

# One-pot synthesis of some novel N-aryl-1,4-dihydropyridines derivatives bearing nitrogen mustard

P. M. Singala, V. H. Shah\*

Department of Chemistry, Saurashtra University, Rajkot - 360005, India

\*E-mail address: drvireshshah@gmail.com

## ABSTRACT

The synthesis of a novel dihydropyridine, bearing carbethoxy groups at C(3) and C(5), respectively, has been achieved by applying three component Hantzsch-type condensation. The products were assayed for their *in vitro* biological assay antibacterial activity against with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs.

**Keywords:** 1,4-Dihydropyridine; Hantzsch synthesis; antimicrobial activity

## 1. INTRODUCTION

1,4-Dihydropyridine derivatives (1,4-DHPs) form a class of heterocyclic compounds with interesting pharmacological and biological properties [1]. It is well known that the 1,4-DHP nucleus serves as the scaffold of important cardiovascular drugs and it has been well established that the calcium modulator activity of this family of compounds is determined by structural requirements [2]. The systematic structural modification of the 1,4-DHP ring yields different compounds used in the treatment of TB and cancer [3]. Recently, studies showed that 3,5-dicarbamoyl derivatives of 1,4-dihydropyridine (DHP) with lipophilic groups have considerable antitubercular activity against *M. tuberculosis* H37Rv [4-8]. It was also observed that esters or substituted isosters of pyridine and pyrazine carboxylic acids (such as tetrazoles) have been more active than the parent acids especially against resistant strains. These esters are presumably activated by an esterase to parent acid [9-12]. Indeed, esters of pyrazinoic acids have been shown to possess activity against pyrazinamide-resistant isolates which has been attributed to a deficiency of nicotinamidase [9-12]. In addition, pyrazoles exhibited significant antitubercular activity [12]. Moreover, Nitrogen mustard is another important class bio-active scaffolds. In addition, nitrogen mustard and isosteric to pyridine and are associated with interesting biologic activities such as anti TB, antibacterial, anticancer, antiviral, cardiotoxic etc.

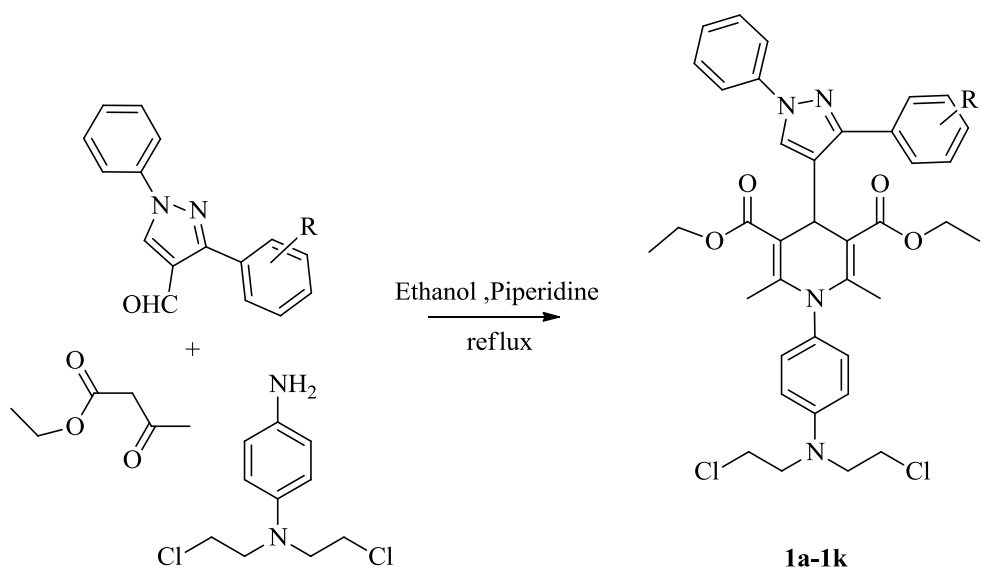
Over the years, molecular hybridization based drug design approach [13, 14] has been exploited by many researchers in order to develop some promising new hybrid chemical entities (NHCEs), displaying significant therapeutic values. The combination of two pharmacophores into a single molecular skeleton is a well established approach for designing more potent drugs with significant increase in activity. A hybrid molecule acting on manifold targets is considered to be a better drug candidate than drug combinations, since administration of single drug will have more predictable pharmacokinetic and pharmacodynamic properties with improved patient compliance. Owing to their well appreciated antitubercular and anticancer properties, we envisaged to design hybrid structures having substituted N-aryl-1,4-dihydropyridines and nitrogen mustard motifs connected with a linker. However, to the best of our knowledge, biological activity of 1,4-dihydropyridines bearing N-aryl-substitution or C-3 and C-5 acetyl group is not much explored.

