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Synthesis and characterization of new Isoniazid Prodrug as anticancer agent

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Abstract: Isoniazid is considered to be first choice of drug against Tuberculosis disease caused by Mycobacterium Tuberculosis. But derivatives of isoniazid which were designed by modifications in parent molecule have shown increased anti-mycobacterium activity. Along with this activity, these derivatives were shown to have a range of pharmaceutical applications like having anticancer activity, antimicrobial activity etc. In the present work, one such isoniazid derivative has been synthesized called as (3-(dodec-1-enyl)pyrrolidin-1-yl) isonicotinamide. This synthesized compound was evaluated for potential anticancer activity, antibacterial activity and DNA cleavage ability. It was observed that as compared to the standards, this is an efficient anticancer agent and was active against gram negative strain. This compound also has potent DNA cleaving abiltity.
Keywords: Isoniazid derivatives, (3-(dodec-1-enyl)pyrrolidin-1-yl)isonicotinamide, Mycobacterium Tuberculosis, Anti bacterial activity, Anti Cancer activity, DNA Cleavage Studies.

Introduction

Isoniazid, known with the name of Isonicotinylhydrazide (INH), is used as an antibiotic considered to be the first to be used for prevention as well as treatment of tuberculosis, both in latent and active forms of the disease [1]. The available formulations for this drug are in the form of tablet, syrup and injections [1]. This drug mainly acts against mycobacteria, specifically Mycobacterium Tuberculosis (causal organism of tuberculosis disease) and also M. kansasii and M. xenopi [1].

This is basically a prodrug which need to be activated in the host mycobacterial organism. Many studies have proposed that this prodrug is activated which results in formation of a variety of numerous compounds which are highly reactive as they can cause acetylation or oxidation of groups present in proteins [2]. Although many studies have been performed, yet the active form of isoniazid is not known with certainty.

In 1950s this drug was first introduced into the medication and in a short duration clinical isolates were discovered which were found to be resistant to this drug. These resistant organisms were found with commonly found with activity of catalase and peroxidase enzymes missing [3]. In early 1990s, the relationship between these enzymes lost activity and activity of isoniazid was proven with aid of cloning and sequencing of catalase-peroxidase primary gene (katG) in mycobacterial species [4]. Many other related studies have shown that about 42-58% of isoniazid resistant organisms have mutations in the katG gene [5, 6]. Although studies have provided a various number of mutations related to this resistance but the the most common mutation is Ser315Thr which

accounts for almost 40% of isoniazid resistant clinical strains [5-7]. This Ser315Thr mutation in the gene causes production of an enzyme which do not have the ability to activate the isoniazid prodrug but maintains about 50% of its actual function [8]. As a result of such modified enzyme production the organism becomes resistant to isoniazid and also it retains sufficient level of enzyme activity to perform detoxification reactions against radicals produced which will protect the organism from oxidative damage. The resistant clinical isolates which have mutations which are less common are studied to have variable levels of resistance to isoniazid and enzyme actual activity [5-8]. Majority of studies suggest that isoniazid mainly stops the synthesis pathway of mycolic acids required in cell wall of the envelope of mycobacterium. Till now two intracellular targets have been identified and are being studied [9, 10]. They are: the fatty-acid enoyl-acyl carrier protein reductase (InhA), and a complex of an acyl carrier protein (AcpM) and a b-ketoacyl-ACP synthase (KasA). These two enzymes are a part of mycolic acid synthesis pathway and mutations are frequently seen in promoter region; organisms showing less isoniazid resistance were found to have mutations in genes which code for these enzymes namely, inhA, acpM and kasA [5]. Studies propose that resistant phenotype is shown by organism when these particular enzymes are expressed in excess. As the kasA mutations were found both in resistant and susceptible strains, its actual importance in resistance has not yet been confirmed; organisms having resistance phenotype were also found containing mutations in katG and inhA genes [11, 12]. In about 10% resistant organisms, mutations were seen in promoter of gene that codes for alkyl hydroperoxidasereductase (ahpC) in which mutations were also found in katG gene [5, 6, 13]. As a result of mutated gene, enzyme is expressed in excess and this enzyme's activity make up for the lost activity of catalase-peroxidase enzyme [14].

