



# Journal of Applicable Chemistry

2014, 3 (5): 1973-1979

(International Peer Reviewed Journal)



## Synthesis, Characterization And Pharmacological Evaluation of Novel Substituted Amides Containing Pyrimidine Nucleus as Antibacterial Agents

S.R.Hareesh<sup>1</sup>, V.Venkatalakshmi<sup>2\*</sup>, K.E.Manoj Kumar<sup>3</sup>, D.N.Vinay<sup>2</sup> and R.Arunachalam<sup>4</sup>

1. Department of Chemistry, Sambhrama College of Arts, Science and Commerce, Vidhyaranyapura, Bangalore, Karnataka-560092, INDIA & Department of Chemistry, AMCEngineering College, Bangalore-560083, **INDIA**

2. Department of Chemistry, AMC Research Centre, AMC Engineering College, Bangalore-560083, **INDIA**

3. Department of Chemistry, KLE'S S. Nijalingappa College, Rajainagar II Block, Bangalore, Karnataka- 500010, **INDIA**

4. Department of Chemistry, Sri Paramakalyani College, Alwarkuruchi, Tamilnadu, **INDIA**

Email: [laxmimurthy@rediffmail.com](mailto:laxmimurthy@rediffmail.com), [harreesr@gmail.com](mailto:harreesr@gmail.com)

Accepted on 9<sup>th</sup> September 2014

### ABSTRACT

*The paper presents the synthesis of some new amides containing pyrimidine as core moiety 6a-e by the reaction between acid 4 with different substituted amines in the presence of base. The key intermediate 4 was synthesized from compound 1 via Suzuki coupling and then hydrolysis reaction. The structures of compounds were confirmed by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and CHN elemental analysis. The newly synthesized compounds were evaluated for their antibacterial activity. It was found that few amides exhibited significant antibacterial activity.*

**Keywords:** Suzuki coupling, pyrimidine, dikis, cesium carbonate, 3-(3-dimethylaminopropyl) carbodi imide hydrochloride, hydroxybenzotriazole, antibacterial activity.

