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Synthesis and Biological Evaluation of Novel Variants of Pyridyl Thiazoles for Antimicrobial, Anti-Candidal and Anti-Mycobacterial Activity

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Abstract

Background: Thiazoles are one of the most studied 5-membered aromatic heterocyclic. Many natural and synthetic thiazoles, as well as their derivatives, exhibited biological activity. Thiazole derivatives have antibacterial action against a variety of bacteria and diseases due to their unique characteristics. Because of its immense biological significance, scientists are working hard to develop novel biologically active thiazole compounds

Method: In the present study the synthesis of some derivatives of the 2-pyridyl/3-pyridyl and 4-pyridyl substituent at C-2, and the aryl substituent at C-2 (Scheme 1) design and the desired target molecules 2a-2g, 3a-3g and 4a-4g were synthesized via the classic Hantzsch thiazole synthesis.

Result: The synthesized compounds were confirmed on the basis of IR, 1H-NMR and Mass analyses. The newly synthesized compounds were evaluated for their Antimicrobial, Anti-Candidal and Anti-Mycobacterial Activity by *S. aureus* and *E. coli;* Anticandidal activity of test compound was assessed against *Candida albicans* and *Mycobacterium smegmatis* for Anti-Mycobacterial Activity.

Conclusion: The biological importance of pyridyl-thiazole derivatives is reported here in the clubbed pyridyl-thiazole derivatives as possible antimycobacterial drugs, as part of our hunt for new antitubercular medicines.

Keywords: Antimicrobial, anti-candidal, anti-mycobacterial, pyridyl thiazole

1. Introduction

Tuberculosis (TB) is a highly contagious airborne disease caused primarily by Mycobacterium tuberculosis (Mtb), a Mycobacterium species. Indeed, para-aminosalicylic acid (1946), pyrazinamide (1952), isoniazid (1952), ethambutol (1961), and rifampin (1965) were all discovered in quick succession after the discovery of TB drugs. The introduction of these antibiotics resulted in a dramatic reduction in tuberculosis (TB) cases in developing countries. Researchers used a variety of screening techniques to find promising compound series for the development of anti-TB drugs. High-throughput phenotypic screening assays [1-3] have identified a large number of compound series. The pyridyl-thiazole (PT) and amino-thiazole (AT) series are promising for their activity among the compound classes identified through such phenotypic screens [3].

Courtney C. Aldricha and Co-workers [4] discovered Structure-activity correlations of 2-aminothiazoles efficient against Mycobacterium tuberculosis (I). (The paper demonstrates that analogues with a 2-pyridyl substituent are substantially more effective in GAST media, with MIC values of 0.39-0.78 M, which is consistent with HTS results (0.35 M for 18-19). The core aminothiazole, the 2-pyridyl substituent at C-4, and the aryl substituent at N-2 make up the lead structure. SAR revealed that the 2-pyridyl substituent at C-4 is required for efficacy, whereas N-2 aryl substituents are tolerated. Tharanikkarasu Kannan and colleagues [5] reported on the synthesis, characterisation, and *in vitro* and in silico analyses of 2-aminothiazole derivatives as antimycobacterial drugs (II). 4-Halophenyl at the second position of 2-aminothiazole derivatives increases activity, while 2-Pyridyl at the second position of 2-aminothiazole derivatives decreases activity, according to the structure activity report. To further understand the mechanism of antimycobacterial action, docking experiments of these compounds with Mtb's-Ketoacyl-12 ACP Synthase (KasA) protein were performed.

Tanya parish and Co-workers [6] has screened 2-aminothiazoles with excellent activity against *M. tuberculosis* (III). They conducted an SAR assessment of different substitutions at the C-2 and C-4 positions, as well as possible replacements for the thiazole core. 2-pyridyl moiety at the C-4 position is essential for bacterial activity, as replacement of

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the pyridine ring resulted in a loss of activity. The efforts around the C-2 position indicate flexibility to various modifications with amine and amide all showing activity. 4-(Pyridin-2-yl)-N-(pyridin-3-yl) thiazol2-amine was the most potent analogue prepared.

Kelly Chibale and Co-workers [7] reported a series of compounds derived from the 2-amino-4-(2-pyridyl) thiazole scaffold (IV) and tested for *in vitro* antimycobacterial activity against the Mycobacterium tuberculosis H37Rv strain, antiplasmodial activity against the chloroquine sensitive NF54 Plasmodium falciparum strain and cytotoxicity on a mammalian cell line. Optimal antimycobacterial activity was found with compounds with a 2-pyridyl ring at position 4 of the thiazole scaffold, a substituted phenyl ring at the 2-amino position, and an amide linker between the scaffold and the substituted phenyl. The antiplasmodial activity was best with compounds that had the phenyl ring substituted with hydrophobic electron withdrawing groups.

