Original Article

Synthesis And Evaluation Of Antitubercular Activity Of Novel N-Cyclohexyl-5-Phenyl-1, 3, 4-Thiadiazol-2-Amine Derivatives

Sujit Kumar Mohanty[4], N.Yella Subbaiah[1], M. Venu Madhavi[1], Kiran Manda[4], Kotte Raju[2], E. Jajili[2], Duggirala Naga Lakshmi Divya[3], B. Naga Sudha[*1]

[1]Department of Pharmaceutical Chemistry, CES College of pharmacy, JNTU Ananthapuramu, Kurnool, Andhra Pradesh, India.
[2]Department of Pharmaceutical Chemistry, Sir C. R. Reddy College of Pharmaceutical Sciences, Santhi Nagar, Eluru, India
[3]Department of Pharmaceutical Analysis, Shri Vishnu College of Pharmacy, Bhimavaram, India.
[4]Department of Pharmaceutical Chemistry, Shri Vishnu College of Pharmacy, Bhimavaram, India.

Correspondence to Author:
Dr. B. Naga Sudha, Associate Professor, Department of Pharmaceutical Chemistry, Creative Educational Society's College of Pharmacy, NH-7, Kurnool-518218. Andhra Pradesh, India, E-mail: drbnsudha@gmail.com
DOI: 10.47750/pnr.2022.13.S09.582

Abstract

Background: In current era the wing of innovative therapeutic molecules i.e hybrid molecules synthesis, which comprises of linking two or more therapeutically verified molecules has gained wide appreciation. It was established that, when one heterocyclic system is attached to one more heterocyclic system, superior biological activity was produced. On basis of this important biological activity profile of several thiadiazoles, we intended to synthesize and evaluate the antimycobacterial activity of novel fused cyclohexylamine 1, 3, 4-thiadiazoles derivaties (C_{1-5}).

Materials and Methods: A series of compounds titled N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazole-2-amine derivatives (C_1-C_5) are synthesized using appropriate synthetic procedure and characterized by using IR.1NMR, Mass & Elemental analysis. All the thiadiazole amines (C_{1-5}) were tested for their antimycobacterial activity against M.tuberculosis H37 RV strain.

Results: The tested compounds showed significant antimycobacterial activity. At 1.6µg/ml, compound C_5 shoewd excellent activity against M.tuberculosis H37 RV strains and compound C_1 exhibited good antitubercular activity at 3.12µg/ml.

Conclusion: Compounds bearing the electron donating groups at 4^{th} position were found to possess excellent antitubercular activity, the substitution on phenyl ring was found to enhance the potentiality of the pharmacophoric moiety.

Keywords: Thiosemicarbazide, 1, 3, 4-thiadiazol, chlorocyclohexane, antimycobacterial activity.

INTRODUCTION:

Tuberculosis (TB) is the oldest documented infectious disease. It is a chronic necrotizing bacterial infection with a wide variety of manifestations caused by Mycobacterium tuberculosis, which has plagued humans throughout recorded and archeological history[1], as per the recent report it has been estimated that approximately one-third of the world's population is infected with this microorganism.

Despite the ready availability of effective treatments, tuberculosis remains a major public health threat worldwide. The emergence of drug-resistant strains of Mycobacterium tuberculosis, particularly multiple drug resistant strains [1-4], has complicated treatment protocols and raises the concern that tuberculosis may once again become an incurable disease. For this reason, it is critical to discover new drugs acting with a mechanism different from those presently in use.

The treatment of mycobacterial infections especially the tuberculosis, has become an important problem due to the emergence of monodrug and multidrug-resistant strains of M. tuberculosis[2]. Therefore, there is a need for new drugs of new structural classes and with a novel mechanism of action other than isoniazid (INH), rifampicin (RIF) and pyridazinamide (PZA). For this reson it is critical to discover new drugs acting with a mechanism different from those presently in use.