Therefore, in view of the above facts and in continuation of our search on biologically active hybrid molecules [15-16], herein we report the synthesis of novel N-aryl-1,4-dihydropyridines hybrid analogs (**1a-1k**).

## 2. RESULTS AND DISCUSSION

### CHEMISTRY

Several methods are well known for the synthesis of pyrimidine derivatives. In the current study, we have utilized Hantzsch pyridine reaction for the synthesis of substituted pyridine derivatives. Synthesis of diversified Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(1,3-diphenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate was accomplished by condensing aromatic heterocyclic aldehydes with 1,3 diketone and aniline nitrogen mustard derivatives in ethanol (Scheme 1).



Where R = H, Cl, F, Br etc

**Scheme 1.**

### 3. EXPERIMENTAL SECTION

#### General Procedures

Melting points of all the compounds are uncorrected and have been recorded by open capillary method. Room temperature, wherever mentioned, normally corresponds to 280 - 330C. Silica gel-G was used for preparing the TLC plates using different solvent systems. Infra red spectra of all the compounds were scanned on SHIMADZU-FOURIER TRANSFORM INFRA RED (FTIR) - 8400 Spectrophotometer using KBr pellet method. PMR Spectra were recorded on BRUKER Spectrophotometer (300 MHz) using TMS as a internal standard and CDCl<sub>3</sub> as solvent. Electron Impact (EI) mass spectra were recorded on GCMSQP2010 Mass Spectrometer.

#### Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(1,3-diphenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1a)

Yield (68%), mp 134 °C; IR (KBr): 3314, 3016, 2928, 1705, 1594 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ = 1.39 (s, 6H, -CH<sub>3</sub>), 2.304 (s, 6H, -CH<sub>3</sub>), 3.78 (s, 8H, -CH<sub>2</sub>), 4.27-4.31 (q, 4H, CH<sub>2</sub>), 5.229 (s, 1H, -CH), 6.34-6.38 (d, 1H, Ar-H), 6.64-6.66 (d, 1H, Ar-H), 7.248-7.285 (t, 1H, Ar-H), 7.365-7.401 (t, 1H, Ar-H), 7.747-7.785 (t, 4H, Ar-H), 7.743-7.763 (d, 2H, Ar-H), 7.958-7.992 (t, 3H, Ar-H), 8.829 (s, 1H, -NH); Anal. Calcd for C<sub>38</sub>H<sub>40</sub>Cl<sub>2</sub>N<sub>4</sub>O<sub>4</sub>: C, 66.37; H, 5.86; N, 8.15; Found: C, 66.48; H, 5.95; N, 9.18; MS: m/z 687.

#### Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(3-(2-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1b)

Yield (52%), mp 122 °C; IR (KBr): 3328, 3011, 2936, 2853, 1706, 1597 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ = 1.39 (s, 6H, -CH<sub>3</sub>), 2.304 (s, 6H, -CH<sub>3</sub>), 3.78 (s, 8H, -CH<sub>2</sub>), 4.27-4.31 (q, 4H, CH<sub>2</sub>), 5.225 (s, 1H, -CH), 6.34-6.38 (d, 1H, Ar-H), 6.64-6.66 (d, 1H, Ar-H), 7.225 (s, 1H, Ar-H), 7.385-7.426 (dd, 3H, Ar-H), 7.584-7.648 (m, 1H, Ar-H), 7.724-7.759 (d, 2H, Ar-H), 7.783 (s, 1H, Ar-H), 7.845-7.934 (m, 2H, -Ar-H), 8.832 (s, 1H, -NH); Anal. Calcd for C<sub>38</sub>H<sub>39</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>4</sub>: C, 63.21; H, 5.44; N, 7.76; Found: C, 63.27; H, 5.49; N, 7.88; MS: m/z 720.

#### Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(3-(2-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1c)

Yield (76%), mp 119 °C; IR (KBr): 3327, 3015, 2929, 2852, 1708, 1593 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ = 1.39 (s, 6H, -CH<sub>3</sub>), 2.304 (s, 6H, -CH<sub>3</sub>), 3.78 (s, 8H, -CH<sub>2</sub>), 4.27-4.31 (q, 4H, CH<sub>2</sub>), 5.175 (s, 1H, -CH), 6.34-6.38 (d, 1H, Ar-H), 6.64-6.66 (d, 1H, Ar-H), 7.184-7.234 (t, 1H, Ar-H), 7.353-7.384 (m, 3H, Ar-H), 7.543-7.586 (m, 1H, Ar-H), 7.684-7.698 (d, 2H, Ar-H, j=5.6), 7.735 (s, 1H, Ar-H), 7.826-7.895 (m, 2H, -Ar-H), 8.827 (s, 1H, -NH); Anal. Calcd for C<sub>38</sub>H<sub>39</sub>Cl<sub>2</sub>FN<sub>4</sub>O<sub>4</sub>: C, 64.68; H, 5.57; N, 7.94; Found: C, 64.69; H, 5.58; N, 7.75; MS: m/z 704.

#### Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(3-(2-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1d)

Yield (63%), mp 144 °C; IR (KBr): 3328, 3012, 2930, 2856, 1706, 1598 cm<sup>-1</sup>; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ = 1.39 (s, 6H, -CH<sub>3</sub>), 2.304 (s, 6H, -CH<sub>3</sub>), 3.78 (s, 8H, -CH<sub>2</sub>), 4.27-4.31 (q, 4H, CH<sub>2</sub>), 5.204 (s, 1H, -CH), 6.34-6.38 (d, 1H, Ar-H), 6.64-6.66 (d, 1H, Ar-H), 7.186 (t, 1H, Ar-H), 7.227-7.316 (m, 3H, Ar-H), 7.446 (d, 1H, Ar-H), 7.543 (t, 1H, Ar-H), 7.636 (t, 1H, -Ar-H), 7.768-7.901 (m, 3H, -Ar-H), 8.832 (s, 1H, -NH); Anal. Calcd for C<sub>38</sub>H<sub>39</sub>BrCl<sub>2</sub>N<sub>4</sub>O<sub>4</sub>: C, 59.54; H, 5.13; N, 7.31; Found: C, 59.71; H, 5.16; N, 7.55; MS: m/z 764.

**Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1e)**

Yield (73%), mp 131 °C; IR (KBr): 3324, 3016, 2924, 1705, 1596  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  = 1.39 (s, 6H, -CH<sub>3</sub>), 2.304 (s, 6H, -CH<sub>3</sub>), 3.78 (s, 8H, -CH<sub>2</sub>), 4.27-4.31 (q, 4H, CH<sub>2</sub>), 5.189 (s, 1H, -CH), 6.34-6.38 (d, 1H, Ar-H), 6.64-6.66 (d, 1H, Ar-H), 7.223-7.285 (m, 1H, Ar-H), 7.294-7.324 (m, 1H, Ar-H), 7.423-7.451 (d, 2H, Ar-H), 7.530-7.562 (d, 2H, Ar-H,  $j=12.8$ ), 7.652-7.672 (t, 2H, -Ar-H), 7.918 (s, 1H, -Ar-H), 8.829 (s, 1H, -NH); Anal. Calcd for C<sub>38</sub>H<sub>39</sub>Cl<sub>3</sub>N<sub>4</sub>O<sub>4</sub>: C, 63.21; H, 5.44; N, 7.76; Found: C, 63.26; H, 5.48; N, 7.79; MS:  $m/z$  720.

**Diethyl 1-(4-(bis(2-chloroethyl)amino)phenyl)-4-(3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (1f)**

Yield (66%), mp 120 °C; IR (KBr): 3319, 3017, 2923, 1706, 1596  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  = 1.39 (s, 6H, -CH<sub>3</sub>), 2.304 (s, 6H, -CH<sub>3</sub>), 3.78 (s, 8H, -CH<sub>2</sub>), 4.27-4.31 (q, 4H, -CH<sub>2</sub>), 5.196 (s, 1H, -CH), 6.34-6.38 (d, 1H, Ar-H), 6.64-6.66 (d, 1H, Ar-H), 7.166-7.188 (t, 3H, Ar-H), 7.291-7.337 (m, 2H, Ar-H), 7.493-7.545 (m, 2H, Ar-H), 7.634-7.658 (d, 1H, Ar-H,  $d=9.6$ ), 7.722-7.763 (m, 2H, -Ar-H), 8.818 (s, 1H, -NH); Anal. Calcd for C<sub>38</sub>H<sub>39</sub>Cl<sub>2</sub>FN<sub>4</sub>O<sub>4</sub>: C, 64.68; H, 5.57; N, 7.94; Found: C, 64.74; H, 5.59; N, 7.96; MS:  $m/z$  704.