Boja Pujari and co-workers [8] *et al* reported the synthesis of thiazole-based hydrazides (V) as anti-inflammatory and antimicrobial agents. The *in vitro* anti-inflammatory study of the target compounds revealed that these compounds are significant inhibitors. The *in vitro* anti-inflammatory activity of these compounds was evaluated by denaturation of the bovine serum albumin method.

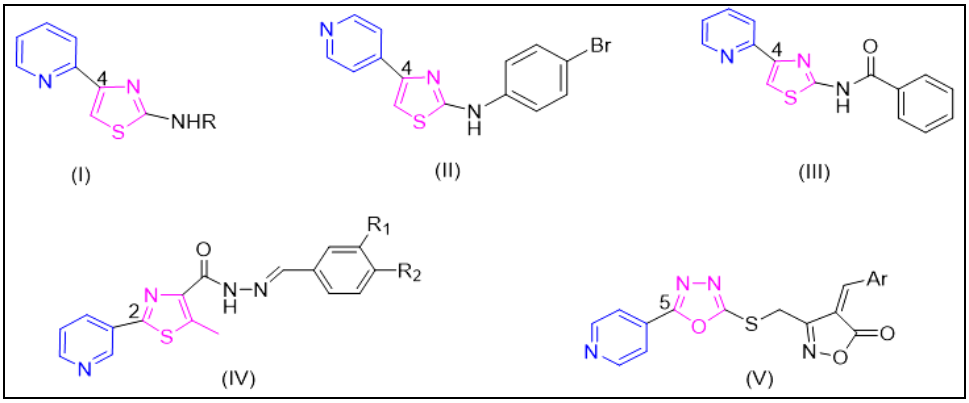

Fig 1: Pyridyl thiazole as an antimycobacterial agents

Keeping in mind, the biological significance of pyridyl-thiazole derivatives and in continuation of our search for new antitubercular agents, we report here in the clubbed pyridyl-thiazole derivatives as potential antimycobacterial agents.

2. Materials and Methods

2.1. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualised with UV light. Merck silica gel (60-120 mesh) was used for column chromatography. The UV and fluorescence were recorded on Shimadzu UV-1800 spectrophotometer. ^{1}HNMR (400MHz) and ^{13}CNMR(100MHz) spectra were recorded on a Brucker Avance II 400 MHz NMR spectrometer in $CDCl_3$/DMSO-d6 solution using TMS as an internal standard. All chemical shifts were recorded in δ(ppm) using TMS as an internal standard. The mass spectra were recorded on Waters, Q-TOF Micromass/ESI-MS at 70eV.

General Procedure for the Synthesis of Isomeric Pyridine Carbothioamide Acid (2, 3, 4)

A solution of pyridine carbonitriles (5gm/5ml) in pyridine (15ml) and triethyl amine (3ml) was stirred for 15min. H_2S gas was then passed into the reaction mixture. Colour of the solution turns green. The reaction was monitored on TLC. After stirring for about 2hrs the solution turns to greenish yellow solid. On completion of the reaction the mixture was poured into crushed ice and stirred for the complete precipitation. The crude thioamide product was filtered and washed extensively with water. The product was re-crystallized from ethanol to obtain almost pure product. Yield: 80-90%.

General Procedure for the Synthesis of Isomeric 4-Phenylthiazol-2-Pyridyl Derivatives (2a-h/3a-h/4a-h)

To a solution of thioamide (2/3/4) (1gm, 7.2 mmol) in ethyl alcohol (4ml), 4-substituted phenacyl bromide (7.2 mmol) was added and the reaction was refluxed on the hot plate. After the completion of the reaction as monitored on TLC (3hr), the reaction mixture was poured in cold water. The solid product obtained was filtered, washed with water, dried and recrystallized from ethanol.