Heterocycles bearing nitrogen, sulfur and oxygen have been under investigation for a long time because of their broad spectrum of pharmacological properties. 1,3,4-Thiadiazole ring is an important scaffold known to be associated with several biological activities including antimicrobial[3-5], antituberculosis[6], antiviral[7],analgesic[8], antidepressant, and anxiolytic[9], anti hypertensive[10], anticonvulsant[11], anti-inflammatory[12], local anesthetic[13] and kinesin inhibitors[14].

On basis of this important biological activity profile of several thiadiazoles, we intended to synthesis, spectral studies and evaluate the antimycobacterial activity of a few fused cyclohexylamine 1, 3, 4-thiadiazoles.

MATERIALS AND METHODS:

Chemistry:

Melting points of the compounds are finished in open capillary tubes using Arson digital melting point apparatus. ^{1}H NMR spectra of resulted compounds were recorded on BRUKER-DR 400MHz in DMSO-d_6 using TMS as an internal standard at SRM University. IR spectra were recorded on BRUKER Alpha FT-IR spectrometer by using universal sampling model with KBr pellets in CESCOP, Kurnool. Purity of compounds was done by Thin Layer Chromatography (TLC) using silica gel precoated plates (GF_{254}) and spots were detected by UV chamber. All solvents and chemicals were used of LR grade and obtained from the SD fine chem. Limited.

General Procedure

Preparation of 2-benzylidenehydrazine-1-carbothioamide (A_{1-5})

Equal quantities (41 mmoles) of thiosemicarbazide and various substituted aromatic aldehydes were weighed and taken in to RBF, and to it added 35 ml of methanol. And then acidify the reaction mixture to PH 4-5 by the addition of glacial acetic acid and reflux the reaction mixture on water bath at a temperature 70-75˚C for 24 hrs.

Preparation of 5-phenyl-1, 3, 4-thiadiazol-2-amine (B_{1-5})

Methanolic solution of 2-benzylidenehydrazine-1-carbothioamide **1** (19.5 mmoles) and 59 mmoles of $FeCl_3$ was refluxed under stirring for an hour. Then added aqueous solution of 60 mmoles of citric acid, 30 mmoles of sodium citrate and again refluxed for 3 hours. The completion of reaction was judged by TLC. The solution was neutralized

to PH 4-5 with 10% aqueous solution of ammonia, then the reaction mixture was added in to crushed ice, filtered, washed with cold water. The solid thus separated was collected, dried and recrystallized from ethanol: methanol in 1: 1 ratio.

General procedure for the synthesis of N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazol-2-amine (C_{1-5})

Solution of 0.96 mmoles of 5-phenyl-1, 3, 4-thiadiazol-2-amine **2** in 1.5 ml of DMF, 3 ml of TEA and 2 ml of Cyclohexyl chloride was taken in to an RBF and refluxed under stirring at 120˚C for 2 hours and the completion of reaction was judged by TLC. The reaction mixture was concentrated and the residue was extracted with $CHCl_3$. The mixture was washed with 5% HCl and water then, the $CHCl_3$ layer was separated, concentrated and recrystallized from methanol to yield the title compounds.