The frequent side effect seen with use of isoniazid is that the hepatic enzymes levels were increased in blood which are upto an extent do not show any toxic effect. Less frequent and comparatively more toxic effects of isoniazid usage are nerves inflammation, numbness in arms and legs and also liver inflammation.

Felipe A. R. Rodrigues et al., 2014 [15] synthesised thirty two derivatives of isoniazid which were evaluated for their potent activity as anticancer agents. The structure activity relationship studies have shown that the number of substituents linked, their attachment positions and their types are important for the compound's biological activity. Out of thirty two compounds, one compound showed potent anticancer activity when compared to standard drug doxorubicin. Similarly isoniazid derivatives were synthesised by Gilani et al., 2014 [16] to evaluate their antifungal activity. It was found that these compound have potential antifungal activity with decreased toxicity as compared to parent isoniazid molecule.

So, it is essential that isoniazid derivatives should be synthesised which have decreased toxic effects and have more potency.

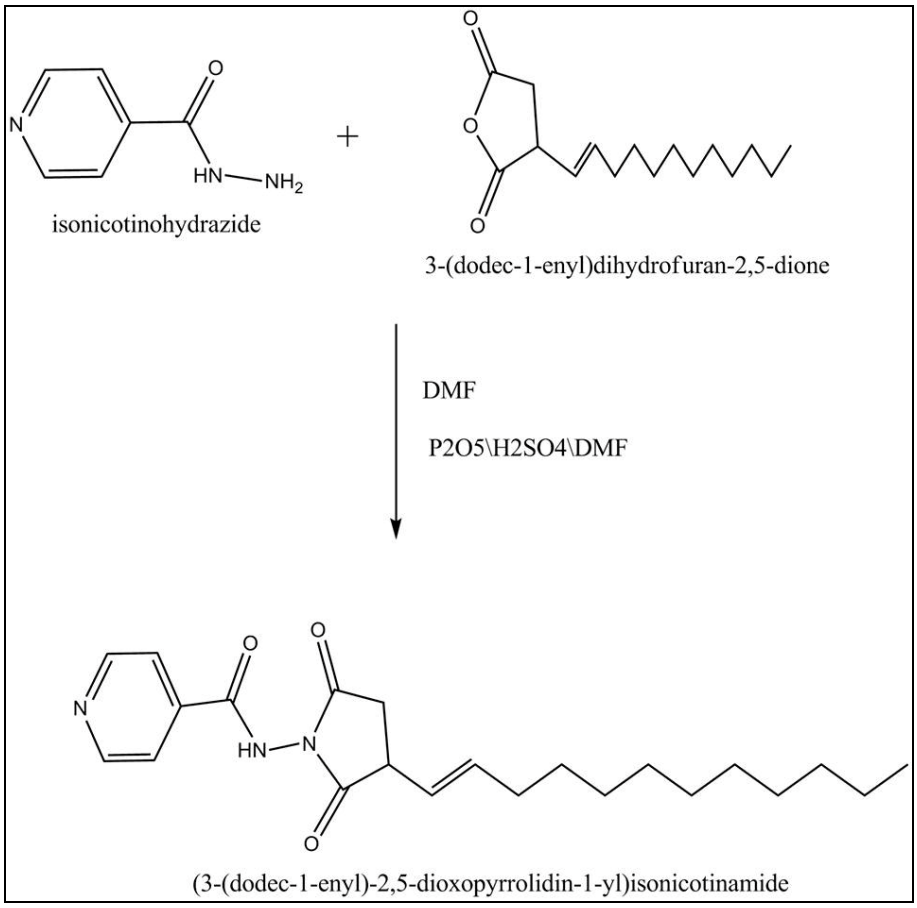
Methodology:

