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Synthesis and evaluation of antibacterial activity of some new N,N'-(5-(6-(4-substitutedphenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)-pyrimidine-2,4-diyl)diacetamide derivatives

Ramappa M. Sankangoud*, Nandini B. Chatni, Prakash S. Goudanavar

K.L.E's College of Pharmacy, Nipani, Karnataka, India

Abstract

*A series of new N,N'-(5-(6-(4-substitutedphenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4-diyl)diacetamide derivatives were synthesized and tested for their antibacterial activity. Guanidine carbonate & ethyl (ethoxymethylene) cyanoacetate are used as the starting materials for the preparation of ethyl-2, 4-Diaminopyrimidine-5-carboxylate (**I**). The reactive amino groups are acetylated using acetic anhydride in presence of DMF to form ethyl 2, 4-diacetamidopyrimidine-5-carboxylate (**Ia**). Efforts without masking the reactive amino groups on pyrimidine at this stage did not yield the target compounds. Further ethyl group from position 5 is removed as ethanol by refluxing **Ia** with 10% alcoholic NaOH for 10min to form 2, 4-diacetamidopyrimidine-5-carboxylic acid (**IIa**). Hence formed acid is refluxed for 4 hours with thiosemicarbazide and phosphorous oxychloride to get N, N'-(5-(5-amino-1, 3, 4-thiadiazol-2-yl) pyrimidine-2, 4-diyl) diacetamide (**IIIa**). Further target compounds were synthesized using different substituted phenacyl bromides. The structures of the newly synthesized compounds were confirmed by IR and ^{1}H NMR spectroscopy. All the synthesized compounds were tested for their antibacterial activities using cup-plate-agar-diffusion method. Result of antibacterial activity reveals that the compounds **IVb, IVe** and **IVf** have shown potent activity against both gram-positive and gram-negative microorganisms as compared to the standard drug methotrexate.*

Key words: Antibacterial activity, thiosemicarbazide, gram-positive, gram negative

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INTRODUCTION

The approach to the practice of medicinal chemistry has developed from an empirical one involving synthesis of new organic compounds based largely on modifications of chemical compounds of known biological activity could be better explored. It is well established that

slight alterations in the structure of certain compounds are able to bring drastic changes to yield better drug with less toxicity to the host. It is observed that chemical modification not only alters physicochemical properties but also pharmacological activities [1].

In view of the general observation several enzymes involved in the biosynthesis of nucleic acid precursors have become attractive biochemical target sites over the past twenty years. In particular, DHFR, a crucial NADPH-linked dehydrogenase that catalyses the reduction of dihydrofolate to tetrahydrofolate has attracted considerable attention. 1,3,4-thiadiazole nucleus is associated with a broad spectrum of biological activity due to presence of toxophoric N-C-S moiety and its non-carcinogenic nature coupled with high polarity (μ=3,25D). The advent of sulfur drugs and the later discovery of mesionic compounds greatly accelerated the rate of progress in the field of thiadiazoles. Thiadiazoles carrying mercapto, hydroxyl and amino substituents can exist in many tautomeric forms and this property is being intensively studied using modern instrumental method [2].

Objective of the present study is to synthesize some new N,N'-(5-(6-(4-substituted phenyl)imidazo[2,1-b] [1,3,4]-thiadiazol-2-yl) pyrimidine-2,4-diyl) diacetamide derivatives. Confirming synthesis of compounds on the basis of IR and NMR spectral data and screen these newly synthesized compounds for antibacterial activity.

RESULTS AND DISCUSSION

The title compounds were synthesized as shown in the scheme. The physico chemical and spectral data of the synthesized compounds shown that the electron withdrawing groups are essential for antibacterial activity. The results of the antimicrobial evaluation of the tested compounds are presented in Table1. They showed that compounds **IVb, IVe** and **IVf** have potent activity against both gram-positive and gram-negative microorganisms with respect to the standard drug methotrexate.