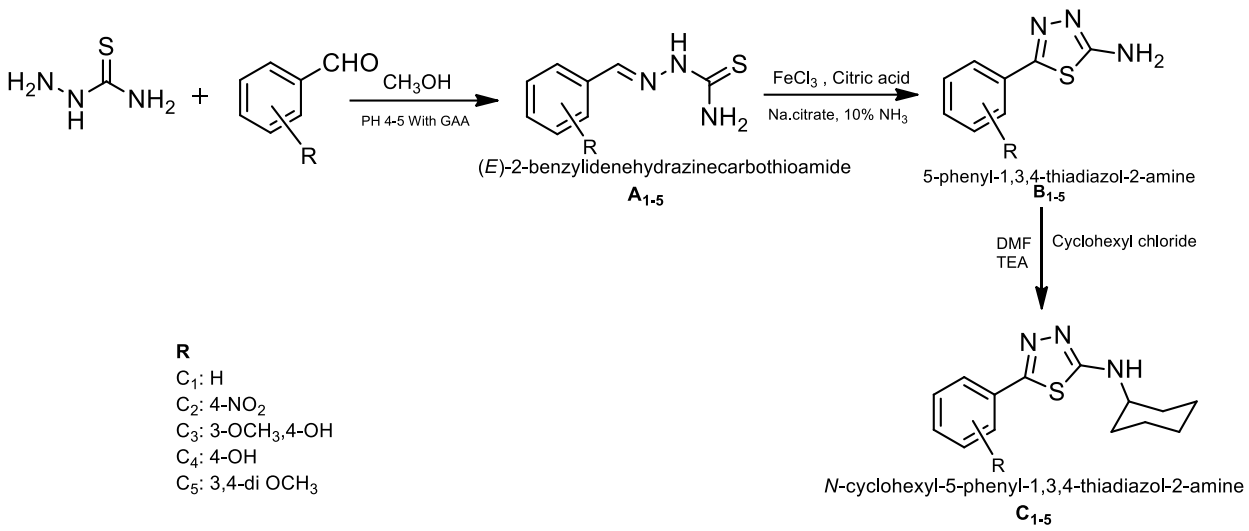
C_1. **N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazole-2-amine:** 30% yield; m.p. 120-124ºC; **IR (KBr, cm^{-1}):** 3414 (NH str), 2929 (C-H str), 1654 (C=C str), 1449 (C=N str), 669 (C-S-C str); **^{1}H NMR (400 MHz, DMSO,ppm) δ:** 7.505 (m, 2H, Ar-H); 7.915-7.954 (m, 3H, Ar-H); 3.588 (s, 1H, NH); 3.360 (m, 2H, Cyclohexane); 2.732-2.891 (m, 2H, Cyclohexane); 1.66-1.707 (m, 2H, Cyclohexane); 1.597 (m, 2H, Cyclohexane); 1.522-1.543 (m, 2H, Cyclohexane); 1.231-1.251 (m, 2H, Cyclohexane); 1.142-1.186 (m, 2H, Cyclohexane); MS [M + 1] Peak calculated for $C_{14}H_{17}N_3S$: m/z 259.37, found 260.42; Elemental analysis for $C_{14}H_{17}N_3S$: C, 64.83; H, 6.61; N, 16.20; S, 12.36; Found: C, 64.80; H, 6.59; N, 16.25; S, 12.40%.

C2. **N-Cyclohexyl-5-(4-nitrophenyl)-1, 3, 4-thiadiazole-2-amine:** 45% yield; m.p. 172-177ºC; **IR (KBr, cm^{-1}):** (N-H str) 3423.55 cm^{-1}; (C-H str) 2924.33 cm^{-1};(O-N=O str) 1636.26 cm^{-1}; (C=C str) 1457.04 cm^{-1}; (C-N str) 1018.34 cm^{-1}; (C-S-C str) 669.27 cm^{-1}; **^{1}H NMR (400 MHz, DMSO,ppm) δ:** 8.288 (m, 1H, Ar-H); 8.006 (m, 1H, Ar-H); 7.382-7.728 (m, 1H, Ar-H); 7.107 (m, 1H, Ar-H); 3.349 (s, 1H, NH); 1.692 (m, 5H, Cyclohexane); 1.216 (m, 5H, Cyclohexane); MS [M + 1] Peak calculated for $C_{14}H_{16}N_4O_2S$: m/z 304.37, found 305.57; Elemental analysis for C14H16N4O2S: C, 55.25; H, 5.30; N, 18.41; O, 10.51; S, 10.53; Found: C, 55.29; H, 5.28; N, 18.44; O, 10.54; S, 10.57%.

