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Synthesis of new dihydropyridazin-3(*2H*)-ylidene)hydrazine carbothioamide derivatives as an antituberculosis agent

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ABSTRACT

A series of new carbothioamide derivatives containing pyridazine ring have been synthesized by acid catalyzed condensation of thiosemicarbazide with corresponding substituted pyridazinone. The compounds have been characterized by IR, NMR and spectral analysis. All the synthesized compounds are evaluated for antituberculosis activity. Some of these derivatives are found to be active against the Mycobacterium tuberculosis H37Ra (MTCC 300).

Keywords: Thiosemicarbazide, pyridazine, carbothioamide, antituberculosis.

INTRODUCTION

Tuberculosis or TB is a very common and often deadly infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* (MTB). TB is the leading worldwide cause of mortality resulting from an infectious bacterial agent.

The World Health Organization (WHO) estimates that almost one-third of the world's population is infected with MTB, with two billion of the World's inhabitants are infected by MTB (WHO 2010). [1] WHO designed a proposal in 2006 named "The Stop TB Strategy". The main aim of this proposal is to reduce the global burden of TB till 2015. The mission also supports the development of new and effective tools to prevent, diagnose and treat TB. The main objective is an eradication of TB as a public health problem till 2050 (WHO STB 2006) [2].

Over the past 20 years have seen the worldwide appearance of multidrug-resistant (MDR) TB, followed by extensively drug-resistant (XDR) TB and most recently, strains that are resistant to all antituberculosis drugs. MDR tuberculosis is caused by *M. tuberculosis* that is resistant to at least Isoniazide (INH) and Rifampicin (RIF) and XDR tuberculosis by mycobacteria resistant to rifampicin and isoniazide (INH), any fluoroquinolone and one of the three injectable drugs, capreomycin, kanamycin, and amikacin. Drug resistance severely threatens tuberculosis control, since it raises the possibility of a return to an era in which drugs are no longer effective [3-5]. Consequently, although efficacious anti-TB drugs are available, TB is still a serious global threat to public health and a continued search is imperative for new antimycobacterial agents and therapeutic regimens. Modifications in preexisting antituberculosis drugs are now widely applied, which results in the development of novel, effective and non-toxic antituberculosis agents.

Hydrazone or carbothioamide derivatives are a considerable pharmacophore group for antimicrobial activity, but the first reports on their pharmaceutical applications as drugs against leprosy and tuberculosis were published in the 1950s [6,7]. Hydrazones constitute an important class of biologically active drug molecules, and are the focus of growing interest of medicinal chemists due to their wide range of pharmacological properties [8]. Moreover, number of carbothioamide derivatives have been reported to exert notably antimicrobial, [9] antihypertensive, [10] anticonvulsant, [11] analgesic, [12] anti-inflammatory, [13] antitubercular, [14] antitumoral, [15] antioxidant, [16] antimalarial, [17] antiplatelet, [18] antifungal, [19] and antiviral [20] activities. In addition, to their varied coordinating behavior makes them interesting candidates for metal-based drugs. Generally, the ligands act synergistically with metals towards their biological activity [21, 22]. Several research groups have synthesized these compounds as well as their metal complexes as target structures and evaluated their biological activities [23].

As part of our ongoing research program focused upon the synthesis of biologically active molecules, [24-30] here in the present context, we synthesized (E)-2-(6-(4-substituted phenyl)-2-(piperidin/pyrolidin-1ylmethyl)-4,5-dihydropyridazine-3(2H)-ylidene)hydrazine carbothioamide or hydrazones **(3a-p)** in order to investigate their antimycobacterial activities by using the resazurin micro titer assay plate method against *Mycobacterium tuberculosis H37Ra (MTCC 300)* in LJ 7H9-S medium. The pyridazinone moiety in the present study has been reported to possess a wide variety of biological activities [31-33]. In particular, a large number of pyridazinones are well known as intermediates for drugs and agrochemicals.

MATERIAL AND METHODS

Melting points were determined by an open capillary method and are uncorrected. IR spectra were recorded (KBr cm^{-1}) on the FTIR SHIMADZU spectrometer. ^1H NMR spectra were recorded in DMSO-*d6* on Avance-300 MHz spectrometer using TMS as an internal standard. Chemical shift values are given in δ ppm. The mass spectra were recorded on EI-SHIMADZU GC- Mass spectrometer. Purity of compounds was checked by thin layer chromatography (TLC), silica gel coated on glass plate and visualized in iodine chamber.