MIC of selected compounds are presented in Table 2. At higher concentration i.e. 100μg/ml and 50 μg/ml the synthesized compounds IVe and IVf shows better zone of inhibition with respect to standard drug methotrexate.

Table1. Screening of Compounds at Concentration at 100 μg by cup-plate method (values are in cm)

Compound Name	*E. Coli*	*S. aureus*	*P. aeruginosa*	*B. subtilis*
Methotrexate	2.1	2.3	2.3	2.2
DMSO	0	0	0	0
Contorl	0	0	0	0
III a	1.1	1.1	0.9	0
IVa	1.7	1.5	1.6	1.4
IVb	1.9	1.8	2.0	2.1
IVc	1.4	1.7	1.5	1.8
IVd	1.7	1.9	1.8	1.6
IVe	2.1	2.1	2.0	1.9
Ivf	1.9	2.1	2.2	2.1

Table2. Analysis of minimum inhibitory concentration (MIC) (concentration µg /ml)

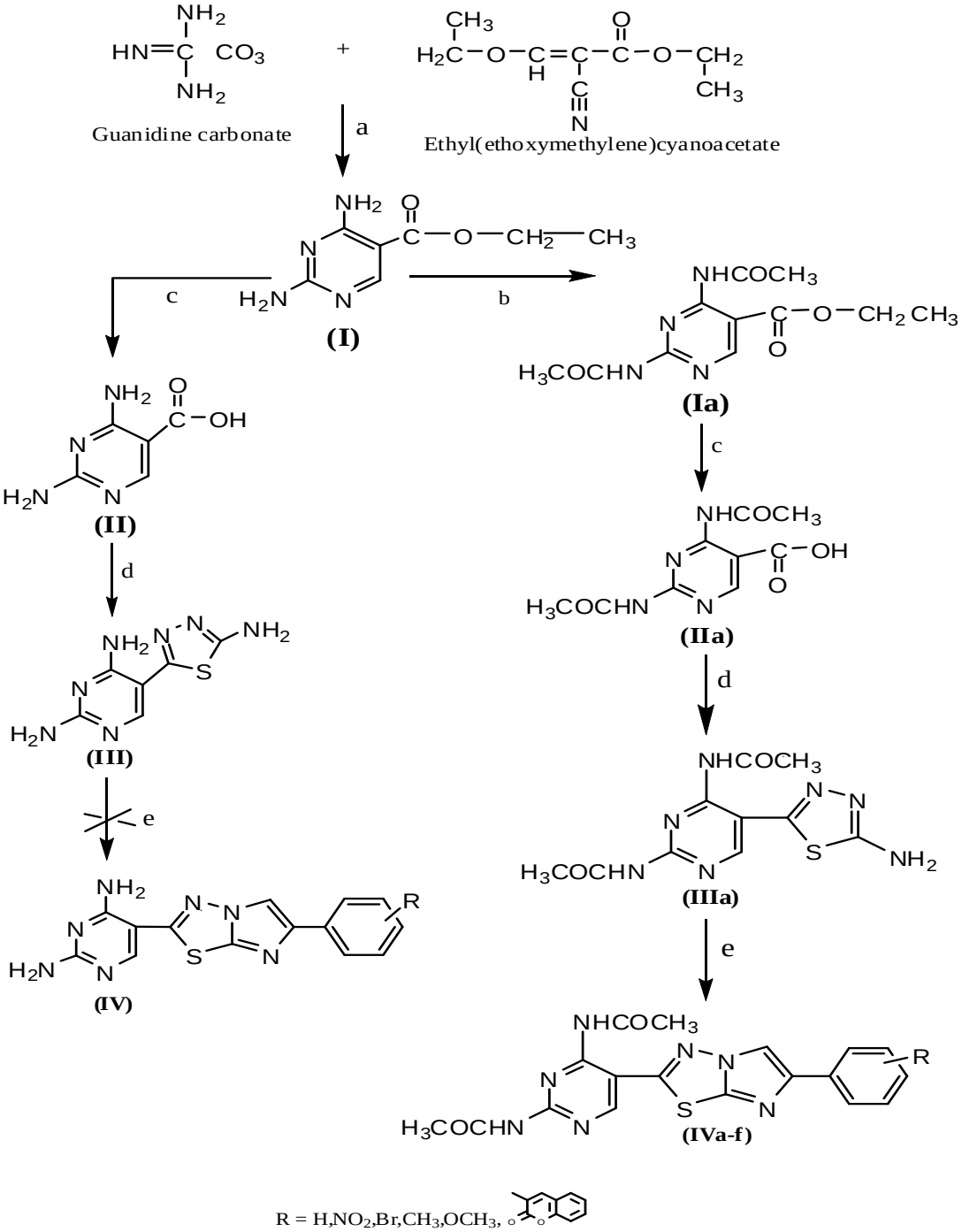
Compound Name	OD at 100 µg	% growth	OD at 50 µg	% growth	OD at 25 µg	% growth	OD at 12.5 µg	% growth	OD at 6.25 µg	% growth	OD at 3.125 µg	% growth	OD at 1.5 µg	% growth	organism
Control	0.58	100	0.58	100	0.58	100	0.58	100	0.58	100	0.58	100	0.58	100	
Methotrexate	0.06	10.34	0.1	17.24	0.16	27.58	0.22	37.93	0.28	48.27	0.39	67.24	0.45	77.58	
DMSO	0.58	100	0.58	100	0.58	100	0.58	100	0.58	100	0.58	100	0.58	100	S.Aureus
IVe	0.04	12.79	0.11	19.41	0.18	29.48	0.24	40.82	0.30	52.89	0.41	74.58	0.58	91.37	
IVf	0.05	11.23	0.12	10.34	0.11	18.96	0.23	39.65	0.29	50.0	0.33	56.89	0.48	82.75	
Control	0.61	100	0.61	100	0.61	100	0.61	100	0.61	100	0.61	100	0.61	100	
Methotrexate	0.08	10.34	0.13	21.31	0.91	31.14	0.26	42.62	0.32	52.45	0.43	70.49	0.54	88.52	