C3. **N-Cyclohexyl-5-(3-methoxy,4-hydroxy phenyl)-1, 3, 4-thiadiazole-2-amine:** m.p. 194-200ºC; **IR (KBr, cm^{-1}):** (N-H str) 3434.08 cm^{-1}; (C-H) 2924.57cm^{-1}; (C-O-CH_3 str) 2853.69 cm^{-1}; (C=C str) 1452.22 cm^{-1}; (C-OH str) 1254.88 cm^{-1};(C-N str) 1018.19 cm^{-1}; (C-S-C str) 669.08 cm^{-1}; **^{1}H NMR (400 MHz, DMSO,ppm) δ:** 7.919-7.944 (m, 1H, Ar-H); 7.460-7.495 (m, 1H, Ar-H);7.329- 7.411(m, 1H, Ar-H); 6.889 (s, 1H, Ar-OH); 3.882(s, 2H, NH); 3.853 (s, 3H, OCH_3); 1.705 (m, 2H,Cyclohexane); 1.231 (m, 4H, Cyclohexane); 1.181 (m, 4H, Cyclohexane); 1.156 (m, 2H, Cyclohexane); Elemental analysis for C15H19N3O2S: C, 58.99; H, 6.27; N, 13.76; O, 10.48; S, 10.50; Found: C, 58.94; H, 6.30; N, 13.77; O, 10.50; S, 10.56%.

C4. **N-Cyclohexyl-5-(4-hydroxy phenyl)-1, 3, 4thiadiazole-2-amine:** m.p. 115-119ºC; **IR (KBr, cm^{-1}):** (C-NH str) 3409.68 cm^{-1}; (OH str) 3346.71 cm^{-1}; (C-H str) 2927.64 cm^{-1}; (C=C str); 1456.91 cm^{-1}; (C=N str) 1506.93 cm^{-1}; (C-S-C str) 841.77 cm^{-1}; **^{1}H NMR (400 MHz, DMSO,ppm) δ:** 7.790 (m, 2H, Ar-H); 6.878 (m, 2H, Ar-H); 3.361 (s, 1H, NH); 1.727 (m, 6H, Cyclohexane); 1.237 (m, 6H, Cyclohexane); 10.057 (s, 1H, Ar-OH); Elemental analysis for C14H17N3OS: C, 61.06; H, 6.22; N, 15.26; O, 5.81; S, 11.64; Found: C, 61.08; H, 6.25; N, 15.27; O, 5.84; S, 11.69%.

C5. **N-Cyclohexyl-5-(3, 4-dimethoxy phenyl)-1, 3, 4-thiadiazole-2-amine:** m.p. 190-192ºC; **IR (KBr, cm^{-1}):** (C-NH str) 3345.34 cm^{-1}; (C-H str) 2927.94 cm-1; (- C=O str) 1654.81 cm^{-1}; (C=C str); 1541.07 cm^{-1}; (C=N str) 1449.52 cm^{-1}; (C-S-C str) 668.61 cm^{-1}. **^{1}H NMR (400 MHz, DMSO,ppm) δ:** 7.211-7.250 (m, 1H, Ar-H); 7.02 (m, 1H, Ar-H); 6.921 (m, 1H, Ar-H); 4.027 (s, 1H, NH); 1.603 (m, 5H, Cyclohexane); 1.127 (m, 5H, Cyclohexane); Elemental analysis for $C_{16}H_{21}N_3O_2S$: C, 60.16; H, 6.63; N, 13.16; O, 10.02; S, 10.04; Found: C, 60.18; H, 6.65; N, 13.10; O, 10.12; S, 10.07%.



Scheme 1: Protocol for synthesis of N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazol-2-amine derivatives **(C_{1-5})** .