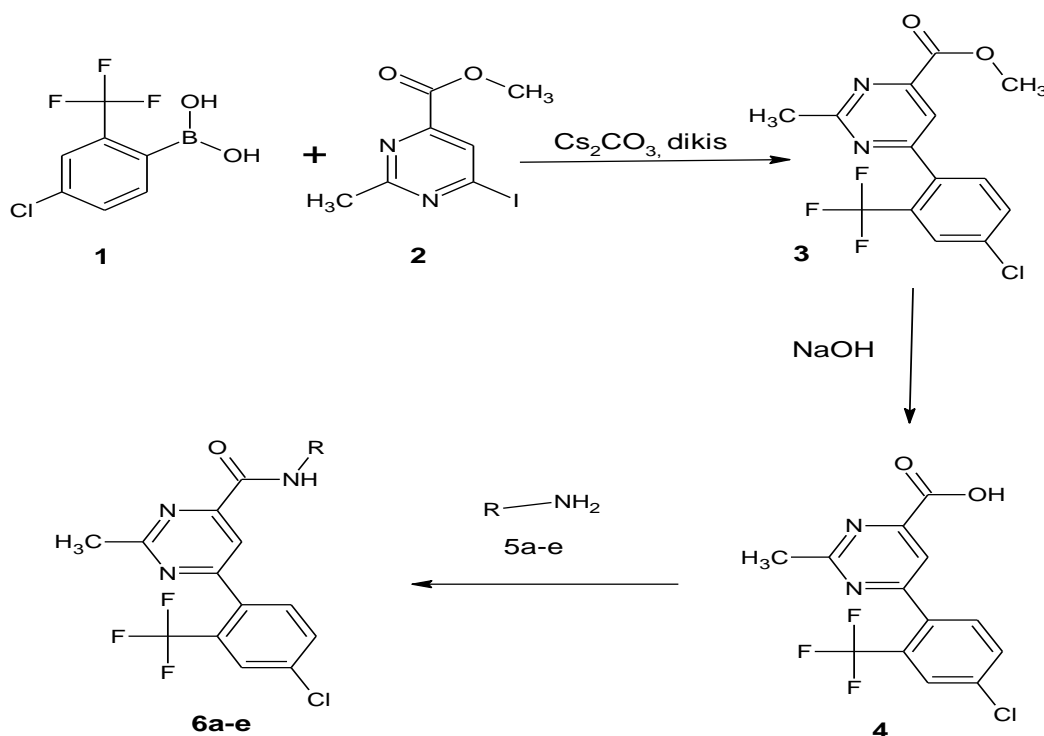
### INTRODUCTION

Pyrimidine is important heterocyclic ring present in a large number of biologically active molecules of different pharmacological classes[1,2]. It is known to have fungicidal, bactericidal and herbicidal activities. Compounds carrying the pyrimidine ring have been reported to demonstrate a wide range of pharmacological activities[3-5], which include antibacterial, antifungal, antitubercular, anticonvulsant, analgesic[6], antihistaminic, and antihypertensive activity[7-9]. Pyrimidines are considered to be important not only because they are integral part of genetic material viz. DNA and RNA as nucleotide and nucleoside but they are also important in numerous biological activities such as bactericidal, fungicidal, insecticidal etc. Biodynamic property of pyrimidine ring system prompted us to account for their pharmacological properties, especially as antibacterial agents[10-18]. In present work some of the new compounds were synthesized and evaluate for their antimicrobial activity.

## MATERIALS AND METHODS

Chemicals were purchased from Merck India, Spectrochem and Sigma–Aldrich. Solvents and chemicals used were of LR grade. The purity of the compounds was confirmed by thin layer chromatography using precoated TLC plates and solvent systems are dichloromethane / methanol (9:1) and petroleum ether / ethyl acetate (6:4) and further purification was done using column chromatography. Melting points were determined in one end open capillary tubes on a liquid paraffin bath and are uncorrected. Mass spectra,  $^1\text{H}$  Nuclear Magnetic Resonance spectra and  $^{13}\text{C}$  nuclear magnetic resonance spectra were recorded for the compounds on Agilent Mass spectrometer, Bruker model advance II (400 MHz,  $^1\text{H}$  NMR) and Bruker model advance II (100 MHz,  $^{13}\text{C}$  NMR) instruments respectively. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard, physical characterization data are given in table 1.

**Experimental:** Synthetic route for the preparation of amides **6a-e** is shown in **Scheme 1**

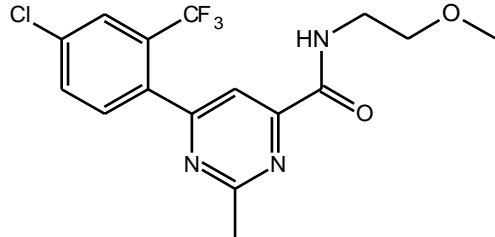
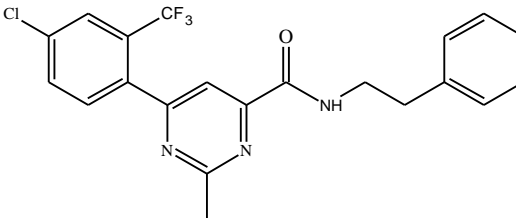
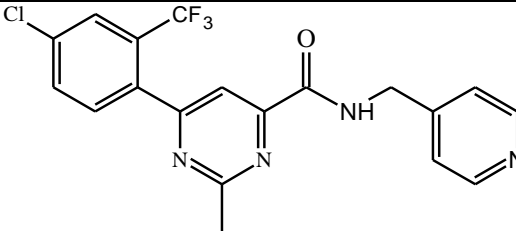