#### 4. BIOLOGICAL EVALUATION

##### Antimicrobial evaluation

All of the synthesized compounds (1a to 1k) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method [11-12] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [11]. Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations of 1.56, 3.12, 6.25, 10, 12.5, 25, 50, 62.5, 100, 125, 250, 500 and 1000  $\mu\text{g mL}^{-1}$ . The tubes were inoculated with  $10^8$  cfu  $\text{mL}^{-1}$  (colony forming unit/mL) and incubated at 37 °C for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1. Compounds 1e and 1f are display broad spectrum antibacterial activities against both gram-positive and gram-negative bacteria as compared with ciprofloxacin. As observed with high antibacterial potency of 1e and 1f a may

be attributed to the presence of electron withdrawing substituents such as chloro and fluoro at 4<sup>th</sup> position of phenyl ring of pyridine compounds. In comparison to the standard drug griseofulvin, the *in vitro* antifungal activity results indicated that compound 1f substituted with fluoro group at 4<sup>th</sup> position of phenyl ring was found to be the most potent against *A. niger*, *A. clavatus* and *C. albicans* respectively.

**Table 1.** Antibacterial and antifungal activity of synthesized compounds 1a to 1k.

Code	Minimum inhibition concentration ( $\mu\text{g mL}^{-1}$ )						
	Gram-positive		Gram-negative			Fungal species	
	<i>S.a.</i>	<i>S.p.</i>	<i>E.c.</i>	<i>P.a.</i>	<i>C. a.</i>	<i>A. n.</i>	<i>A.c.</i>
<b>1a</b>	500	1000	500	100	500	>1000	1000
<b>1b</b>	100	250	500	1000	>1000	500	1000
<b>1c</b>	500	100	250	125	500	250	1000
<b>1d</b>	100	500	250	250	>1000	1000	>1000
<b>1e</b>	50	50	100	100	500	500	250
<b>1f</b>	50	100	50	100	50	100	>250
<b>1g</b>	500	1000	100	250	500	500	250
<b>1h</b>	500	250	100	250	500	500	250
<b>1i</b>	100	100	100	250	500	500	250
<b>1j</b>	100	500	250	100	500	1000	>1000
<b>1k</b>	500	250	250	250	500	250	250
<b>Ampicillin</b>	250	100	100	100	-	-	-
<b>Chloramphenicol</b>	50	50	50	50	-	-	-
<b>Ciprofloxacin</b>	50	50	25	25	-	-	-
<b>Norfloxacin</b>	10	10	10	10	-	-	-
<b>Nystatin</b>	-	-	-	-	100	100	100
<b>Griseofulvin</b>	-	-	-	-	500	100	100

## 5. CONCLUSIONS

In the present paper, we report the synthesis, spectral studies and antimicrobial activity of various pyridine derivatives. The high bioactivity of these compounds makes them suitable tools for additional *in vitro* and *in vivo* evaluations, in order to develop new class of antimicrobial drugs or prodrugs with potential antimicrobial activity in the treatment of several diseases. Further studies and modification in this area are in progress in our laboratory.

### Acknowledgments

We acknowledge the support of UGC through Grant no. 41-198/ 2012(SR), New Delhi, India, for financial support and National Facility for Drug Discovery (NFDD) for providing instrumental support. I also wish to acknowledge Professor and Head, Department of Chemistry, Saurashtra University Rajkot for providing research facility. P. M. Singala is thankful to RGNF for meritorious Fellowship.