4-Phenyl-2-(Pyridin-2-yl) Thiazole (2a)

Yield 87%, Yellowish green, M.P.198oC. 1H NMR (400MHz, CDCl3): δ 7.07-7.09 (m, 1H, phenyl), 7.10-7.12 (dd, J = 8Hz, J = 7.6 Hz, 2H, phenyl), 7.33-7.34 (ddd, J=7.8 Hz, J=4.6 Hz, J=1.06Hz, 1H, pyridine), 7.42 (s, 1H, thiazole), 7.76-7.79 (dt, J =1.0 and J = 7.8 Hz, 1H, pyridine), 7.85-7.86 (dd, J = 8Hz, J= 2.3Hz, 2H, phenyl), 8.10-8.20 (dd, J = 7.8Hz, J =1.06 Hz, 1H, pyridine), 8.33-8.34 (dd, J = 4.6Hz, J = 1.0 Hz, 1H, pyridine)

13C NMR (100 MHz, CDCl3): δ 116.80, 117.29, 117.40, 121.90, 125.60, 129.02, 129.12, 139.06, 146.36, 151.43, 157.70, 164.20.

4-(4-Fluorophenyl)-2-(Pyridin-2-yl) Thiazole (2b)

Yield 89%, Green, M.P.114oC, 1H NMR (400 MHz, CDCl3): δ 7.12-7.16 (dd, J=10.64 Hz, J=8 Hz, 2H, phenyl), 7.32-7.36 (ddd, J=7.6 Hz, J=4.8 Hz, J=1.0 Hz, 1H, pyridine), 7.53 (s, 1H, thiazole), 7.80-7.85 (dt, J=1.0Hz, J=7.6 Hz, 1H, pyridine), 7.95-7.99 (dd, J=8Hz, J=7.44 Hz, 2H, phenyl), 8.30-8.32 (dd, J=7.92Hz, J=1.0 Hz, 1H, pyridine), 8.62-8.63 (dd, J=4.8Hz, J=1.0 Hz, 1H, pyridine)

13C NMR (100 MHz, CDCl3) δ: 114.86, 115.58, 115.79, 119.86, 124.59, 128.06, 128.14, 137.07, 149.48, 155.67. m/z (70 eV): 257.0612 [M+1].

4-(4-Chlorophenyl)-2-(Pyridin-2-yl) Thiazole (2c)
Yield 81%, Light Brown, M.P.136oC, 1H NMR (400MHz, CDCl3): δ 7.14-7.16 (d, J=7.8 Hz, 2H, phenyl), 7.42-7.45 (ddd, J=7.6Hz, J=4.4Hz, J=1.02 Hz, 1H, pyridine), 7.59 (s, 1H, thiazole), 7.86-7.87 (dt, J=0.98Hz, J=7.6 Hz, 1H, pyridine), 7.91-7.94 (d, J=7.8 Hz, 2H, phenyl), 8.33-8.34 (dd, J=7.6Hz, J=1.02 Hz, 1H, pyridine), 8.65-8.66 (dd, J=4.4Hz, J=0.98 Hz, 1H, pyridine)
13C NMR (100 MHz, CDCl3): δ 114.86, 117.29, 118.35, 122.29, 126.77, 130.45, 132.39, 140.87, 149.38, 152.24, 158.43, 165.16.

4-(4-Bromophenyl)-2-(Pyridin-2-yl) Thiazole (2d)
Yield 52%, Cream, M.P. 132oC, 1H NMR (400MHz, CDCl3): δ 7.13-7.17 (d, J=7.7 Hz, 2H, phenyl), 7.46-7.47 (ddd, J=7.5 Hz, J=4.2 Hz, J=1.04 Hz, 1H, pyridine), 7.60 (s, 1H, thiazole), 7.87-7.88 (dt, J=1.02Hz, J=7.5 Hz, 1H, pyridine), 7.9-7.92 (d, J=7.7Hz, 2H, phenyl), 8.34-8.35 (dd, J=7.5Hz, J=1.04 Hz, 1H, pyridine), 8.64-8.65 (dd, J=4.2 and J=1.02 Hz, 1H, pyridine)
13C NMR (100 MHz, CDCl3): δ 114.72, 117.08, 118.14, 121.18, 125.60, 129.39, 131.28, 138.26, 148.23, 150.00, 157.22, 164.05.

