Haya: The Saudi Journal of Life Sciences (SJLS)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2415-623X (Print)
ISSN 2415-6221 (Online)

MORE Generated Isatinyl Thiazole Derivatives as Anti-*Mycobacterium Tuberculosis* Agents and *d*TDP-Rhamnose Inhibitors

Rajendra D. Dighe[1*], Sheetal R. Dighe[2], Prashant D. Dighe[3], Rahul Y. Pagar[1], Rupesh K. Deore[1], Rakesh M. Bachhav[1], Pratik D. Patil[1], Yogesh T. Sonawane[1], Vinod A. Bairagi[1]
[1]K.B.H.S.S Trust's Institute of Pharmacy, Malegaon-Camp, Malegaon, Dist- Nashik, Maharashtra, India
[2]Mylan Labs Ltd, Sinner, Dist-Nashik, M.S.-422103, India
[3]Sun Pharma Industries Ltd, Tandalja, Dist-Vadodara, G.S.-390020, India

Original Research Article

***Corresponding author**
Rajendra D. Dighe

Article History
Received: 13.10.2018
Accepted: 27.10.2018
Published: 30.10.2018

DOI:
10.21276/haya.2018.3.10.7

Abstract: In last few decades, though significant progress has been made in the treatment and control strategies of tubercular infections by introducing new diagnostic and monitoring tools and combination therapy, it still continues to be severe problem. Thus with the aim of developing novel molecule with improved potency for treating *Mycobacterium tuberculosis* H37Rv strain infections and with decreased probability of developing drug resistance, herein we report the synthesis of isatinyl thiazole derivatives, starting from ethyl acetoacetate , by microwave organic reaction enhancement method (MORE) and results of investigations of their antimycobacterial and antimicrobial activities. Many compounds have shown promising activity while others were inactive.

Keywords: Isatin, thiazole, thiazolidinone derivatives, azetidinone derivative, well diffusion method, broth microdilution assay.

INTRODUCTION

Infections especially microbial remain the major cause of death over the world. Emergence of multi-drug resistant to different infectious organisms like *M. tuberculosis* made the condition most alarming [1, 2]. Therefore, there is an urgent demand for a new class of antimicrobial agent with a different mode of action and it led medicinal chemists to explore a wide variety of chemical structures. A *novel* structural design has demonstrated that the thiazole derivatives especially with carbonyl group scaffold inhibit an enzyme *RmlC* which is essential component for the biosynthesis of *d*TDP-rhamnose. *d*TDP-rhamnose is found in a variety of different glycol-conjugates in the cell walls of pathogenic bacteria [3].

While reports [5, 6] are available stating emergence of thiazole as potent antibacterial agent. β-Lactams are the most successful antimicrobials [7-10] till recent days, unless microorganisms producing β-lactamase. Isatin derivatives are reported to show biological activity like antibacterial activity [11]. After extensive literature search, it was observed that, till date enough efforts have not been made to combine the moieties as a single molecular scaffold and to study its biological activity [12, 13]. This initiated us constructing compounds containing both the isatinylthiazole, azetidinone and thiazolidinone ring systems in the same matrix to serve as a new scaffold.

Microwave assisted reactions [14] using dry media [15] have attracted much interest because of the simplicity in operation, greater selectivity and rapid synthesis of variety of heterocyclic compounds [16]. Thus it was thought worthwhile to synthesize titled compounds using Green Route that is MORE method.

MATERIALS AND METHODS

Experimental

Scheme (Figure-1) for synthesis of 2-amino-4-methylthiazole-5-carboxylic acid ethyl ester (**1**) [4] and its derivatives (**2-6**) [4, 16]. The nucleus and its derivatives were analyzed by different ways. The melting points were recorded on electrothermal apparatus and are uncorrected. (IR) spectra were determined on Bruker IFS-66 FTIR (Bruker Bioscience, USA) using KBr pallets and wave number (í) was reported in cm^{-1}. ^1H NMR spectra on a Bruker Avance 300 MHz instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts (δ) were reported in ppm with coupling constants (*J*) are given in Hz. Mass spectra were recorded on a Finning LCQ mass spectrometer. Microwave irradiation was carried out in Raga Scientific Microwave Systems, Model RG31L at 2450 MHz. Elemental analysis was performed on a Hera- cus CHN-Rapid Analyser. Analysis indicated by the symbols of the elements of functions was within ±0.4% of the theoretical values.