**Procedure for the preparation of methyl-6-(4-chloro-2-(trifluoromethyl)phenyl)-2-methylpyrimidine-4-carboxylate (**3**):** Compound 4-chloro-2-(trifluoromethyl)phenylboronic acid (**1**) (20g, 71.94 mmol) was taken in 200 mL of 1,4-dioxane, ethanol and water solvent system (2;2;1) at room temperature under nitrogen atmosphere. The reaction mixture was degassed with argon for 20 min and  $\text{Cs}_2\text{CO}_3$  (58.45 g, 179.85mmol), dikis (2.21g, 3.16mmol) were added and degassed for 30 min. Methyl 6-iodo-2-methylpyrimidine-4-carboxylate (20.49g, 79.13mmol) (**2**) was added and the reaction mixture was heated at  $65^\circ\text{C}$  for 5 h. Reaction mixture was allowed to cool to room temperature and diluted with ethyl acetate filtered over celite, washed with ethyl acetate. The filtrate was washed with water and brine solution. The ethyl acetate was dried over anhydrous  $\text{MgSO}_4$  and concentrated to get the crude product which was further purified by column chromatography using pet ether and ethyl acetate as eluent to get the title compound (**3**) as off white solid. The structure of the compound was confirmed by IR & NMR data as given below. IR:  $\nu_{\text{max}}/\text{cm}^{-1}$ : 1673.2 (CO).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 8.06 (s, 1H, Ar-H), 7.83 (s, 1H, Ar-H), 7.69 (d, 1H, Ar-H), 7.50 (d, 1H, Ar-H), 3.79 (s, 3H, O-CH<sub>3</sub>), 2.81(s, 3H, Ar-CH<sub>3</sub>) MP:  $199\text{--}200^\circ\text{C}$ . Yield: 64%.

**Procedure for the preparation of 6-(4-chloro-2-(trifluoromethyl)phenyl)-2-methylpyrimidine-4-carboxylic acid (4):** Methyl-6-(4-chloro-2-(trifluoromethyl)phenyl)-2-methylpyrimidine-4-carboxylate (5) (20g, 10.66mmol) and sodium hydroxide (41.81g, 181.81mmol) taken in aqueous THF (200mL) and heated the reaction mixture at 60 °C for 1 h. Cool the reaction mixture, adjusted to pH 6 using 1.5 N HCl, precipitate was filtered and dried to get title compound (6). <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ ppm: 8.00 (s, 1H, Ar-H), 7.83 (s, 1H, Ar-H) 7.69 (d, 1H, Ar-H), 7.50 (d, 1H, Ar-H) 2.89 (s, 3H, Ar-CH<sub>3</sub>). LCMS: 415.12 (M+1). MP: 221-222 °C. Yield 90 %.

**General Procedure for the preparation for final compounds (6a-e):** 6-(4-chloro-2-(trifluoro methyl) phenyl)-2-methylpyrimidine-4-carboxylic acid (4) (1.5g, 4.74mmol), different substituted amines (5a-e) (5.69mmol), 3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) (33.01g, 117.12mmol), hydroxybenzotriazole (HOBT) (0.317g, 2.35mmol) and triethylamine (21.8g, 141.00mmol) were stirred in dry ethylene dichloride (16mL) under nitrogen atmosphere at room temperature for 12 h. The reaction mixture was washed with 10% NaHCO<sub>3</sub>, the organic phase was washed with water and brine, then dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. Residue was purified by neutral alumina column chromatography using MDC / MeOH as a eluent (9:1) to get title compounds (6a-e) in moderate yield. Physical data of all the final compounds and IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR values are tabulated in table 1 and table 2 respectively.

**Table 1:** Physical data of final molecules 6a-e

Compound	MP (°C)	Yield (%)	LCMS (M+1)	Structures	Calculated (Found) %		
					C	H	N
6a	198	55	374.1		50.08 (50.07)	3.64 (3.62)	11.68 (11.67)
6b	182	62	420.1		60.08 (60.05)	4.08 (4.06)	10.01 (10.00)
6c	176	61	407.8		56.10 (56.07)	3.47 (3.46)	13.77 (13.75)

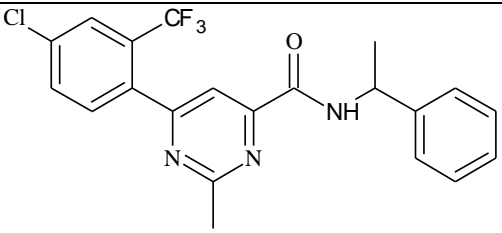
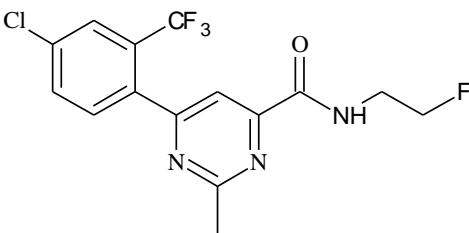
6d	210	76	420.1		60.08 4.08 10.01 (60.06)(4.07)(10.02)
6e	188	52	362.7		49.81 3.34 11.62 (49.80)(3.32)(11.62)

Table 2: IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR of the final compounds 6a-e