General procedure of (E)-2-(6-(4-substituted phenyl)-2-(piperidin/pyrrolidin-1ylmethyl)-4,5-dihydro pyridazine-3(2H)-ylidene)hydrazine carbothioamide (3a-p):
A mixture of pyridazine ketone i.e. 6-(4-substituted phenyl)-2-(piperidin/pyrolidin-1-ylmethyl)-4,5-dihydropyridazin-3-(2H)-one (1mol) and thiosemicarbazide (1mol) was taken in of acetic acid (20 mL), catalysed by 3-4 drops of conc. H_2SO_4. The reaction mixture was heated for 3 hours at 60-70°C. The progress of the reaction was monitored by thin layer chromatography (TLC) time to time. After completion of the reaction, the reaction mass was cooled and poured into a beaker containing water (100 mL) with stirring. The obtained solid product was filtered, washed using water (2 X 20 ml). It was then dried and recrystallized from methanol.

Microbiology
One clinical isolate is used in this study from sputum sample collected from local hospital and *M. tuberculosis* H37Ra (MTCC 300) was used as the susceptible control. All strains were freshly sub cultured on LJ medium before use and were tested by the different methods.

Rifampicin (RIF) was obtained from Sigma-Aldrich (Bornem, Belgium). The stock solution was prepared in advance at a concentration of 1 mg/ml in 0.1 N NaOH, filter sterilized and kept in -20°C for no more than 1 month. The stock solution of compounds was prepared 1mg/ml in DMF. A stock solution of the resazurin sodium salt powder (Himedia, Mumbai) was prepared at 0.01% in distilled water, filter sterilized, and kept on -4°C. The resazurin micro titer assay plate method was carried out as described by Anandi Martin et al. Briefly, the inoculum was prepared from fresh LJ medium to 7H9-S medium (consisting of Middlebrook7H9 broth containing 0.1% Casitone and 0.5% glycerol and supplemented with oleic acid, albumin, dextrose, and catalase [OADC; Becton Dickinson], adjusted to a no. 1 McFarland tube, and diluted 1:20; 100µl was used as the inoculum. The Rifampicin stock solution was thawed and diluted in 7H9-S medium to four times higher final concentration tested. Serial twofold dilutions of Rifampicin were prepared directly in a sterile 96-well flat-bottom micro titer plate (Merck, Banglore) by using 100µl of 7H9-S. The range of concentrations tested for Rifampicin and the tested compounds (3a-3p) was 1.56 to 200 µg/ml. A growth control containing no antibiotic and a sterile control without inoculation were also prepared on each plate. Two hundred micro liters of sterile water were added to all perimeter wells to avoid evaporation during incubation. The plate was covered with its lid, replaced in the original bag, and incubated at 37°C under a normal atmosphere. After 7 days of incubation, 30 µl of resazurin solution was added to each well

and the plate was re-incubated overnight. Then, at day 8, a change in color from blue (oxidized state) to pink (reduced state) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in color. [34]