Antitubercular activity:

The antimycobacterial activity of synthesized compounds were assessed against M. tuberculosis using Microplate Alamar Blue Assay (MABA).[15,16] This is a non-toxic method, uses a thermally stable reagent and it is suitable for random screening of the antimycobacterial activity. The titled compounds were tested by serial dilution method in the range of 100 to 0.2µg/ml by using the 100 µl of the middlebrook 7H9 broth. A blue colour in the well was interpreted as no bacterial growth, and pink colour as bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration required inhibiting 90% of bacterial growth. Pyrizinamide (3.125 µg/ml), streptomycin (6.25 µg/ml) and ciprofloxacin (3.125 µg/ml) was uesd as reference standards. The results of activity studies are depicted in Table 1.

Table 1: Antitubercular results of N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazol-2-amine (C_{1-5}).

S.No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	C1	S	S	S	S	S	S	**R**	**R**
2	C2	S	S	S	S	S	**R**	**R**	**R**
3	C3	S	S	S	S	S	**R**	**R**	**R**
4	C4	S	S	S	S	S	**R**	**R**	**R**
5	C5	S	S	S	S	S	S	S	**R**

R- Resistant, **S**- Sensitive

Strain used: M.tuberculosis (H37 RV Strain): ATCC No- 27294.

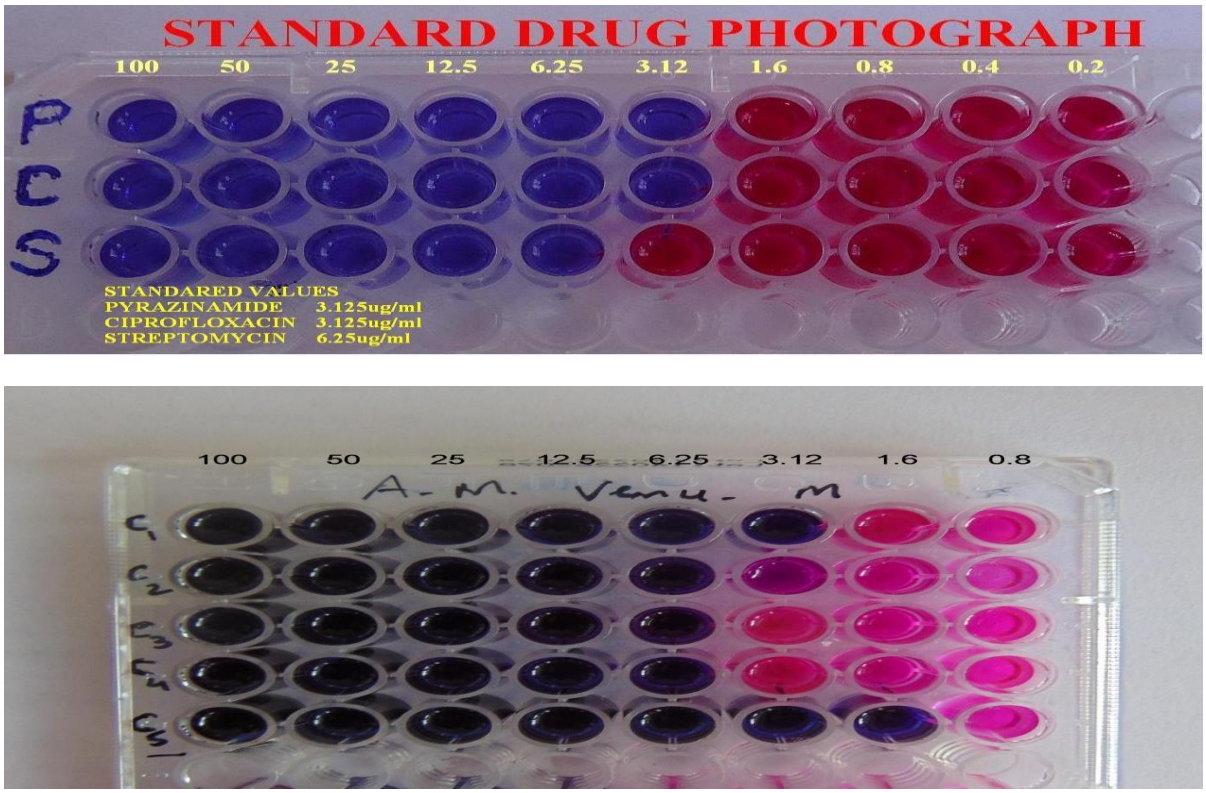


Fig 1: Images from Anti-tubercular activity of N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazol-2-amine (C_{1-5}).