DMSO	0.61	100	0.61	100	0.61	100	0.61	100	0.61	100	0.61	100	0.61	100	E.Coli
IVe	0.05	8.19	0.11	18.03	0.22	36.06	0.29	47.54	0.37	60.65	0.49	80.32	0.56	91.8	
IVf	0.1	16.39	0.16	26.22	0.23	37.7	0.35	57.37	0.42	68.85	0.55	90.16	0.61	100	
Control	0.75	100	0.75	100	0.75	100	0.75	100	0.75	100	0.75	100	0.75	100	
Methotrexate	0.03	4.0	0.09	12.0	0.17	22.66	0.25	33.33	0.37	49.33	0.49	65.33	0.66	88.0	
DMSO	0.75	100	0.75	100	0.75	100	0.75	100	0.75	100	0.75	100	0.75	100	P.Aeruginosa
IVe	0	0	0.05	6.66	0.12	16.0	0.19	25.33	0.29	38.66	0.36	48.0	0.53	70.66	
IVf	0	0	0.07	9.33	0.13	17.33	0.19	25.33	0.3	40.0	0.37	49.33	0.59	78.66	
Control	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	
Methotrexate	0.07	10.6	0.13	19.69	0.23	34.84	0.35	53.03	0.42	63.63	0.56	84.84	0.66	100	
DMSO	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	0.66	100	B.Subtilis
IVe	0.13	19.69	0.22	33.33	0.33	50.0	0.49	74.24	0.59	89.39	0.63	95.45	0.66	100	
IVf	0	0	0.08	12.12	0.16	24.24	0.29	43.93	0.38	57.57	0.51	77.27	0.62	93.93	

OD: Outer diameter, DMSO: Dimethyl sulfoxide

MATERIALS AND METHODS

Chemicals used in the synthesis of the compounds described were purchased from Sigma aldrich Ltd and s.d. fine Chem. Ltd. They were ethyl (ethoxymethylene) cyanoacetate **(**EMCE), guanidine carbonate, thiosemicarbazide. p-nitroacetophenone, p-methoxyacetophenone, p-chloroacetophenone, p-methyl acetophenone, p-bromoacetophenone.