Compound	Spectral Data
3a	IR (KBr): 3378 (N-H Stretching), 3030 (Ar-H Stretching), 1610 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.41 (s, 1H, -NH), 7.33-7.28 (m, 5H, Ar-H), 7.01 (s, 2H, $-NH_2$), 4.49 (s, 2H, N-CH_2-N), 2.92 (t, 2H –CH_2), 2.44 (t, 2H, –CH_2) protons of pyridazine ring, 2.47-1.58 (m, 10H, protons of piperidine). ppm; Mass (m/z), 344 (M+ ion).
3b	IR (KBr): 3370 (N-H Stretching), 3029 (Ar-H Stretching), 1605 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.40 (s, 1H, -NH), 7.38-7.30 (m, 4H, Ar-H), 7.10 (s, 2H, $-NH_2$), 4.51 (s, 2H, N-CH_2-N), 2.91 (t, 2H), 2.40 (t, 2H) protons of pyridazine ring, 2.36-1.57 (m, 10H, protons of piperidine) ppm; Mass (m/z), 378 (M+ ion).
3d	IR (KBr): 3380 (N-H Stretching), 3029 (Ar-H Stretching), 1603 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.42 (s, 1H, -NH), 7.39-7.33 (m, 4H, Ar-H), 7.08 (s, 2H, $-NH_2$), 4.50 (s, 2H, N-CH_2-N), 2.90 (t, 2H), 2.42 (t, 2H) protons of pyridazine ring, 2.34-1.56 (m, 10H, protons of piperidine). ppm; Mass (m/z), 423 (M+ ion).
3h	IR (KBr): 3383 (N-H Stretching), 3031 (Ar-H, Stretching), 1609 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6):δ 7.43 (s, 1H, -NH), 7.41-7.39 (m, 4H, Ar-H), 6.98 (s, 2H, $-NH_2$), 4.52 (s, 2H, N-CH_2-N), 2.95 (t, 2H), 2.41 (t, 2H) protons of pyridazine ring , 2.33 (t, 4H), 1.51-1.55 (m, 10H protons of piperidine). ppm; Mass (m/z), 389 (M+ ion).
3i	IR (KBr): 3372 (N-H Stretching), 3031 (Ar-H, Stretching), 1617 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.54 (s, 1H, -NH), 7.32-7.39 (m, 5H, Ar-H), 6.99 (s, 2H, $-NH_2$), 4.50 (s, 2H, N-CH_2-N), 2.90 (t, 2H), 2.81(t, 2H) protons of pyridazine ring, 2.38-2.33(m, 8H, protons of piperidine). ppm; Mass (m/z), 330 (M+ ion).
3j	IR (KBr): 3374 (N-H Stretching), 3030 (Ar-H, Stretching), 1639 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.54 (s, 1H,-NH), 7.32-7.39 (M, 4H, Ar-H), 6.99 (s, 2H, $-NH_2$), 4.50 (s, 2H, N-CH_2-N), 2.93 (t, 2H), 2.84(t, 2H) protons of pyridazine ring, 2.40-2.30(m, 8H, protons of piperidine). ppm; Mass (m/z), 374 (M+ ion).
3l	IR (KBr): 3371 (N-H Stretching), 3032 (Ar-H, Stretching), 1605 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.51 (s, 1H,-NH), 7.22-7.10 (m, 4H, Ar-H), 6.91 (s, 2H, $-NH_2$), 4.49 (s, 2H, N-CH_2-N), 2.90 (t, 2H), 2.86 (t, 2H) protons of pyridazine ring, 2.39-2.28(m, 8H, protons of piperidine). ppm; Mass (m/z), 410 (M+ ion).
3p	IR (KBr): 3377 (N-H Stretching), 3028 (Ar-H, Stretching), 1606 (C=N), cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d6): δ 7.50 (s, 1H, -NH), 7.37-7.33 (M, 4H, Ar-H), 7.02 (s, 2H, $-NH_2$,), 4.51 (s, 2H CH_2-N), 2.88 (t, 2H), 2.80 (t, 2H) protons of pyridazine ring, 2.48-2.31(m, 8H, protons of piperidine). ppm; Mass (m/z), 375 (M+ ion).

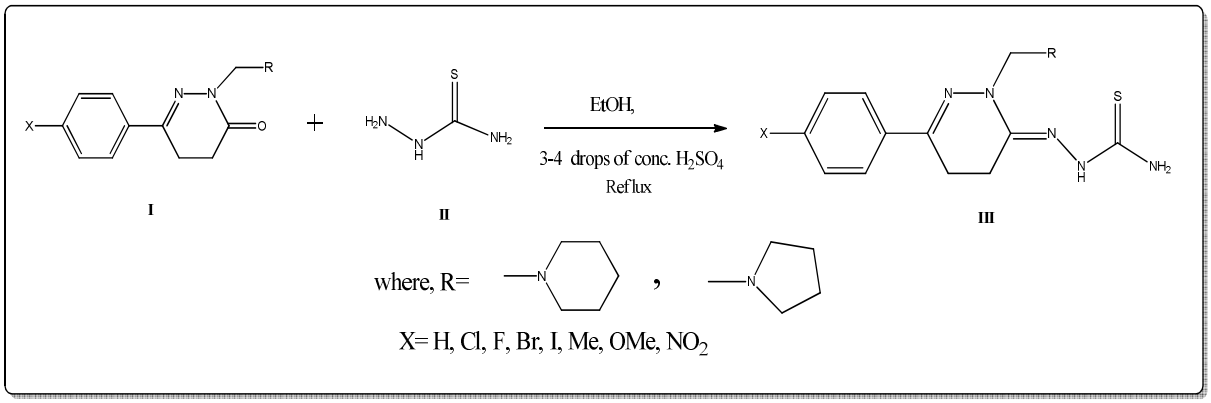
RESULTS AND DISCUSSION

The pyridazinone derivatives i.e (I) were synthesized by the reported method as shown in Scheme with following sequence of reactions:

(i) Friedel Crafts acylation of substituted benzene with succinic anhydride in the presence of anhydrous aluminium chloride yielded substituted benzoyl propionic acid.