2-(Pyridin-2-yl)-4-(p-Tolyl) Thiazole (2e)
Yield 73%, Yellow, M.P. 110oC 1H NMR (400MHz, CDCl3): δ 6.98-7.0 (d, J=8.4 Hz, 2H, phenyl), 7.30-7.31 (ddd, J=7.0 Hz, J=4.42 Hz, J=1.03 Hz, 1H, pyridine), 7.56 (s, 1H, thiazole), 7.79-7.83 (dt, J=1.01Hz, J=7.9 Hz, 1H, pyridine), 7.92-7.94 (d, J=8.4 Hz, 2H, phenyl), 8.31-8.33 (dd, J=7.9Hz, J=1.03 Hz, 1H, pyridine), 8.62-8.64 (dd, J=4.42Hz, J=1.01 Hz, 1H, pyridine)
13C NMR (100 MHz, CDCl3): δ 21.32 (-CH3), 113.50, 114.20, 119.80, 124.31, 125.51, 125.61, 135.82, 148.30, 150.40, 155.39, 158.20, 166.90.

4-(4-Methoxyphenyl)-2-(Pyridin-2-yl) Thiazole (2f)
Yield 90%, Dark yellow, M.P. 108oC, 1H NMR (400MHz, CDCl3): δ 6.96-6.99 (d, J=8.8 Hz, 2H, phenyl), 7.29-7.32 (ddd, J=7.76Hz, J=4.84 Hz, J=1.08 Hz, 1H, pyridine), 7.45 (s, 1H, thiazole), 7.78-7.82 (dt, J=1.0Hz, J=7.76 Hz, 1H, pyridine), 7.91-7.93 (d, J=8.8 Hz, 2H, phenyl), 8.30-8.32 (dd, J=7.76Hz, J=1.08 Hz, 1H, pyridine), 8.60-8.62 (dd, J=4.84Hz, J=1.0 Hz, 1H, pyridine)
13C NMR (100 MHz, CDCl3): δ 55.10 (-OMe), 113.60, 114.14, 119.85, 124.43, 127.51, 127.66, 136.99, 149.44, 151.55, 156.50, 159.72, 168.61. m/z (70 eV): 269.0817 [M+1].

4-(4-Nitrophenyl)-2-(Pyridin-2-yl) Thiazole (2g)
Yield 92%, Yellow, M.P. 178oC, 1H NMR (400MHz, CDCl3): δ 7.22-7.23 (d, J= 8 Hz, 2H, phenyl), 7.42-7.44 (ddd, J=7.6 Hz, J=4.42 Hz, J=1.01 Hz, 1H, pyridine), 7.92(s, 1H, thiazole), 7.98-7.99 (dt, J=1.0Hz, J=7.6 Hz, 1H, pyridine), 8.02-8.03(d, J=8 Hz, 2H, phenyl), 8.42-8.44 (dd, J=7.6 Hz, J=1.01 Hz, 1H, pyridine), 8.66-8.67 (dd, J=4.42 and J=1.0 Hz, 1H, pyridine)
13C NMR (100 MHz, CDCl3): δ 116.77, 117.10, 117.11, 120.82, 126.72, 130.03, 130.20, 139.12, 147.40, 152.30, 158.10, 165.20.

4-(2-(Pyridin-2-yl) Thiazol-4-yl) Benzonitrile (2h):
Yield 93%, White, M.P. 168oC, 1H NMR (400MHz, CDCl3): δ 7.21-7.22 (d, J=7.9 Hz, 2H, phenyl), 7.43-7.44 (ddd, J=7.2 Hz, J=4.32 Hz, J=1.02 Hz, 1H, pyridine), 7.86 (s, 1H, thiazole), 7.78-7.79 (dt, J=1.0Hz, J=7.2 Hz, 1H, pyridine), 8.00-8.10 (d, J=7.9 Hz, 2H, phenyl), 8.20-8.21 (dd, J=7.2Hz, J=1.02 Hz, 1H, pyridine), 8.65-8.66 (dd, J=4.32Hz, J=1.0 Hz, 1H, pyridine).
13C NMR (100 MHz, CDCl3): δ 114.02, 114.25, 115.27, 118.70 (-CN), 124.07, 129.12, 129.24, 138.17, 146.2, 151.25, 157.06, 164.23.

4-Phenyl-2-(Pyridin-3-yl) Thiazole (3a)
Yield 98%, Buff white, M.P. 270oC, 1H NMR (400MHz, CDCl3):δ7.90-8.00(m, 1H, pyridine), 8.00-8.10(d, J=8Hz, 2H, phenyl), 8.00-8.40(m, 1H, pyridine), 8.00-8.90(d, J=8Hz, 2H, phenyl), 8.27(s, 1H, Thiazole), 8.50-8.80(m, 1H, phenyl), 8.97(m, 1H, pyridine), 9.51(m, 1H, pyridine).
13CNMR(100MHz, CDCl3):δ115.00, 122.00, 128.00, 128.00, 131.50, 132.00, 139.00, 142.10, 144.10, 155.10, 157.10, 161.10.