Scheme:



a: sodium ethoxide; b: DMF, Ac_2O, 80ºC, 6hrs; c:10% alcoholic NaOH, 10min reflux;
d: thiosemicarbazide, $POCl_3$, 4hrs reflux; e: p-substituted phenacylbromide, DMF,80ºC 8hrs.

Melting points of synthesized compounds were determined on thermonik melting point apparatus and are uncorrected. IR spectra were recorded on a ThermoNicolet FT/IR Spectrometer using KBr pellets. The ^{1}H NMR spectra were recorded on bruker avance II NMR 400 MHz instruments using DMSO as solvent and TMS as internal standard, chemical shifts are expressed as δ values (ppm) downfield from tetramethylsilane. Reagents and solvents were used as obtained from the suppliers without further purification. Yields have not been optimized.

Synthesis:

The title compounds were synthesized as per scheme. Ethyl 2, 4-Diaminopyrimidine-5-carboxylate (**I**) was prepared by reaction with guanidine carbonate & ethyl (ethoxymethylene) cyanoacetate. Which on acetylation in presence of DMF & acetic anhydride, ethyl 2, 4-diacetamidopyrimidine -5-carboxylate (**Ia**) was obtained. Upon hydrolysis with alcoholic NaOH, **(Ia)** gave 2, 4-diacetamidopyrimidine-5-carboxylic acid (**IIa**). The formed acid was condensed with thiosemicarbazide in presence of phosphoryloxychloride to get N, N'-(5-(5-amino-1, 3, 4-thiadiazol-2-yl) pyrimidine-2, 4-diyl) diacetamide (**IIIa**). Further which on condensation with different substituted phenacyl bromides gave N,N'-(5-(6-(4-substitutedphenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4 diyl)diacetamide (**IVa-f**).

1. Synthesis of ethyl 2, 4-diaminopyrimidine-5-carboxylate (I) [3]:

Sodium ethoxide was prepared by adding 4.1gm (0.176 mol) of sodium metal into the 120ml absolute alcohol. To the sodium ethoxide solution 15.8gm (0.088mol) guanidine carbonate was added and refluxed for about 1 hour. After cooling, the precipitated sod. carbonate was filtered off and to the filtrate 7.5g (0.044 mol) of EMCE was added, shaken well and allowed to stand overnight. The crystals were collected by filtration and purified by recrystallization from ethanol to yield ethyl 2, 4-Diaminopyrimidine-5-carboxylate.

Yield 95%, mp. 213-214^{o}C, mol. formula: $C_7H_{10}N_4O$, IR (KBr, cm^{-1}): 3362.16 (NH str), 3153.21(CH str), 1672.42(C=O str), 1263.46(C-O str). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 7.85 (s, ArH, 1H), 6.80, 6.08 (s, $ArNH_2$, 2H), 4.15 (q, CH_2, 2H), 1.23 (t, CH_3, 3H).

2. Synthesis of ethyl 2, 4-diacetamidopyrimidine-5-carboxylate (Ia) [4]:

A mixture of 6.5g (0.036 mol) **I,** 40ml of acetic anhydride and 40ml of DMF was heated on a steam bath for 6hr with occasional stirring. The solution was kept at –5^{0}C overnight. The separated product was collected and washed with water. It was purified by recrystallization from chloroform to obtain colorless needles of ethyl 2, 4-diacetamidopyrimidine-5-carboxylate.

Yield 70%, mp. 195^{o}C, mol. formula: $C_{11}H_{14}N_4O_3$, IR (KBr, cm^{-1}): 3180.12 (N-H str), 1684.54(C=O str), ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 10.85, 10.62 (s, $NHCOCH_3$, 1H), 8.86(s, ArH, 1H), 4.24(q, CH_2, 2H), 1.27(t, CH_3, 3H).