Synthesis of 3-(dodec-1-enyl)pyrrolidin-1-yl)isonicotinamide:

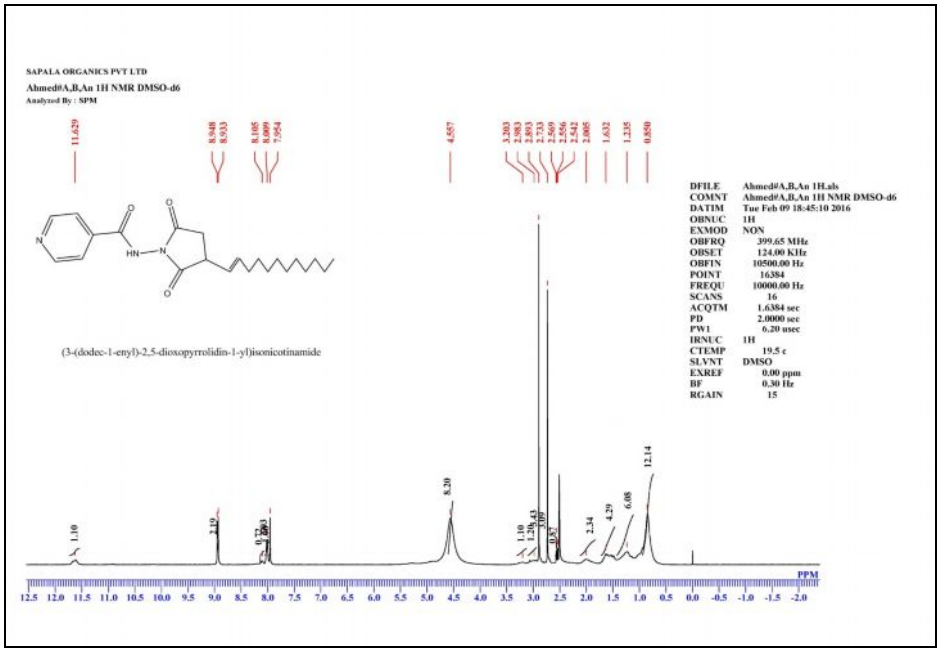
In a round bottom flask Isoniazid (0.15mM) was dissolved in Dimethylformamide. Dodecenyl succinic anhydride (0.15mM) dissolved in Dimethylformamide was added to this solution which yielded solution A and B. Solution B was added dropwise into solution A to give solution C. This solution was kept in a water bath at 20oC and was stirred until completely dissolved. To this solution, Phosphorus pentoxide (P2O5) 12 grams dissolved in H2SO4 (10 ml) and DMF (50ml) was added.

This solution was stirred for 2 hours at 70oC; then this mixture was kept on ice bath and poured into cold water. Resulting product was then filtered to obtain the desired compound. The compound was purification by column chromatography