The purity of the compounds was checked on silica gel coated Al plates (Merck).

Synthesis of 4 - Methyl – 2 - (2-oxo-1, 2-dihydro-indol-3-ylideneamino)-thiazole -5 -carboxylic acid ethyl ester (2):

A solution of Ethyl-2-amino–4-methylthiazol–5-carboxylate **(1)** (0.02 mol) with isatin (0.02 mol) was prepared in 10 ml ethanol containing 1 ml glacial acetic acid. To this acidic alumina (10 g) was added. Ethanol then was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 15 min at the power level of 300 W. The mixture was cooled and poured on ice. The solid thus separated was filtered and extracted with ether. Ether was distilled off and product obtained was crystallized from hot ethanol-chloroform mixture to furnish the product (2).

Synthesis of 4 – Methyl - 2-(2-oxo-1, 2-dihydro-indol-3-ylideneamino)-thiazole - 5 -carboxylic acid hydrazide (3):

A solution of 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid ethyl ester (2) (0.01 mol) with 5 ml (0.01 mol) hydrazine hydrate (98%) was prepared in 10 ml ethanol. To this acidic alumina (10 g) was added. Ethanol then was evaporated invacuo, and mixture was kept inside the alumina bath and irradiated for 5-6 min at the power level of 300 W. The mixture was cooled and the product was extracted with ether. Ether was distilled off and product 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid hydrazide (3) was obtained. The product was recrystallized from n-hexane–carbon tetrachloride mixture.

General procedure for the synthesis of 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid benzylidene substituted-hydrazide (4a-4j):

A solution of 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid hydrazide (3) (0.01 mol) with aryl aldehyde (0.01 mol) was prepared in 10 ml ethanol. To this acidic alumina (10 g) was added. Ethanol then was evaporated in vacuo, and mixture was kept inside the alumina bath and irradiated for 1 min at the power level of 300 W. The mixture was cooled and poured on ice. The solid thus separated was filtered and extracted with ether. Ether was distilled off and product obtained was crystallized from hot ethanol to furnish the product (4a-4j).

General procedure for the synthesis of 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid (3-chloro-4-oxo-2-substitutedphenyl-azetidin-1-yl)-amide (5a-5j):

4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylicacid benzylidene substituted-hydrazide Schiff base (4) (0.01 mol) was mixed with triethylamine (0.02 mol) and chloroacetyl chloride (0.02 mol) was added drop wise over a period of 30 min. Acidic alumina was added to the above solution at room temperature. The reaction mixture was absorbed, dried and kept inside the alumina bath and irradiated for 40-80 seconds. The mixture was cooled and then product was extracted with absolute ethanol and poured onto crushed ice. The solid thus separated was filtered, washed thoroughly with water and recrystallised from aqueous ethanol. The product obtained was filtered washed with water and recrystallized. Other azetidine-2-one were obtained in similar manner and number as (5a-5j).

General procedure for the synthesis of 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid (4-oxo-2-substitutedphenyl-thiazolidin-3-yl)-amide (6a-6j):

4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylicacid benzylidene substituted-hydrazide (4) Schiff base (0.01 mol) was added to mercaptoacetic acid (0.02 mole) anhydrous alumina chloride (0.05 g) acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed adsorbed, dried and kept inside the alumina bath and irradiated for 40-80 sec. The reaction mixture was then cooled and triturated with an excess of 10% sodium bicarbonate solution. The product obtained was filtered, wash several times with water and crystallized with isopropanol. Other thiazolidin-4-one were obtained in similar manner and number as (6a-6j).

Antitubercular Activity

The compounds 5a-5j and 6a-6j were screened for antitubercular and antimicrobial activities against different strains. Primary screening was conducted at 6.25 µg mL^{-1} against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [17]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [18]. Compounds showing more than/95% inhibition in the primary screening were considered active and then re-tested at a lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual Minimum Inhibitory Concentration (MIC), using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 95% with respect to the controls. Rifampin (RMP) was used as the reference compound (RMP MIC =/0.015-0.125 mg mL^{-1}). We also have done cytotoxicity analysis of the above-synthesized compounds, using neutral red uptake by using Vero-C-1008 cell line at various concentrations (6.25 µg/mL to 50 µg/mL), none of them were found toxic. Hence the activities of the above-synthesized compounds were not due to cytotoxicity.