(ii) cyclization of substituted benzoyl propionic acid to react with hydrazine hydrate to form 6-(4-substitutedphenyl)-4,5-dihydro pyridazin-3(2H)-one (pyridazinone ring)

Mannich bases of different pyridazinones were prepared by the reaction with paraformaldehyde and piperidine or pyrrolidine under reflux in a mixture of ethanol and 0.5 mL of conc. HCl. The Mannich reaction product was condensed with thiosemicarbazides in acetic acid catalysed by 3-4 drops of conc. H_2SO_4. A series of (E)-2-(6-(4-substituted phenyl)-2-(piperidin/ pyrolidin-1yl methyl)-4,5-dihydro pyridazine-3(2H)-ylidene) hydrazine carbo -thioamide (3a-p) were synthesized as depicted in scheme.

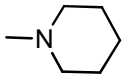
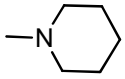
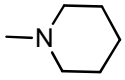
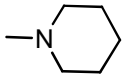
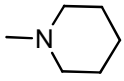
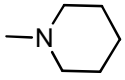
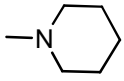
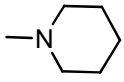
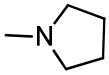
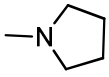
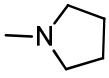
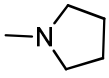
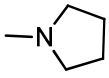
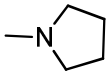
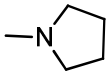
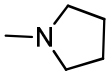


Scheme1

The structures of the synthesized compounds were elucidated through analytical and on the basis of spectral data. The ^{1}H NMR spectrum showed the presence of singlets at δ 4.53-4.56 ppm for 3a-3h and δ 3.48-4.10 ppm for 3i-3p corresponding to the N-CH_2-N group, one singlet of –NH proton at δ 7.41 ppm, one singlet of –NH_2 proton at δ 7.00 ppm, two triplets at near about δ 2.92-2.44 ppm corresponding to protons of pyridazine moiety. The aromatic protons were observed at the expected chemical shift and integral values.

The IR spectrum of **3a** exhibited sharp band at 3378, 3324 cm^{-1} for N-H and NH_2, a characteristic band at 1610 cm^{-1} for -C=N, that clears the disappearance of -C=O band at 1670 cm^{-1} confirms the condensation of thiosemicarbazide with pyridazinone. Mass spectrum of compound 3a showed a molecular ion peak *m/z* at 344.

Table 1. Antimycobacterial activity results of the synthesized compounds (3a-3p)

Sr. No.	R	X	M.P in °C	MIC (µg/ml)	Zone of inhibition in Mm 150 µg/ml
3a		H	178	3.2	21
3b		4-Cl	180	3.4	29
3c		4-F	180	3.0	25
3d		4-Br	188	2.7	27
3e		4-I	155	2.5	23
3f		4-Me	162	2.8	23
3g		4-OMe	154	2.4	25
3h		4-NO_2	190	1.5	30
3i		H	167	1.9	16
3j		4-Cl	144	2.8	18
3k		4-F	176	2.5	17
3l		4-Br	169	2.7	23
3m		4-I	187	2.8	25
3n		4-Me	163	2.4	21
3o		4-OMe	146	2.3	22
3p		4-NO_2	155	1.7	31
Rifampicin	-	-		1.1	38

[a]*Isolated yield*

The synthesized series of hydrazone derivatives with pyridazine moiety were screened for antitubercular activity against *Mycobacterium tuberculosis H37Ra (MTCC 300)* in LJ 7H9-S medium using the resazurin micro titer assay plate method. With the resazurin assay, colorimetric test results were available after an average of 8 days of incubation. These results are presented in **Table 1**. From the data obtained by antitubercular screening, it reveals that several compounds showed inhibitory activity against *M. tuberculosis* in primary screening assays at a concentration 1.56-200 µg/ml. The compounds 3h, 3n, 3m, 3g, 3j, 3l and 3p shown to be active against the *M. tuberculosis*, where as the other compounds 3a, 3b, 3c, 3d, 3 f, 3i, 3k, 3e and 3o are found to be moderately active against *M. tuberculosis*. The potency of the compounds can be explained on the basis substitution pattern of the aromatic nucleus and presence of five member heterocycles. The pyrrolidine nucleus with an electron donating group exhibits a synergic effect against *M. tuberculosis*. This is may be due to increase in the liophilicity of the compounds.