Chemical reaction



H[1]NMR



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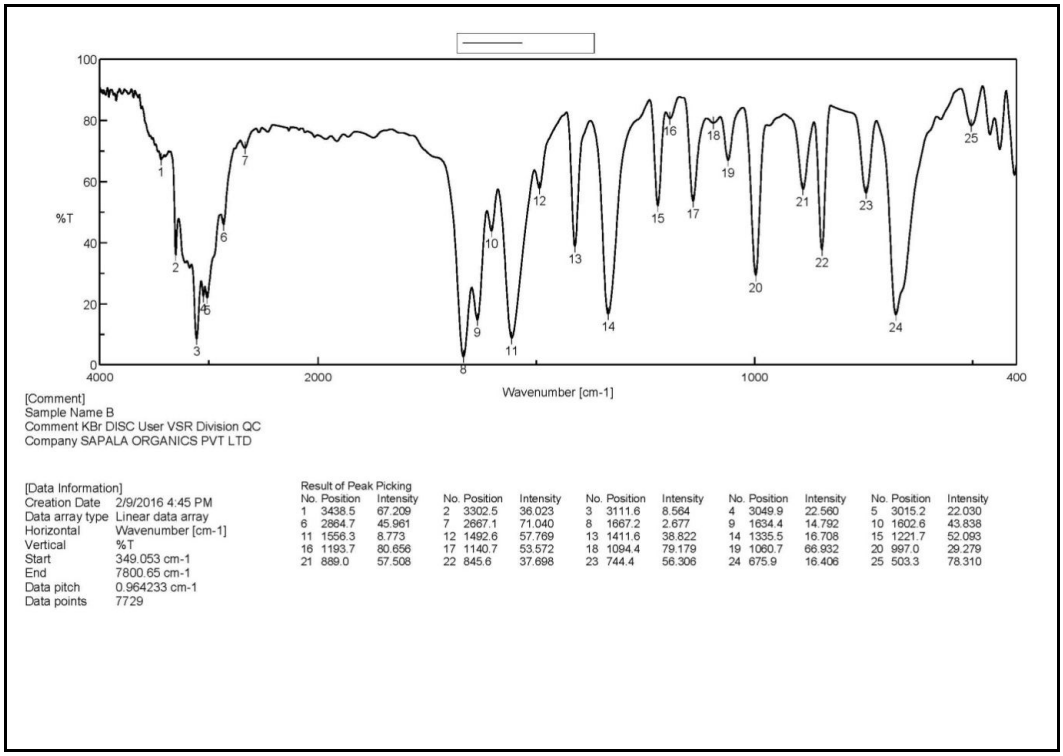


Anti Cancer Activity:

Maintenance of cell line:

The Hela cervical cancer cell lines were purchased from NCCS, Pune. The cells were maintained in DMEM supplemented with 10% FBS with addition of antibiotics penicillin/streptomycin (0.5-1 mL), in atmosphere of 5% CO2/ 95% air at 37°C. For MTT assay, HeLa cells were plated in 96 well plate at 5.0 X 10^3 cells were per well in culture medium and incubated overnight at 37°C.

Hela cell viability:

Cell viability was evaluated by MTT Assay with three independent triplicate experiments of six concentrations of compound (5, 10, 25, 50 75 and 100 µM). After 24 hrs of incubation, each treatment was withdrawn and MTT solution (0.5 mg / mL-1) was added to each well and plates were incubated at 37°C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophoreformazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 560 nm on a microplate reader.

Antibacterial activity:

Mueller Hinton agar plates were prepared by sterilizing the medium first. Antibacterial assay was performed using agar well diffusion assay. In this, wells were prepared in agar plates using sterilized cork borer. Indicator organism was spread under aseptic conditions. In the wells prepared, 10ul, 25ul, 50ul, 100ul and 150ul of the compound was added. The plates were then incubated for 24 hours at 37°C. After incubation, the resultant zone of inhibition was measured which was compared with the respective standards.

DNA Cleavage Studies:

The compounds were dissolved in DMSO and added separately to the CT-DNA (Calf Thymus DNA) sample and Hydrogen Peroxide was added. The samples mixtures were incubated at 37ºC for 1 hour. The electrophoresis of the samples was done according to the following procedure. Weigh 0.25grams of agarose and dissolve it in 25 ml of 1x TAE buffer (121.1g Tris base, pH 8.0, 0.5 M EDTA, 57.1ml of Glacial acetic acid for 1 ltr) by boiling. When the gel attains approximately 55ºC, pour it into the gel cassette fitted with comb. Let the

gel to solidify. Carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer. Load DNA sample with bromophenol blue carefully into the wells, along with standard DNA marker and pass the constant 100 V of electricity till the dye front reaches the end of gel. Remove the gel and carefully stain with ETBR solution (10 µg/ml) for 10-15 min and destain the gel and observe the bands under UV transilluminator.