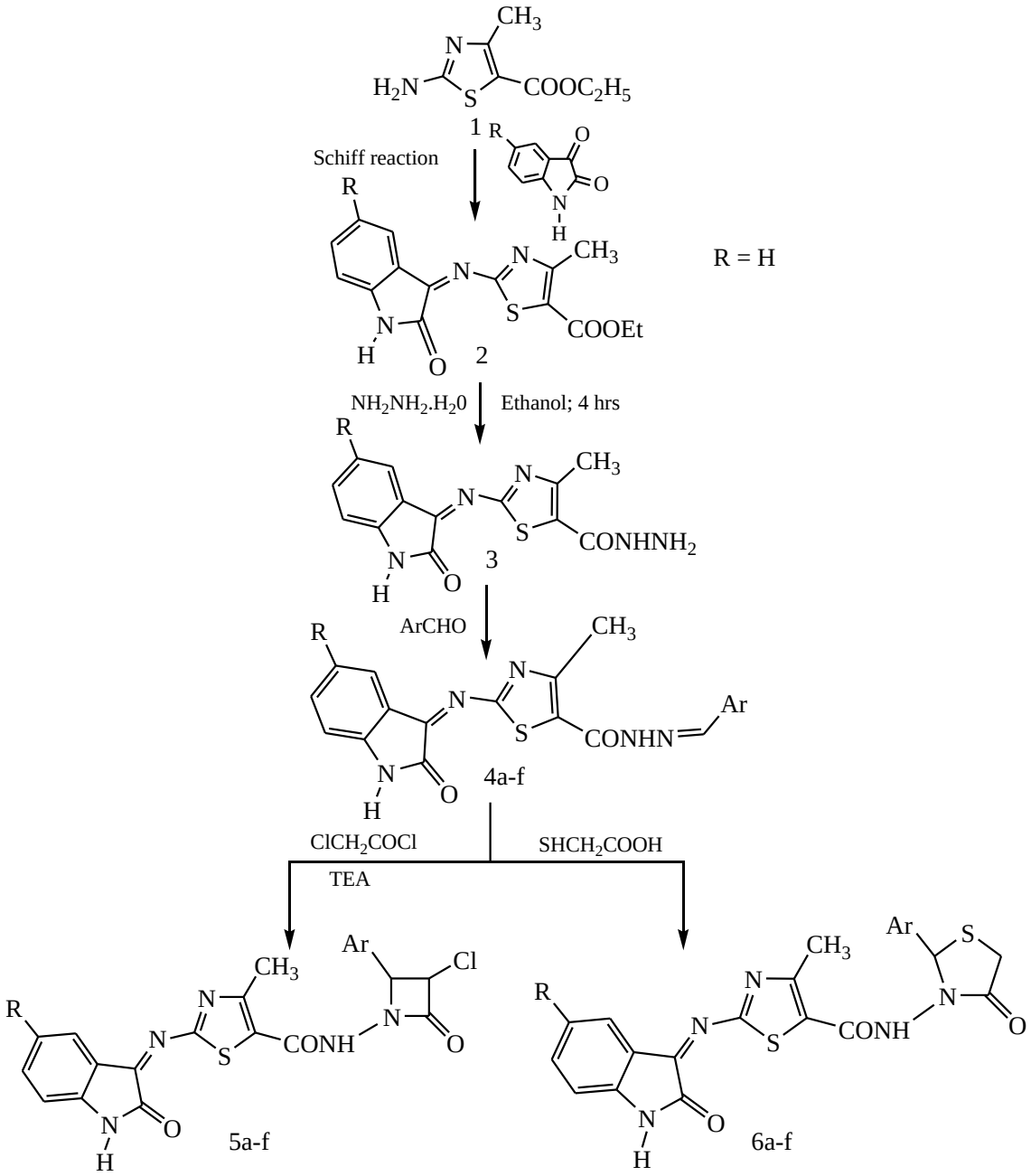

Fig-1: Scheme for synthesis of isatinyl thiazole derivatives

Antimicrobial Activity

Microbial strains- *Staphylococcus aureus* ATCC 23564, *Escherichia coli* ATCC35218, *Pseudomonas aeruginosa* ATCC 25619, *Salmonella typhi* ATCC 10749