RESULTS AND DISCUSSION

Chemistry

The N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazole-2-amine derivatives **(C_{1-5})** were synthesized according to the pathway in scheme 1. The initial material 2-benzylidenehydrazine-1-carbothioamide **A**, was prepared by equimolar quantites of thiosemicarbazide and various substituted aromatic aldehydes in the presence of methanol followed by heating under reflux with glacial acetic acid to afford 2-benzylidenehydrazine-1-carbothioamide **A_{1-5}**, 55% yield. The amide **A** was then treated with ferric chloride and citric acid in sodium citrate was refluxed for 3 hours, which was then basifying with 10% ammonia solution to give 5-phenyl-1, 3, 4-thiadiazol-2-amine **B_{1-5}** in 60% yield. Finally the 5-phenyl-1, 3, 4-thiadiazol-2-amine **B_{1-5}** was then refluxed under stirring with Cyclohexyl chloride, triethylamine in DMF for 2 hours at 120^{o}C to afford titled compounds i.e N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazol-2-amine **C_{1-5}** in moderate yields. Characterization of synthesized cyclohexyl amine thiadiazole derivatives was confirmed by using spectroscopic analysis. The spectral data confirms the synthesis of targeted compounds. The IR spectram of cyclohexyl amine thiadiazole derivatives (**C_{1-5}**) showed absorption bands around (N-H str) 3423.55 cm^{-1}, (C-H str) 2924.33 cm^{-1},(O-N=O str) 1636.26 cm^{-1}, (C=C str) 1457.04 cm^{-1}, (C-N str) 1018.34 cm^{-1}, (C-S-C str) 669.27 cm^{-1}. ^{1}H NMR study of the synthetic compounds gave confirmation about the presence of specific proton in certain environment. Splitting content and area under peak denotes the chemical environment and number of protons. Further confirmation was made on the basis of Mass and Elemental (CHN) analysis data.

In vitro anti-tubercular studies:

A series of novel cyclohexyl amine thiadiazole derivatives (**C_{1-5}**) were evaluated for their anti-TB activity against M.tuberculosis (H37 RV strains): ATCC No: 27294. All the synthesized derivatives C_{1-5} was found to be highly

efficient antitubercular agents in comparison to the standard drugs. Among all compounds, **C_2, C_3, C_4** showed equipotent activity at 6.25µg/ml. The compound **C_1** showed good antitubercular activity at 3.12µg/ml concentation, it showed equivalent activity with standard drugs i.e., Pyrazinamide and Ciprofloxacin (3.12µg/ml). The compound **C_5** had shown greater antitubercular activity at MIC of 1.6µg/ml than standard drugs. **C_5** derivative was found to be the most potent antitubercular agent.

CONCLUSION

A series of N-Cyclohexyl-5-phenyl-1, 3, 4-thiadiazole-2-amine derivatives **(C_{1-5})** were synthesized and characterized with the help of spectral analysis data. All the compounds were evaluated for their antitubercular activiy by standard procedure. All synthesized compounds exibited good antitubercular activity. Compound C5 and C1 containing 3,4-dimethoxy and unsubstituted phenyl ring elicited excellent activity against M.tuberculosis (H37 RV strains) than standard drugs. So, it can be considered as lead molecule for further biological investigation in development of new antitubercular drugs.