---

## References

- [1] (a) W. G. Mayler, *Calcium Antagonist Academic Press, London* (1989) (b) R. A. Janis, P. J. Silver and D. J. Triggle, *Adv. Drug Res.* 16 (1987) 309–391; (c) F. Bossert and W. Vater, *Med. Res. Rev.* 9 (1989) 291–324; (d) N. Marti´n and C. Seoane, *Quim. Ind.*, 36 (1990) 115–127; (e) R. Peri, S. Padmanabhan, A. Rutledge, S. Singh and D. J. Triggle, *J. Med. Chem.* 43 (2000) 2906–2914; (f) S. Tasaka, H. Ohmori, N. Gomi, M. Iino, T. Machida, A. Kiue, S. Naito and M. Kuwano, *Bioorg. Med. Chem. Lett.* 11 (2001) 275–277; (g) J. L. Harper, C. S. Camerini-Otero, A. Li, S. Kim, K. A. Jacobson and J. W. Daly, *Biochem. Pharmacol.* 65 (2003) 329–338; (h) A. A. S. Fernandes, M. S. Santos, J. A. F. Vicente, A. J. M. Moreno, Aa. Veleno, G. Duburs and C. R. Oliveira, *Mitochondrion* 3 (2003) 47–59; (i) T. Okamura, T. Kikuchi, A. Nagamine, K. Fukushi, T. Sekine, Y. Arano and T. Irie, *Free Radical Biol. Med.* 38 (2005) 1197–1205.
- [2] (a) U. Eisner and J. Kuthan, *Chem. Rev.* 72 (1972) 1–42; (b) D. M. Stout and A. I. Meyers, *Chem. Rev.* 82 (1982) 223–243; (c) F. Bossert, H. Meyer and E. Wehinger, *Angew. Chem., Int. Ed. Engl.* 1981, 20, 762–769; (d) J. Kuthan and A. Kurfu´rst, *Ind. Eng. Chem. Prod. Res. Dev.* 21 (1982) 191–261; (e) R. J. Chorvat and K. J. Roring, *J. Org. Chem.*, 53 (1988) 5779–5781; (f) A. Guzman, M. Romero, A. Maddox and J. Muchowski, *J. Org. Chem.* 55 1990 5793–5797; (g) C. O. Kappe, *Tetrahedron* 49 (1993) 6937–6963; (h) L.M. Yagupolskii, I. I. Maletina, K. L. Petko, D. V. Fedjuk, R. Handrock, S. S. Shavaran, B. M. Klebanov and S. Herzig, *J. Fluorine Chem.* 109 (2001) 87–94; (i) C. Vela´zquez and E. E. Knaus, *Bioorg. Med. Chem.* 12 2004 3831–3840. Vela´zquez and E. E. Knaus, *Bioorg. Med. Chem.* 12 (2004) 3831–3840.
- [3] D. h. Sriram; P. Yogeewari; K. Madhu, *Bioorg. Med. Chem. Lett.* 15 (2005) 4502.
- [4] B. Desai, D. Sureja, Y. Naliapara, A. Shah, A. K.Saxena, *Bioorg. Med. Chem.* 9 (2001) 1993.
- [5] M. Khoshnevizadeh, N. Edraki, K. Javidnia, A. Alborzi, B. Pourabbas, J. Mardenah, R. Miri, *Bioorg. Med. Chem.* 17 (2009) 1579.
- [6] A. T. Manvar, R. R. Pissurlenkar, V. R. Virsodia, K. D. Upadhyay, D. R. Manvar, A. K. Mishra, H. D. Acharya, A. R. Parecha, C. D. Dholakia, A. K. Shah, E. C. Coutinho, *Mol. Divers.*, 14 (2010) 285.
- [7] P. S. Kharkar, B. Desai, H. Gaveria, B. Varu, R. Loriya, Y. Naliapara,; A. Shah, V. Kulkarni, *M. J. Med. Chem.* 45 (2002) 4858.
- [8] K. Sirisha,; G. Achaiiah,; V. M. Reddy, *Arch. Pharm.* 6 (2010) 342.
- [9] M. H. Cynamon, S. P. Klemens, T. S. Chou, R. H. Gimi, J. T. Weleh, *J. Med. Chem.* 35 (1992) 1212.
- [10] M. H.Cynamon, R. H. Gimi, F. Gyenes, C. A. Sharpe, K. E. Bergmann, H.-J. Jan, L. B.Gregor, R. Rapolu, G. Luciano, J. T. Weleh, *J. Med. Chem.* 38 (1995) 3902.
- [11] R. J. Speirs, J. T. Weleh, M. H. Cynamon, *Antimicrob. Agents Chemother.* 39 (1995) 1269. 12. G. A.Wachter, M. C. Davis, A. R. Martin, S. G. Franzblau, *J. Med. Chem.* 41 (1998) 2436.
- [12] C. Viegas-Junior, A. Danuello, B.V. da Silva, E.J. Barreiro, C.A. Fraga, *Curr. Med. Chem.* 14 (2007) 1829-1852.

- 
- [13] V. R. Solomon, C. Hua, H. Lee, *Bioorg. Med. Chem.* 17 (2009) 7585-7592.
- [14] A. R. Trivedi, D. K Dodiya, B. H. Dholariya, V. B. Kataria, V. R. Bhuva, V. H. Shah, *Bioorganic & medicinal chemistry letters* 21 (18) (2011) 5181-5183.
- [15] A. Trivedi, D. Dodiya, B. Dholariya, V. Kataria, V. Bhuva, V. Shah, *Chemical biology & drug design* 78 (5), 2011, 881-886.
- [16] *Vogel's Textbook of Practical Organic Chemistry*, Fifth Edition, ELBS, Longman Scientific and Technical: England. (1989) Reprinted (1994) 1150.
- [17] National Committee for Clinical and Laboratory Standards, Method for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically Approved Standard, fourth ed. NCCLS, Villanova, Italy, *Document M 100-S7*. (1997) S100-S157.

( Received 04 February 2015; accepted 17 February 2015 )