The compounds listed in the Table-3 were screened for the antimicrobial activity against different microorganisms using well diffusion method [19, 20], where 50µM and 100 µM concentrations were taken for activity in nutrient agar medium. Chloroform was used as solvents and antibiotic Gentamycin was used as standard. The culture was kept for 24 h. The nutrient agar medium, 20 mL was poured into the sterile petri dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24 h (at 24-28 °C) subcultured bacteria was inoculated in the petri-plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured into the wells with varying concentrations ranging from 50 & 100 µM using a micropipette. The plates were left over for 24 h at 24-28 °C. The antibiotic Gentamycin was used as a standard for comparative study. The percentage of inhibition was calculated by the formula; percent Inhibition = Diameter of the inhibition zone x 100.

RESULTS

Antitubercular Activity

Table-1: First antituberculosis screening: MIC and % Growth Inhibition of synthesized compounds tested against *M. tuberculosis* H37Rv strain

Comp	Ar	MIC (µg/mL^{-1})[a]	GI (%)[b]
5a	-C_6H_5	<6.25	----
5b	-4-F -C_6H_4	<6.25	100
5c	-3,4,5-CH_3O -C_6H_2	<6.25	----
5d	-4-$(CH_3)_2N$-C_6H_4	<6.25	----
5e	-2-F -C_6H_4	<6.25	100
5f	-4-Cl -C_6H_4	<6.25	96
5g	-3-NO_2 -C_6H_4	<6.25	----
5h	-4-OH -C_6H_4	<6.25	----
5i	-2-Cl -C_6H_4	<6.25	98
5j	-2-NO_2 -C_6H_4	<6.25	----
6a	-C_6H_5	<6.25	----
6b	-4-F -C_6H_4	<6.25	98
6c	-3,4,5-CH_3O -C_6H_2	<6.25	----
6d	-4-$(CH_3)_2N$-C_6H_4	<6.25	----
6e	-2-F -C_6H_4	<6.25	100
6f	-4-Cl -C_6H_4	<6.25	97
6g	-3-NO_2 -C_6H_4	<6.25	----
6h	-4-OH -C_6H_4	<6.25	----
6i	-2-Cl -C_6H_4	<6.25	97
6j	-2-NO_2 -C_6H_4	<6.25	----

[a] MIC of rifampin: 0.015-0.125 mg mL^{-1} versus *M. tuberculosis* H37Rv (97% inhibition).
[b] Growth inhibition of virulent H37Rv strain of *M. tuberculosis.*

Table-2: Second level antituberculosis assays: MIC of synthesized compound which showed higher % growth inhibition in primary screening

Sr. No.	MIC (µM)[a]
5b	6.25
5e	1.56
5f	3.13
5i	0.39
6b	3.13
6e	6.25
6f	6.25
6i	0.78

[a] Actual minimum inhibitory concentration (MABA assay).

During the preliminary screening 20 compounds 5a-5j and 6a-6j were tested (Table 1) at 6.25 µg/mL concentration for their antimycobacterial activity, eight compounds 5b, 5e, 5i, 6b, 6e, 6f and 6i have exhibited more than 96% inhibition at this concentration while other compounds exhibited less than 90% inhibition at the same concentration. SAR of the synthesized compounds suggests that most of these compounds are very much similar to each other, differing in the substitutions on the aryl ring. And it can be seen that compounds having halogen are more potent than other. On the other hand, in secondary screening (Table-2), only 5e, 5i and 6i were found to have promising antimycobacterial activity. Other compounds are not as active as the earlier ones. Although we have not been able to substantially enhance the activity of these compounds in the present study, the data presented here are encouraging and deserve further investigation.