Results:

Compound was synthesized in the laboratory and was evaluated for antibacterial activity, anticancer activity and DNA Cleavage potential. The results of the activity are mentioned below.

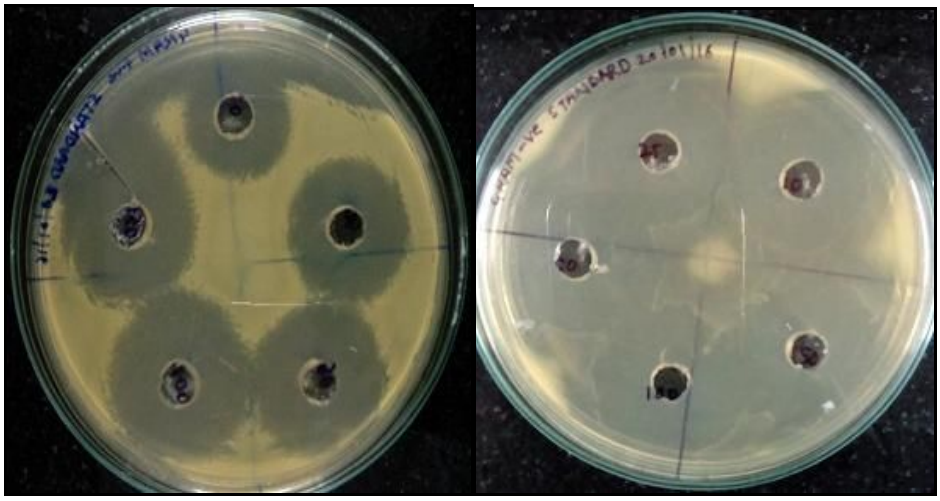
Anti Cancer activity:

The compound showed a potential anticancer activity when used against HeLa cells. Standard drug used was Cisplatin. The potent activity can be depicted by comparison of IC50 values.

S.No	Compound	IC 50 µM
1	2G	7.38
2	Cisplatin standard	4.6

Antibacterial activity:

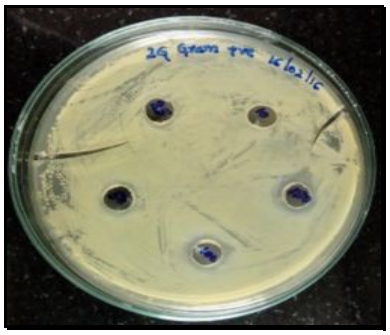
Antibacterial activity was measured against one gram positive strain: Staphylococcus aureus and one gram negative Strain E.Coli bacteria.



Gram Positive **Gram Negative**

Strain/ Concentration (µg)	10 Zone of Inhibition (mm)	25 Zone of Inhibition (mm)	50 Zone of Inhibition (mm)	100 Zone of Inhibition (mm)	150 Zone of Inhibition (mm)
Gram Positive	9	10	12	13	13
Gram Negative	10	10	11	11	12

Gram Positive Bacteria:

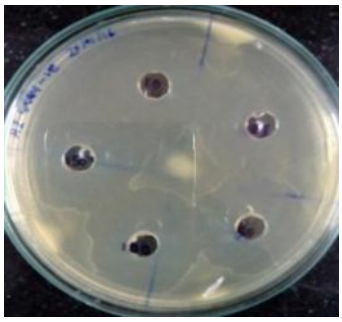


A

A: The compound did not show antibacterial activity in the Gram Positive bacteria

Compound/ Concentration (µg)	10 Zone of Inhibition (mm)	25 Zone of Inhibition (mm)	50 Zone of Inhibition (mm)	100 Zone of Inhibition (mm)	150 Zone of Inhibition (mm)
2G	-	-	-	-	-

Gram Negative bacteria:



B

B: In the above compound when compared to standard at 150ug concentration the zone of inhibition was observed high. In the below table the inhibition was mentioned in mm.