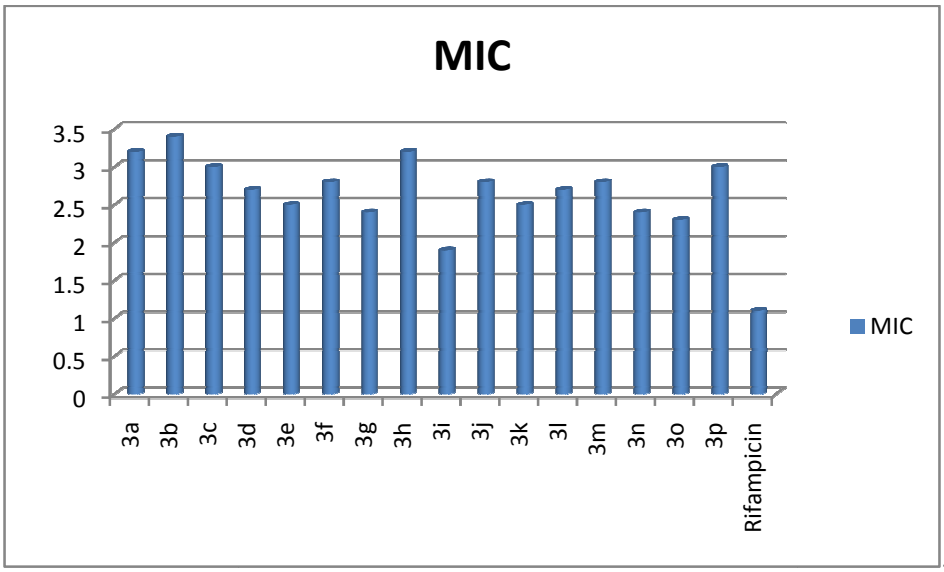


Figure 1: Graphical Antituberculosis activity of Compounds 3a-3p against *Mycobacterium tuberculosis* H37Ra (MTCC 300)

CONCLUSION

In the present study, a series of dihydropyridazin-3(2H)-ylidene)hydrazine carbothioamide derivatives were synthesized and screened for their antituberculosis activities. The structures of the compounds were established on the basis of satisfactory spectral analysis. Some of the synthesized compounds showed inhibitory activity against *M. tuberculosis* in primary screening assays. The compounds 3h, 3n, 3m, 3g, 3j and 3e found to be active against the *M. tuberculosis.* The dihydropyridazin-3(2H)-ylidene)hydrazine carbothioamide derivatives.

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REFERENCES

[1] Global tuberculosis control: a short update to the 2010 report.
http://whqlibdoc. who.int/publications/**2010**/9789241564069_eng.pdf.
[2] The stop TB Strategy, WHO http://whqlibdoc.who.int/hq/2006/WHO_HTM_STB_**2006**. 368_eng.pdf.
[3] Logu AD, Onnis V, Saddi B, Congiu C, Schivo ML, Cocco MT, *J. Antimicrob. Chemoth*, **2002**, 49, 275.
[4] Takahashi K, Hasegawa Y, Abe T, Yamamoto T, Nakashima K, Imaizumi K, Shimokata K, *Tuberculosis*, **2008**, 88, 52.