3. Synthesis of 2, 4-diacetamidopyrimidine-5-carboxylic acid (IIa) [5]:

A mixture of 7.1 g (0.027 mol) of **Ia** in 50 ml 10% alcoholic NaOH was refluxed for 10 min to precipitate the sodium salt of 2,4-diacetamidopyrimidine-5-carboxylic acid, which was collected by filtration. Further the residue was dissolved in water and acidified with AcOH to get colorless crystals of 2, 4-diacetamidopyrimidine-5-carboxylic acid (**IIa**). It was purified by recrystallization from ethanol to get colorless needles.

Yield: 65%, mp. 195^{o}C, mol. formula: $C_{11}H_{14}N_4O_3$, IR (KBr, cm^{-1}): 3340.27(N-H str), 2557.65 (O-H str), 1881.96(C=C str), 1705.75(C=O str). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 12.05 (s, OH, 1H), 10.59, 10.36(s, $NHCOCH_3$, 1H), 8.26(s, ArH, 1H), 1.94, 1.87(s, CH_3, 3H).

4. Synthesis of N, N'-(5-(5-amino-1, 3, 4-thiadiazol-2-yl) pyrimidine-2, 4-diyl) diacetamide (IIIa) [6]:

A mixture of 2.38g (0.01 mol) of **IIa,** 0.91g (0.01 mol) of thiosemicarbazide and 3.5ml phosphorus oxychloride was refluxed for 4 hours the reaction mixture was filtrated if not clear solution. The filtered solution was neutralized with aqueous solution of potassium hydroxide to precipitate N, N'-(5-(5-amino-1, 3, 4-thiadiazol-2-yl) pyrimidine-2, 4-diyl) diacetamide (**IIIa**), which was purified by recrystallization from ethanol to give needles of **IIIa**.

Yield: 50%, mp. 245 ^{o}C, mol. formula: $C_{10}H_{11}N_7O_2S$. IR (KBr, cm^{-1}): 3329.21, 3172.75 (N-H str), 2853.39(C-H str), 1623.65(C=O str). ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 11.40, 11.05(s, $NHCOCH_3$, 1H), 7.48 (s, ArH, 1H), 6.82(s, $ArNH_2$, 2H), 2.49, 2.18(s, CH_3, 3H).

5.General Procedure for the Synthesis of N,N'-(5-(6-(4-substitutedphenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4 diyl)diacetamide: (IVa-f) [7]:

A mixture of equimolar quantities of (0.030mol) N, N'-(5-(5-amino-1, 3, 4-thiadiazol-2-yl) pyrimidine-2, 4-diyl) diacetamide **(IIIa)** and various substituted (0.030 mol) α-bromoketones in DMF solvent was heated at 80^{0}C for about 8hours. The reaction mixture was then poured into water, hydrobromide salts separates out. The formed hydrobromide salts were neutralized with cold aqueous solution of sodium carbonate to yield the corresponding free bases. Further the compound (IVa-f) was purified by recrystallization from ethanol.

N,N'-(5-(6-phenyl imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4 diyl)diacetamide **IVa**: Yield 40%, mp. 248^{0}C, mol. formula: $C_{18}H_{17}N_7O_2S$. IR (KBr, cm^{-1}): 3413.18(NH str), 2922.50, 2855.37(C-H str), 1656.37(C=O str); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 10.85, 10.62(s, $NHCOCH_3$, 1H), 8.86(s, ArH, 1H), 8.41(s, ArH, 1H), 7.19(m, ArH, 5H), 2.20, 2.07(s, CH_3, 3H).

N,N'-(5-(6-(4-nitro-phenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4-iyl)diacetamide **IVb**: Yield 55%, mp.234^{0}C, mol. formula: $C_{18}H_{14}N_8O_4S$. IR (KBr, cm^{-1}): 3419.60(N-H str), 2922.77, 2855.28(C-H str), 1646.00(C=O str);

N,N'-(5-(6-(4-methylphenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4-diyl)diacet-amide **IVc**: Yield: 65%, mp. 248^{0}C, mol. formula: $C_{19}H_{19}N_7O_2S$. IR (KBr, cm^{-1}): 3413.94(N-H str), 2925.67, 2857.91(C-H str), 1601.77(C=O str).