Antimicrobial Activity

Table-3: Inhibitory Zone Diameter (mm) of synthesized compounds against tested bacterial strains

Comp.	Organisms					Comp.	Organisms			
	Sa	Pa	Ec	St			Sa	Pa	Ec	St
5a	18	17	14	12		**6a**	18	16	10	12
5b	18	16	15	14		**6b**	20	16	10	10
5c	22	20	18	14		**6c**	22	22	20	16
5d	25	22	20	16		**6d**	26	24	22	18
5e	20	20	18	14		**6e**	36	34	30	30
5f	16	16	20	16		**6f**	24	22	20	18
5g	16	10	10	18		**6g**	22	11	24	24
5h	11	17	15	22		**6h**	22	22	20	20
5i	32	32	30	36		**6i**	36	38	32	32
5j	16	16	12	12		**6j**	16	16	12	14
Gent	34	35	31	30		Gent	34	35	31	30

Sa: *Staphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, St: *Salmonella typhosa*, Gent: *Gentamycin*

It has been found that all the compounds tested showed broad spectrum of inhibitory properties. From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed. Looking at the structure activity relationship, marked inhibition in bacteria was observed in the compounds 5i, 6i and 6e whereas 5c, 5d, 6d, 6f, 6g and 6h have shown moderate activity and others showed least activity (Table-3).

DISCUSSION

2-Amino-4-methyl-thiazole-5-carboxylic acid ethyl ester (1) was synthesized by cyclization of 2-bromo-ethylacetoacetate with thiourea. The internal bromination of ethylacetoacetate was achieved by treatment with N-bromosuccinamide. The reactive amino group was then protected, by Schiff reaction process to yield 4-Methyl-2-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-thiazole-5-carboxylic acid ethyl ester (2). This was confirmed on the basis of ^{1}H NMR wherein, the δ value for free amino group 2.03 was loss due to conversion into C=N group. Chemical transformation of compound (2) to hydrazide derivative (3) was achieved. The free amino group of hydrazide was condensed to schiff's base (4a-4j), by reacting with arylaldehydes in ethanol, which on treatment with mercaptoacetic acid and chloroacetyl chloride gave thiazolidinone (6a-6j) and azetidinone (5a-5j) derivatives respectively.

Recent studies have pointed to the essential nature of rhamnose in some cell walls and capsules. L-Rhamnose is a 6-deoxyhexose that is found in a variety of different glycol-conjugates in the cell walls of pathogenic bacteria. The precursor of L-rhamnose is dTDP-L-rhamnose, which is synthesised from glucose-1-phosphate and deoxythymidine triphosphate (dTTP) via a pathway requiring enzymes. Significantly this pathway does not exist in humans so the enzyme there for represent potential therapeutic targets. The thiazole as potent antibacterial agent and its structure has been refined to a crystallographic R-factor of 20.4% and an R-free value of 24.9% with good stereochemistry. Production of β-lactamase in microorganism takes place. Appreciation of these finding towards the development of novel antimicrobial agents. A novel analogs form by clubbing together two or three nuclei having different sites or mechanism of action.

Hence the synthesized and identified compounds possess antimycobacterium potential as well as are *d*TDP-rhamnose inhibitors. Finally, systematic evaluation of metabolic pathways of host and pathogen can be extended to other pathogens of clinical interest.

CONCLUSION

Isatinthiazole derivatives particularly with carbonyl group scaffold inhibit an enzyme *RmlC*, which is an essential component for the biosynthesis of *d*TDP-rhamnose and produce good antimycobacterium and antimicrobial activity.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Shinde Sashikant, Assistant Vice President and Dr. Khire Jitendra, Hr Head Mylan Lab. LTD, Nashik for providing necessary facilities to carry out this research work. Authors are thankful to Dr. Das Ashitosh, CSIR, IIIM Jammu for help during biological activity of the compounds.

REFERENCES

1. Ania, A. E., Idokob, J., Dalyopc, Y. B., & Pitmangd, S. L. (2009). Drug resistance profile of *mycobacterium tuberculosis* isolates from pulmonary tuberculosis patients in Jos, Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 103, 67-71.
2. Said, H. A., Abdel, G. S., Morlock, G., & Cooksey, R. C. (2009). Molecular identification of mutations

associated with anti-tuberculosis drug resistance among strains of mycobacterium tuberculosis. *International Journal of Infectious Diseases*, 13(6), 673-678.