[5] Caminero JA, Sotgiu G, Zumla A, Migliori GB, *Lancet Infect. Dis*, **2010**, 10, 621.
[6] Bavin, EM, Rees, RJW, Robson JM, Seiler M, Seymour DE, Suddaby D, *J. Pharm. Pharmacol*, **1950**, 2, 764.
[7] Koch O, Stuttgen G, *Arch. Exp. Pathol. Pharmakol*, **1950**, 210, 409.
[8] Seleem HS, El-Inany GA, El-Shetary BA, Mousa MA, *Chemistry Central Journal*, **2011**, 5:2, 1.
[9] Rollas S, Kucukguzel SG, Molecules, **2007**, 12, 1910.
[10] Minami M, Togashi H, Sano M, Saito I, Morii K, Nomura A, Yoshioka M, Saito H, *Hokkaido Igaku Zasshi*, **1985**, 60(6), 856.
[11] Dimmock JR, Vasishtha SC, Stables JP, *Eur. J. Med. Chem*, **2000**, 35(2), 241.
[12] Lima PC, Lima LM, Silva KC, Leda PH, Miranda ALP, Fraga CAM, Barreiro E J, *Eur. J. Med. Chem*, **2000**, 35(2), 187.
[13] Todeschini AR, Miranda AL, Silva CM, Parrini SC, Barreiro EJ, *European Journal of Medicinal Chemistry*, **1998**, 33(3), 189.
[14] Janin Y, *Bioorganic & Medicinal Chemistry*, **2007**, 15(7), 2479.
[15] Cocco MT, Congiu C, Lilliu V, Onnis V, *Bioorganic & Medicinal Chemistry*, **2006**, 14(2), 366.
[16] Jagadeesh PK, Himajaa M, Malib SV, Ranjithaa R, Karigarc A, Sikarward M, *Journal of Pharmacy Research*, **2010**, 3(10), 2460.
[17] Gemma S, Kukreja G, Fattorusso C, Persico M, Romano M, Altarelli M, *Bioorg. Med. Chem. Lett*, **2006**, 16, 5384.
[18] Silva GA, Costa LMM, Brito FCB, Miranda ALP, Barreiro EJ, Fraga CAM, *Bioorganic & Medicinal Chemistry*, **2004**, 12 (12), 3149.
[19] Loncle C, Brunel JM, Vidal N, Dherbomez M, Letourneux Y, *Eur. J. Med. Chem*, **2004**, 39, 1067.
[20] Galaiko NV, Tolmacheva IA, Grishko VV, Volkova LV, Prevozchikova EN, Pestereva SA, *Bioorg Khim*, **2010**, 36(4), 556.
[21] Chaudhuri P, *Coord. Chem. Rev*, **2003**, 243, 143.
[22] Kurtoglu M, Baydemir SA, *J. Coord.Chem*, **2007**, 60, 655.
[23] Patil SD, Kamble RD, Hese SV, Acharya AP, Dawane BS, Kote JR, Gachhe RN, *Der Pharmacia Sinica*, **2013**, 4(2):171.
[24] Dawane BS, Gacche RN, Yemul OS, Kamble RD, Patil SD, Mogle PP, Hese SV, More RA, Kamble SS, Kote JV, **2014**, A process for the preparation of thiosemicarbazone encapsulated metal nano particles, *Indian Patant Application No*. 86/MUM/2014.
[25] Dawane BS, Konda SG, Mandawad GG, Shaikh BM, *Eur. J. Med. Chem*, **2010**, 45, 387.
[26] Chobe SS, Kamble RD, Patil SD, Acharya AP, Hese SV, Yemul OS, Dawane BS, *Med Chem Res*, **2013**, 22, 5197.
[27] Acharya AP, Kamble RD, Patil SD, Hese SV, Yemul OS, Patil SG, Hallale SN, Dawane BS, *Chemical Papers*, **2013**, 68 (5) 719.
[28] Kamble RD, Jawadwar GV, Patil SD, Hese SV, Acharya AP, Dawane BS, Pekamwar SS, *Org. Commun*, **2013**, 6, 2, 95.
[29] Shaikh BM, Konda SG, Mehare AV, Mandawad GG, Chobe SS, Dawane BS, *Der Pharma Chemica*, **2010**, 2(4): 25.
[30] Dawane BS, Chobe SS, Mandawad GG, Shaikh BM, Konda SG, Patil SD, *Der Pharmacia Sinica*, **2010**, 1 (3), 140.
[31] Samanta KC, Asif M, Pooja, Garg V, Sharma P, Singh R, *E-Journal of Chemistry*, **2011**, 8(1), 245.
[32] Dogruer DS, Onkol T, Ozkan S, Ozgen S, Sahin MF, *Turk J Chem*, **2008**, 32, 469.
[33] Khaidem S, Sarveswari S, Gupta R, Vijayakumar VV, *International Journal Of Research In Pharmacy And Chemistry*, **2012**, 2(2), 258.
[34] Martin A, Palomino JC, Portaels F, *J. Clin. Microbiol*, **2005**, 43, 1612.