold which may lead to a promising antibacterial drug candidate.

Table-1 Results of Antibacterial Evaluation of Hydrazone derivatives of 4-(4-chlorophenyl)cyclohexanecarboxylic acid **4a – 4k**

Compound[a]	Zone of inhibition (mm)				Minimum inhibitory concentration (MIC-µg mL^{-1})			
	E.coli	*P.aeruginosa*	*S.aureus*	*B.subtilis*	*E.coli*	*P.aeruginosa*	*S.aureus*	*B.subtilis*
4a	23	22	25	25	128	128	128	128
4b	22	22	24	24	128	128	128	128
4c	21	23	22	21	128	128	128	128
4d	22	21	23	22	128	128	128	128
4e	23	21	23	24	128	128	128	128
4f	25	25	27	26	32	32	64	64
4g	25	25	27	26	32	32	64	64
4h	25	25	27	26	32	32	64	64
4i	18	17	20	20	256	256	256	256
4j	17	17	19	20	256	256	256	256
4k	19	18	20	20	256	256	256	256
Ciprofloxacin	25	25	27	26	5	5	5	5

a. Concentration at 4.0mg mL^{-1}

APPLICATIONS

The novel hydrazones derivatives **4a-4k** synthesized in the present study were evaluated for antibacterial activity and are found to be as active pharmacophore. Further chemical and biological studies are undergoing to explore the various applications of these hydrazones derivatives.

CONCLUSIONS

The present paper in summary describes the synthesis and antibacterial activity of novel hydrazone derivatives **4a-4k** derived from 4-(4-chlorophenyl)cyclohexanecarboxylic acid **1** as starting material. The structures of the synthesized compounds were confirmed by ^{1}H NMR and Mass spectral data. The antibacterial activity of newly synthesized 4-(4-chlorophenyl)cyclohexanecarbohydrazide derivatives was evaluated by agar well diffusion method by measuring zone of inhibition. Within the series of hydrazone derivatives **4a-4k**, compounds **4f-4h** exhibited equipotent activity, compounds **4a-4e** showed good activity while compounds **4i-4k** displayed moderate activity. Some more relevant literature available to this work [17-20].

REFERENCES

[1] H. S. Seleem, G.A.El-Inany, B. A. El-Shetary, and M. A. Mousa, *Chemistry Central Journal.*, **2011**, 5, 2.
[2] B. F. Abdel-Wahab, G. E. A. Awad, F. A. Badria. *Eur.J.Med. Chem.*, **2011**, 46, 1505–1511.
[3] A. S. Abu-Surrah, K. A. Abu Safieh, I. M. Ahmad. *Eur.J.Med. Chem.*, **2010**, 45, 471–475.
[4] O. O. Ajani, C. A. Obafemi, O. C. Nwinyi, D. A. Akinpelu. *Bioorg. Med. Chem.*, **2010**, 18, 214–221.
[5] M. S. Al-Said, M. S. Bashandy, S. I. Al-Qasoumi, M. M. Ghorab. *Eur.J.Med. Chem.*, **2011**, 46, 137–141, 2011.
[6] M. A. S. Aslam, S.-U. Mahmood, M. Shahid, A. Saeed, J. Iqbal. *Eur.J.Med. Chem.*, **2011**, 46, 5473–5479.
[7] Z. Cui, Y. Li, Y. Ling. *Eur.J.Med. Chem.*, **2010**, 45, 5576–5584.
[8] D. Kaushik, S. A. Khan, G. Chawla, S. Kumar. *Eur.J.Med. Chem.*, **2010**, 45, 3943–3949.

[9] M. M. Edrees, T. A. Farghaly, F. A. A. El-Hag, M. M. Abdalla. *Eur.J.Med. Chem.*, **2010**, 45, 5702–5707.
[10] I. Chopra, C. Schofield, M. Everett, A. Oneill, K. Miller, M. Wilcox, J.M. Frere, M. Dawson, L. Czaplewski, U. Urleb, P. Courvalin. *Lancet Infect. Dis.*, **2008**, *8*, 133-139.
[11] A. Khalafi-Nezhad, M.N.S. Rad, H. Mohabatkar, Z. Asrari, B. Hemmateenejad. *Bioorg. Med. Chem.*, **2005**, *13*, 1931-1938.
[12] N.R. Bhairab, P.S. Girij, L.S. Piyush, K.R. Manoj, R. Mitra, A. Trivedi. *Indian. J. Chem.*, **2013**, 52B, 1299-1312.
[13] J.M. Andrews. *Antimicrob. Chemother*., **2001**, *48*, 5-16.
[14] I. Ahmad, A.J. Beg. *J. Ethnopharmacol*., **2001**, *74*, 113-123.
[15] NCCLS, Method for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically Approved Standards, 5th Edn., National Committee for Clinical Laboratory Standards, Villanova, PA, **2000.**
[16] N. Rajasekhar, K.B. Chandrasekhar, M. Sandeep, B. Balram, *Letters in Drug Design & Discovery*., **2013**, 10, 620-624.
[17] Rajasekhar Narisetty, K.B. Chandrasekhar, Sandeep Mohanty, M. Rameswara Rao, B. Balram.. *Journal of Applicable Chemistry.*, **2013**, 2 (6): 1489-1498.
[18] T. Ravi Sankar, P. Venkata Ramana. Synthesis, *Journal of Applicable Chemistry.*, **2013**, 2 (6): 1459-1467.
[19] Battula Sreenivasa Rao, Gurrala Srinivasa Rao, B.V.Kiran, K.Siva Nagaraju *Journal of Applicable Chemistry.*, **2012**, 1 (2):232-238.
[20] K. Siddappa, Mallikarjun Kote. Synthesis, *Journal of Applicable Chemistry.*, **2013**, 2 (3): 405-414.