Comp	IR	<sup>1</sup> H NMR	<sup>13</sup> C NMR
6a	IR (KBr,cm-1) : 1650 (C=O), 1301-1165 (CF <sub>3</sub> stretching).	<sup>1</sup> H NMR ; CDCl <sub>3</sub> (ppm): 8.36 (s, 1H, NH); 8.04 (s, 1H, Ar-H); 7.80 (d, 1H, Ar-H); 7.66 (dd, 1H, Ar-H); 7.46 (d, 1H, Ar-H); 3.73 (q, 2H, NCH <sub>2</sub> ); 3.62 (t, 2H, O-CH <sub>2</sub> ); 3.43 (s, 3H, -O-CH <sub>3</sub> ); 2.83 (s, 3H, CH <sub>3</sub> ).	<sup>13</sup> C NMR; CDCl <sub>3</sub> (ppm): 173.4, 170.1, 167.7, 161.5, 155.4, 149.4, 138.5, 130.7, 126.1, 125.0, 120.3 (CF <sub>3</sub> ), 106.0, 64.4, 55.8, 41.5, 20.3.
6b	IR (KBr,cm-1) : 1680 (C=O), 1293-1264 (CF <sub>3</sub> stretching).	<sup>1</sup> H NMR ; CDCl <sub>3</sub> (ppm): 8.56 (s, 1H, N-H); 7.79 (s, 1H, Ar-H); 7.66 (d, 2H, Ar-H); 7.56 (dd, 1H, Ar-H); 7.41-7.39 (m, 5H, Ar-H); 3.37 (q, 2H, -CH <sub>2</sub> ); 2.93 (t, 2H, -CH <sub>2</sub> ); 2.79 (s, 3H, Ar-CH <sub>3</sub> ).	<sup>13</sup> C NMR; CDCl <sub>3</sub> (ppm): 165.4, 163.2, 160.6, 157.3, 140.5, 132.6, 132.5, 130.9, 128.5 (2C), 126.5 (2C), 125.4, 123.6, 123.4, 123.2 (CF <sub>3</sub> ), 115.1, 40.2, 35.1, 24.3.
6c	IR (KBr,cm-1) : 1634 (C=O), 1252-1166 (CF <sub>3</sub> stretching).	<sup>1</sup> H NMR ; CDCl <sub>3</sub> (ppm): 8.76 (s, 1H, N-H); 8.56 (d, 2H, Ar-H); 7.79 (s, 1H, Ar-H); 7.66 (d, 2H, Ar-H); 7.56 (dd, 1H, Ar-H); 7.35 (d, 2H, Ar-H); 4.11 (d, 2H, -CH <sub>2</sub> ); 2.74 (s, 3H, -CH <sub>3</sub> ).	<sup>13</sup> C NMR; CDCl <sub>3</sub> (ppm): 165.9, 162.3, 161.0, 157.9, 149.8 (2C), 147.6, 134.6, 132.6, 130.9, 129.2, 125.4, 123.4, 123.9, 123.4 (CF <sub>3</sub> ), 122.4 (2C), 116.0, 43.7, 24.2.

<b>6d</b>	IR (KBr,cm-1) : 1635 (C=O), 1280-1160 (CF <sub>3</sub> stretching).	<sup>1</sup> H NMR ; CDCl <sub>3</sub> (ppm): 8.87 (s, 1H, N-H); 7.79 (s, 1H, Ar-H); 7.66 (d, 2H, Ar-H); 7.56 (dd, 1H, Ar-H); 7.40-7.31 (m, 5H, Ar-H); 4.97-4.91 (m, 1H, junction proton); 2.78 (s, 3H, Ar-CH <sub>3</sub> ); 1.44 (d, 3H, -CH <sub>3</sub> ).	<sup>13</sup> C NMR; CDCl <sub>3</sub> (ppm): 165.9, 163.4, 160.7, 157.3, 141.5, 134.6, 132.6, 130.9, 128.5 (2C), 126.5 (2C), 126.1, 126.0, 125.4, 123.9, 123.4 (CF <sub>3</sub> ), 116.0, 49.5, 24.4, 21.5.
<b>6e</b>	IR (KBr,cm-1) : 1648 (C=O), 1290-1170 (CF <sub>3</sub> stretching).	<sup>1</sup> H NMR ; CDCl <sub>3</sub> (ppm): 8.55 (s, 1H, N-H); 7.79 (s, 1H, Ar-H); 7.66 (d, 2H, Ar-H); 7.56 (dd, 1H, Ar-H); 4.35 (t, 2H, CH <sub>2</sub> F); 3.16 (q, 2H, NCH <sub>2</sub> ); 2.70 (s, 3H, -CH <sub>3</sub> ).	<sup>13</sup> C NMR; CDCl <sub>3</sub> (ppm): 165.9, 163.4, 160.7, 157.3, 134.6, 130.9, 129.2, 129.1, 123.9, 123.8, 122.4 (CF <sub>3</sub> ), 116.0, 81.0, 38.0, 24.0.