Compound/ Concentration (µg)	10 Zone of Inhibition (mm)	25 Zone of Inhibition (mm)	50 Zone of Inhibition (mm)	100 Zone of Inhibition (mm)	150 Zone of Inhibition (mm)
2G	11	12	12	13	16

DNA Cleavage Studies:

M 1 2

Sl.No	Well	Sample Order
1	M	Marker
2	1	Control (only CT-DNA)
3	2	Compound

DNA Cleavage is measured by relaxation of supercoiled DNA to nicked circular conformation and linear conformation. During electrophoresis process supercoiled DNA will migrate faster when compared with DNA in nicked and linear conformations. The above figure illustrates the gel electrophoresis experiment showing apparent cleavage in the presence of H2O2 when compared with Control.

Discussion:

Isoniazid (Isonicotinylhydrazide (INH)) was initially prescribed only for treatment of tuberculosis but strains resistant to this drug were also found. As a measure to control these resistant strains, new derivatives of isoniazid were prepared which showed efficient antimycobacterial activity [17,18]. But these studies have shown that these derivatives have more biological activities other than antimycobacterial activity like, analgesic, anticancer, antifungal, antidiabetic [19-23] etc. The compound 3-(dodec-1-enyl)pyrrolidin-1-yl)isonicotinamide synthesized in this study has shown potent in vitro anticancer and antibacterial activity. This compound also consists the ability to cleave DNA efficiently which makes it efficient for further studies to be carried out.

References:

1. Berning SE, Peloquin CA. Antimycobacterial agents: Isoniazid. In: Antimicrobial Therapy and Vaccines, Yu V, Merigan T, Barriere S (Eds), Williams and Wilkins, Baltimore 1998.
2. Johnsson K, Schultz PG: Mechanistic studies of the oxidation of isoniazid by the catalase peroxidase from Mycobacterium tuberculosis. J Am ChemSoc 1994, 116:7425–7426.
3. Middlebrook G: Isoniazid resistance and catalase activity of tubercle bacilli. Am Rev Tuberc 1954, 69:471–472.
4. Zhang Y, Heym B, Allen B, Young D, Cole S: The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 1992, 358:591–593.
5. Zhang Y, Telenti A: Genetics of drug resistance in Mycobacterium tuberculosis. In: Molecular Genetics of Mycobacteria. Edited by Hatful GF, Jacobs WR Jr. Washington DC: ASM Press; 2000:235–254.
6. Ramaswamy S, Musser JM: Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuberc Lung Dis 1998, 79:3–29.
7. Marttila HJ, Soini H, Eerola E, Vyshnevskaya E, Vyshnevskiy BI, Otten TF, Vasilyef AV, Viljanen MK: A Ser315Thr substitution in KatG is predominant in genetically heterogeneous multidrugresistant Mycobacterium tuberculosis isolates originating from the St. Petersburg area in Russia. Antimicrob Agents Chemother 1998, 42:2443–2445.
8. Rouse DA, DeVito JA, Li Z, Byer H, Morris SL: Site-directed mutagenesis of the katG gene of Mycobacterium tuberculosis: effects on catalase-peroxidase activities and isoniazid resistance. MolMicrobiol 1996, 22:583–592.