3. Babaoghe, K., Page, M. A., Johns, V. C., Naismith, J. H., & Lee, R. E. (2003). Novel inhibitors of an emerging target in mycobacterium tuberculosis; substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. *Bioorganic & Medicinal Chemistry Letters*, 13(19), 3227-3230.
4. Dighe, R. D., Shiradkar, M. R., Rohom, S. S., & Dighe, P. D. (2011). Microwave Assisted Synthesis and Evaluation of Thiazole Derivatives as M. tuberculosis and Antimicrobial Activity. *International Journal of Drug Design and Discovery*, 2(2), 464-463.
5. Lamotte, J., Dive, G., & Ghuysen, J. M. (1991). Conformational analysis of β and γ-lactam antibiotics. *European Journal of Medicinal Chemistry*, 26(1), 43-50.
6. Khalil, A. M., Berghot, M. A., & Gouda, M. A. (2009). Synthesis and antibacterial activity of some new thiazole and thiophene derivatives. *European Journal of Medicinal Chemistry*, 44(11), 4434-4440.
7. Jacqueline, M. B., Ruth, L., Fabien, D. M., & Jean, M. F. (1988). Synthesis and biological evaluation of new C (4) heterofunctionalized monocyclic β-lactams derived from penicillin G. *European Journal of Medicinal Chemistry*, 23(6), 149-154.
8. Arnoldi, A., Grasso, S., Meinardi, G., & Merlini, L. (1988). Synthesis and anti-fungal activity of simple β lactams. *European Journal of Medicinal Chemistry*, 23(2), 149-154.
9. Luis, J., Nunez, V., & Juan, S. (1988). Measurement of interaction parameters between glutathione and β-lactam antibiotics: a polarographic approach. *European Journal of Medicinal Chemistry*, 23(6), 573-575.
10. Wang, Y., Lambert, P., Zhao, L., & Wang, D. (2002). Synthesis & antibacterial activity of dual-action agents of a β-lactum antibiotic with cytotoxic agent mitozolomide or temozolomide. *European Journal of Medicinal Chemistry*, 37(4), 323-332.
11. Pandeya, S. N., Sriram, D., Nath, G., & DeClercq E. (1999). Synthesis, antibacterial, antifungal and anti HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4-chlorophenyl) thiazol-2-yl] thiosemicarbazide. *European Journal of Pharmaceutical Science*, 9(1) 25-31.
12. Shiradkar, M. R., & Shivaprasad, H. N. (2006). Microwave assisted synthesis of novel azetidin-2-one and thiazolidi-4-one derivatives of triazolyl thiophene. *Asian Journal of Chemistry*, 18(1), 331-338.
13. Shiradkar, M. R., Murahari, K. K., & Reddy, B. H. (2007). Synthesis of new S-derivatives of clubbed triazolyl thiazole as anti-mycobacterium tuberculosis agents, *Bioorganic & Medicinal Chemistry*, 15, 3997–4008.
14. Villemin, D., Martin, B., & Garrigues, B. (1993). Potassium Fluoride on Alumina: Dry Condensation of 3-Phenylisoxazol-5-one with Aldehydes Under Microwave Irradiation. *Synthetic communications*, *23*(16), 2251-2257.
15. Kidwai, M., Bhushan K. R., & Kumar P. (1999). Microwave Induced Synthesis of Ferrocenyl Substituted 1,2,4-s-Triazolo[3,4-b]-1,3,4-thiadiazoles. *Monatshefte für Chemie*, 130(4), 585-588.
16. Kidwai, M., & Negi, N. (1995). Novel synthetic approach to new thiadiazolo-aminotetrazoles. *Acta Pharma*, 45, 511-517.
17. Daisy, J. V., & Paramasivan, C. N. (2004). Evaluation of microplate alamar blue assay for drug susceptibility testing of *Mycobacterium avium* complex isolates. *Diagnostic Microbiology and Infectious Disease*, 49, 179–182.
18. Clark, B. I., & Lowell, S. Y. (1986). Radiometric in vitro susceptibility testing of *mycobacterium tuberculosis. The Antimicrobic Newsletter*, 3(8), 55-62.
19. Rautenbach, M., & du Toit, E. A. (2000). A sensitive standardised micro-gel well diffusion assay for the determination of antimicrobial activity. *Journal of Microbiological Methods*, 42, 159–165.
20. NCCLS. (2002). Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, second ed. NCCLS document M27-A2.