## RESULTS AND DISCUSSION

In the present work we reported the synthesis of novel amide of pyrimidines **6a-e** according to **Scheme 1**. The key intermediate scaffold **4** was generated from commercially available compound **1** via Suzuki coupling[19,20] and hydrolysis reaction. The scaffold **4** underwent condensation reaction[21] with various alkyl, aryl substituted amines in the presence of base, dehydrating agent and coupling agent to yield the desired compounds **6a-e**.

## APPLICATIONS

**Invitro antibacterial activity:** Amides containing pyrimidine derivatives **6a-e** was studied against Gram positive *Staphylococcus aureus* (NCIM-5022) and Gram negative *Klebsiella aerogenes* (NCIM-2098), *Escherichia coli* (NCIM-5051), *Pseudomonas aeruginosa* (NCIM-2242) bacterial strains. All the bacterial strains were procured from CSIR-National Chemical Laboratory (NCL) Pune. Agar well diffusion method[22] was incorporated for the study broth cultures of bacterial strains were incubated for 24 h and were uniformly smeared on sterile nutrient agar medium in each petri plates using sterile L-Shaped glass rod. Five uniform wells with 6 mm diameter were bored using cork borer to accommodate 50 µL of solution in each well. Samples were dissolved in dimethylsulphoxide (DMSO) a negative control which showed no zone of inhibition and Ciprofloxacin (5 µg 50 µL<sup>-1</sup>) was taken as standard drug a positive control, purchased from Himedia, Mumbai. Concentrations of 200 and 400 µg 50 µL<sup>-1</sup> well were used to assess the dose dependent activity. Sterile micropipette tips were used to load the wells with appropriate amount of sample, control and standard. Then the plates were incubated at 37 °C for 36 h. After the incubation period, the diameter of the zone of inhibition of each well was measured in mm, the experiment was performed in triplicates the average values were calculated are given in table 3.

**Table 3:** Antibacterial activity of 6a-e

Samples	Treatment ( $\mu\text{g}/50\mu\text{L}$ )	<i>Klebsiella aerogenes</i> (Mean $\pm$ SE)	<i>E. coli</i> (Mean $\pm$ SE)	<i>Staphylococcus aureus</i> (Mean $\pm$ SE)
Standard	10	12.33 $\pm$ 0.33	15.12 $\pm$ 0.43	12.10 $\pm$ 0.39
6a	200	—	4.86 $\pm$ 0.30**	1.01 $\pm$ 0.15**
	400	—	6.88 $\pm$ 0.51**	3.21 $\pm$ 0.29**
6b	200	—	5.15 $\pm$ 0.81**	2.22 $\pm$ 0.31**
	400	—	7.22 $\pm$ 0.31**	3.94 $\pm$ 0.32**
6c	200	—	4.25 $\pm$ 0.41**	2.44 $\pm$ 0.20**
	400	—	7.88 $\pm$ 0.61**	2.90 $\pm$ 0.14**
6d	200	—	3.68 $\pm$ 0.31**	3.14 $\pm$ 0.1**
	400	—	5.42 $\pm$ 0.26**	5.01 $\pm$ 0.42**
6e	200	4.58 $\pm$ 0.59**	5.15 $\pm$ 0.58**	2.35 $\pm$ 0.26**
	400	6.95 $\pm$ 0.53**	6.28 $\pm$ 0.22**	5.06 $\pm$ 0.41**

Values are the mean  $\pm$  SEM of clear zone. Symbols represent statistical significance, \* $P < 0.05$ , \*\* $P < 0.01$  as compared with the control group.