N,N'-(5-(6-(4-coumarinophenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4-diyl)diacet-amide **IVd**: Yield: 63%, m.p.230^{0}C, mol. formula: $C_{27}H_{19}N_7O_4S$. IR (KBr, cm^{-1}): 3418.91(N-H str), 2923.40, 2369.18(C-H str), 1663.19(C=O str).

N,N'-(5-(6-(4-bromophenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4-diyl)diacet-amide **IVe**: Yield: 55%, m.p.284^{0}C, mol. formula: $C_{19}H_{16}BrN_7O_2S$. IR (KBr, cm^{-1}): 3348.70(N-H str), 3060.58(Ar-CH str), 2922.76(C-H str), 1711.49(C=O str).

N,N'-(5-(6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]-thiadiazol-2-yl)pyrimidine-2,4-diyl)diaceta-mide **IVf**: Yield: 60%, m.p. 224^{0}C, mol. formula: $C_{19}H_{19}N_7O_3S$. IR (KBr, cm^{-1}): 3304.9(N-H str), 3048.7(Ar-C-H str), 2981.6(C-H str), 1692.4(C=O str).

Antibacterial activity:

Media used: Peptone 10 g, NaCl 10g, Yeast extract 5g and Agar 20g in 1000ml of distilled water.

Initially the stock cultures of E-coli (MTCC 1554), Staphylococcus aureus (MTCC 737), Bacillus Subtillis (MTCC 1133) and Pseudomonas Aeruginosa (MTCC 1036) were reviewed by inoculating in broth media and grown at 37^0 C for 18hrs[8].

The agar plates of above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hr old cultures (200 µl) and spread evenly on the plate. After 20 mins, the wells were filled with 100µg of each compound (10mg/ml stock in DMSO). The control plates with antibiotic (10mg/ml stock) and DMSO were also prepared. All the plates were incubated at 37^{0}C for 24hrs and the diameter of inhibition zone were noted .The compounds with significant activity were selected for the determination of MIC [9].

The tubes containing the above media (5ml, without agar) were autoclaved at 121^0 C, 15 lbs. Each tube was added with required volume of the compound enough for concentrations varying from 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml and inoculated with 18hr old cultures (50 µl) and mixed gently. The control tubes were added with antibiotic and DMSO were also prepared. All the tubes were incubated at 37^0 C for 24 hrs and the absorbance of the bio mass was read at 660 nm, against autoclaved media as blank along with compound without inoculum [10].

CONCLUSION

The brief structural activity relationship studies of synthesized compounds revealed that the presence of OCH_3, and halo group on p-position of aryl group of the target nucleus has contributed for greater antibacterial activity.

REFERENCES

[1] S.D Sharma., S Bandari. *Ind.J.Hetero Chem*. **2002**: 11; 221-24.
[2] R.K Kisliuk. "Folates Antagonists as Therapeutic Agents" Academic Press.OrlandoFL.984; 1-68.
[3] N.K Sadao, K Senga, T.T Aida, Y Fumio. *Chem. Pharm. Bull*.**1970**: 5; 1003-1007.
[4] I. Caleta et.al., *J.Org.Chem*.**1960**, 12; 224-227.
[5] J.R. Charles, et.al., *J.Org.Chem*.**1960**, 12; 224-227.
[6] A Andreani, A Leoni, A Locatelli, et,al. *Arzneim Forwsch/Drug Res* **2000**;50(1):550.
[7] P.M.Hawkey, D.A.Lewis, Medical microbiology-a practical approach (Oxford University press, United Kingdom, **1994**) 181-194.
[8] S.H. Gillespie, Medical microbiology-Illustrated, (Butterworth Heimann Ltd: United Kingdom, **1994**) 234-237.
[9] P. Panneerselvam, R.S. Reddy, *Der Pharma Chemica* **2010**, 2 (1): 28-37.