ACKNOWLEDGMENT

B.N.S and authors like to thank Creative Educational Society's College of Pharmacy, Kurnool, Andhra Pradesh, India, for providing laboratory facilities in time to carry out this research work successfully.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

REFERENCES

1. Dutt AK, Stead WW, David S. Tuberculosis and Nontuberculosis Mycobacterial Infections. 4th ed. W.B. Saunders; 1999.
2. Rattan A, Kalia A, Ahmad N. Multidrug-resistant Mycobacterium tuberculosis: molecular perspectives. Emerg Infect Dis. 1998; 4(2):195-209.
3. Amir M, Kumar A, Ali I, Khan SA. Synthesis of pharmaceutically important 1,3,4-thiadiazole and imidazolinone derivatives as antimicrobials. Indian J Chem. 2009; 48B:1288-93.
4. Desai K, Baxi AJ. Studies on 2-azetidinone: part VI synthesis and antimicrobial activity of 5- (2′,4′-dichlorophenoxymethyl)-2-(4″-aryl-3″-chloro-2″-azetidinone-1″-yl)-1,3,4 thiadiazole. Indian J Pharm Sci. 1992; 54:183-88.
5. Salimon J, Salih N, Hameed A, Ibraheem H, Yousif E. Synthesis and antibacterial activity of some new 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives. J Appl Sci Res. 2010; 6(7):866- 70.
6. Gadad AK, Malleshappa N, Noolvi RV, Karpoormath. Synthesis and anti-tubercular activity of a series of 2-sulfonamide/trifluoromethyl6-substituted imidazo-[2,1-b]-1,3,4-thiadiazole derivatives. Bioorg. Med. Chem. 2004; 12:5651-59.
7. Bonina L, Iannello D, Merendino R, Arena A, Mastroeni P. Tumor-dependent resistance of rat peritoneal macrophages to herpes simplex virus. Drugs Exp Clin Res. 1983; 9:577-83.
8. Schenone S, Brullo C, Bruno O. New 1, 3, 4-thiadiazole derivatives endowed with analgesic and anti-inflammatory activities. Bioorg Med Chem. 2006; 14(6):1698-705.
9. Clerici F, Pocar D, Guido M, Loche A, Perlini V, Brufani M. Synthesis of 2-amino-5-sulfanyl- 1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity. J Med Chem. 2001; 44(6):931-36.
10. Turner S, Myers M, Gadie B. Antihypertensive thiadiazoles: Vasodilator activity of some 2-aryl-5-guanidino-1,3,4-thiadiazoles. J Med Chem. 1988; 31(5):907-13.
11. Labanauskas L, Kalcas V, Udrenaite E, Gaidelis P, Brukstus A, Dauksas A. Synthesis of 3- (3,4-dimethoxyphenyl)-1H-1,2,4-triazole-5-thiol and 2-amino-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole derivatives exhibiting anti-inflammatory activity. Pharmazie. 2001; 56(8):617-19.
12. Pattan SR, Desai NS, Rabara PA, Bukitgar AA, Wakale VA. Synthesis and antimicrobial evaluation of some 1, 3, 4-thiadiazole derivatives. Indian J Pharm Ed Res. 2008; 42(4):314-18.
13. Mazzone G, Pignatello R, Mazzone S. Synthesis and local anesthetic activity of alkylaminoacyl derivatives of 2-amino-1,3,4-thiadiazole. Farmaco. 1993; 48(9):1207-224.
14. Siddiqui A, Dai C, Mansoor U, Yang L, Vitharana L, Angeles A. Spirocondensed 1,3,4- thiadiazole derivatives for inhibiting KSP kinesin activity. 2009/052288 WO.
15. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, et al.Rapid, Low-Technology MIC Determination with Clinical Mycobacterium tuberculosis isolates by Using the Microplate Alamar Blue Assay. J. Clin.Microbiol. 1998; 36: 362-66.

16. Carvalho SA, Edson FD, Marcus VN, Maria CS, Felipe RV. Synthesis and antimycobacterial evaluation of new trans-cinnamic acid hydrazide derivatives. Bioorg. Med.Chem. Lett. 2008; 18: 538-41.