## CONCLUSIONS

Some of the new series of compounds were synthesized according to Suzuki and condensation reactions, then screened for their antibacterial activity. Among the tested compounds **6b**, **6c** and **6e** possess significant antibacterial activity, rest of the amide derivatives showed moderate antibacterial activity as compared to standard. It can be concluded that this class of compounds certainly hold great promise for discovering safer antibacterial, anthelmintic and anti-inflammatory agents.

## ACKNOWLEDGMENT

The authors are thanks to AMC research centre, Sambhram Institute of Technology for experimental facilities and also thank full to Sree Siddaganga College of Pharmacy for providing the pharmacological studies.

## REFERENCES

- [1] G.S. Gadaginamath, A.S. Shyadligeri, R.R. Kavali, *Indian J Chem*, **1999**, 38B, 156.
- [2] P. Renukadevi, J.S. Birada, *Indian J Heterocyclic Chem*, **1999**, 9, 107.
- [3] D. Martin, Z. Jan, K. Diana, K. Jiri, S. Michaela, *Molecule* **2009**, 14, 4180-4189.
- [4] M.B. Siddesh, P. Basavaraj, K.S. Thriveni, C. Sandeep, B.C. Goudrashivannanavar, *Journal of Applicable Chemistry*, **2013**, 2(5), 1281-1288.

- [5] K.E. Manojkumar, S. Sreenivasa, N.R. Mohan, T. Madhu Chakrapani Rao, T. Harikrishna, *Journal of Applicable Chemistry*, **2013**, 2 (4), 730-737.
- [6] H. Oza, D. Joshi, H. Parekh, *Indian J Chem*, **1998**, 37 B, 822.
- [7] R.S. Lodhi, S.D. Srivastava, *Indian J Chem*, **1997**, 36B, 947.
- [8] F.N. Arthur, M.S. James, R.M. Robert, C.M. Frank, L.S. Evald, *Appl. Microbiol.* **1969**, 1050-1056.
- [9] N.C. Desai, D. Dave, M.D.Shah, G.D.Vyas, *Indian J Chem*, **2000**, 39 B, 277.
- [10] M.B. Hogle, A.C. Uthale, B.P. Nikam, *Indian J Chem*, **1991**, 30B, 717.
- [11] I.V. Ukrainets, N.L.Bereznyakova, V.A. Parshikov, O.V. Gorokhova, *Chemistry of Heterocyclic Compounds*. 2008. 44(12), 1493-1499.
- [12] N. Pedemonte, T. Diena, E. Caci, J.V. Galletta, *Mol Pharmacol*, **2005**, 68, 1736.
- [13] C.O.Kappe, *Tetrahedron*, **1993**, 49, 6937.
- [14] K. E. Manojkumar, S. Sreenivasa, N. R. Mohan, P. A. Suchetan, C. G. Darshan Raj and H. Raja Naika, 2-[5-(2-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzoic acid: Synthesis, Characterization and Pharmacological Evaluation, *Journal of Applicable Chemistry*, **2014**, 3 (1): 64-73.
- [15] S K Dewan, Punam, A Kuma, *Journal of Applicable Chemistry*, **2013**, 2, 714.
- [16] S K Dewan, Punam, *Journal of Applicable Chemistry*, 2014, 3, 639.
- [17] D.P Mahajan, R S Bendre, *Journal of Applicable Chemistry*, **2014**, 3, 1239.
- [18] Punam, Deepika, Anil Kumar and Sharwan K Dewan, *Journal of Applicable Chemistry*, 2014, 3(4), 1495- 1498.
- [19] Manojkumar K E, Sreenivasa S, Mohan N R, Harikrishna, T. *Molbank*. 2013, M803; doi: 10.3390/M803.
- [20] S. Sreenivasa, K.E. Manojkumar, P.A. Suchetan, N.R. Mohan, B.S. Palakshamurthy, T. Srinivasan, D. Velmurgan, *Acta Cryst.* **2012**. E68, 03370.
- [21] K.E. Manojkumar, S. Sreenivasa, G. Shivaraja, T. Madhu Chakrapani Rao, *Molbank*. **2013**, M803; doi: 10.3390/M803.
- [22] S. Sharma, K.S. Lakshmi, A.Patidar, A.Chaudhary, S. Dhaker, S. *Indian J Phramacol.* **2009**, 41(2), 87-88.