9. Vilchèze C, Morbidoni HR, Weisbrod TR, Iwamoto H, Kuo M, Sacchettini JC, Jacobs WR Jr: Inactivation of the inhA-encoded fatty acid synthase II (FASII) enoyl-acyl carrier protein reductase induces accumulation of the FASI end products and cell lysis of Mycobacterium smegmatis. J Bacteriol 2000, 182:4059–4067.
10. Slayden RA, Lee RE, Barry CE: Isoniazid affects multiple components of the type II fatty acid synthase system of Mycobacterium tuberculosis. MolMicrobiol 2000, 38:514–525.
11. Lee AS, Lim IH, Tang LL, Telenti A, Wong SY: Contribution of kasA analysis to detection of isoniazid-resistant Mycobacterium tuberculosis in Singapore. Antimicrob Agents Chemother 1999, 43:2087–2089.
12. Piatek AS, Telenti A, Murray MR, El-Hajj H, Jacobs WR Jr, Kramer FR, Alland D: Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: Implications for rapid susceptibility testing. Antimicrob Agents Chemother 2000, 44:103–110.
13. Sherman DR, Mdluli K, Hickey MJ, Arain TM, Morris SL, Barry CE III, Stover CK: Compensatory ahpC gene expression in isoniazid-resistant Mycobacterium tuberculosis. Science 1996, 272:1641–1643.
14. Wilson T, de Lisle GW, Marcinkeviciene JA, Blanchard JS, Collins DM: Antisense RNA to ahpC, an oxidative stress defence gene involved in isoniazid resistance, indicates that AhpC of Mycobacterium bovis has virulence properties. Microbiology 1998, 144:2687–2695.
15. Felipe A. R. RODRIGUES , Augusto C. A. OLIVEIRA , Bruno C. CAVALCANTI , Claudia PESSOA , Alessandra C. PINHEIRO , Marcus V. N. DE SOUZA. Biological Evaluation of Isoniazid Derivatives as an Anticancer Class. Sci Pharm. 2014; 82: 21–28.
16. Sadaf Jamal Gilani, DivyaPrakashMaurya, DeeptiKatiyar, RichaGoel, KandasamyNagarajan and Suroor A Khan. Synthesis, Antifungal and Toxicity Screening of Newer Isoniazid Derivatives. Med chem 2014, 4:4.
17. Sinha N, Jain S, Tilekar A, et al. Synthesis of isonicotinic acid N'-arylidene-N-[2-oxo-2-(4-aryl-piperazin-1-yl)-ethyl] hydrazides as antituberculosis agents. Bioorg Med ChemLett. 2005 15; 15(6): 1573-6.
18. Kakimoto S, Yamamoto K. Studies on antitubercular compounds. X. Condensation products of aldehydes and acid hydrazides of pyridine group. Pharm Bull. 1956; 4(1): 4-6.
19. Mohan J, Kumar A (2003) Bridgehead nitrogen heterocyclic systems: Synthesis and antimicrobial activity of imidazo [2,1-b]-1,3,4-thiadiazolo[2,3-c]-s-thiadiazoles and s-triazolo [3,4-b]-1,3,4-thiadiazoles. Indian J HeterocyclChem 12: 189–192.
20. Vigorita MG, OttanÃ R, Monforte F, Maccari R, Monforte MT, et al. (2003) Chiral 3,3'-(1,2-ethanediyl)-bis[2-(3,4-dimethoxyphenyl)-4-thiazolidinones] with anti-inflammatory activity. Part 11: evaluation of COX-2 selectivity and modelling. Bioorg Med Chem 11: 999-1006.
21. Kumar A, Rajput CS, Bhati SK (2007) Synthesis of 3-[4'-(p-chlorophenyl)- thiazol-2'-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethyl]-6-bromoquinazolin-4-ones as anti-inflammatory agent. Bioorg Med Chem 15: 3089–3096.
22. Küçükgüzel G, Kocatepe A, De Clercq E, Sahin F, Güllüce M (2006) Synthesis and biological activity of 4-thiazolidinones, thiosemicarbazides derived from diflunisalhydrazide. Eur J Med Chem 41: 353-359.
23. Pattan SR, Suresh C, Pujar VD, Reddy VVK, Rasal VP, et al. (2005) Synthesis and antidiabetic activity 2-amino[5'(4-sulphonylbenzylidine)-2,4-thiazolidinedione]-7-chloro-6-fluorobenzothiazole. Indian J Chem 44B: 2404-2408.

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