4-(4-Fluorophenyl)-2-(Pyridin-3-yl) Thiazole (3b)
Yield 82%, White, M.P. 120oC, 1H NMR (400MHz, CDCl3): δ 7.72-7.74(m, 1H, pyridine),
8.30-8.50(m, J=8.04Hz, 2H, phenyl), 8.40(m, 1H, pyridine), 8.70(s, 1H, Thiazole), 7.90-8.00(m, J=8.04Hz, 2H, phenyl), 8.70-8.80(m, 1H, pyridine), 9.10(m, 1H, pyridine).
13CNMR(100MHz, CDCl3):δ117.00, 121.00, 127.10, 128.10, 131.00, 132.10, 139.70, 140.10, 144.40, 155.10, 156.40, 162.00.

4-(4-Chlorophenyl)-2-(Pyridin-3-yl) Thiazole (3c)
Yield 69%, Light yellow, M.P.244oC, 1HNMR(400MHz, CDCl3):δ7.40-7.50(d, J=8Hz, 2H, phenyl), 8.20-8.40(d, J=8Hz, 2H, phenyl), 8.30(s, 1H, thiazole), 8.03(m, 1H, pyridine), 8.00-8.10(m, 1H, pyridine), 8.40-8.80(m, 1H, pyridine), 9.30(m, 1H, pyridine).
13CNMR(100MHz, CDCl3):δ116.00, 120.00, 128.00, 129.80, 131.00, 133.40, 138.10, 142.00, 143.50, 154.94, 156.10, 161.00.

4-(4-Bromophenyl)-2-(Pyridin-3-yl) Thiazole (3d)
Yield 48%, Light Brown, M.P. 226oC 1HNMR (400MHz, CDCl3): δ 7.60-7.62(d, J = 8.48Hz, 2H, phenyl), 8.00-8.05(d, J=8.52Hz, 2H, phenyl), 8.06-8.09(m, 1H, pyridine), 8.27(s, 1H, thiazole), 8.92-8.94(m, 1H, pyridine), 8.97(m, 1H, pyridine), 9.51(m, 1H, pyridine).
13CNMR(100MHzCDCl3):δ117.20, 121.88, 127.00, 128.00, 131.51, 132.36, 139.98, 141.32, 144.50, 154.94, 156.00, 160.98. m/z (70 eV): 316.9769 [M+1]and 318.9743 [M+2].

2-(Pyridin-3-yl)-4-(p-Tolyl) Thiazole (3e)
Yield 81%, Light Yellow, M.P. 280oC, 1H NMR (400MHz, CDCl3): δ 7.10-7.15(m, 1H, pyridine), 7.50(s, 1H, thiazole), 7.71-7.79(d, J=8Hz, 2H, phenyl), 7.90-8.00(d, J=8Hz, 2H, phenyl), 8.00-8.09(m, 1H, pyridine), 8.10-8.19(m, 1H, pyridine), 9.20(m, 1H, pyridine).
13CNMR(100MHzCDCl3):δ21.32(CH3), 117.00, 124.10, 125.10, 128.01, 130.01, 134.01, 141.02, 148.40, 152.10, 155.10, 157.0, 164.90.

4-(4-Methoxyphenyl)-2-(Pyridin-3-yl) Thiazole (3f)
Yield 97%, Dark yellow, M.P.204oC, 1HNMR (400MHz, CDCl3): δ 7.40-7.42(m, 1H, pyridine), 7.43-7.46(s, 1H, thiazole), 7.72-7.92(d, J=8.3Hz, 2H, phenyl), 7.90-8.00(d, J=8.3Hz, 2H, phenyl), 8.34-8.36(m, 1H, pyridine), 8.71(m, 1H, pyridine), 9.25(m, 1H, pyridine).

13CNMR(100MHz, CDCl3):δ55.10(OMe), 116.57, 122.00, 124.10, 127.00, 129.30, 132.50, 138.50, 148.00, 151.20, 153.90, 157.0, 166.00.

4-(4-Nitrophenyl)-2-(Pyridin-3-yl) Thiazole (3g)
Yield 80%, Dark Brown, M.P. 158oC, 1H NMR (400MHz, CDCl3):δ7.43-7.46(m, 1H, pyridine), 7.76(s, 1H, thiazole), 8.16-8.19(d, J=8.9Hz, 2H, phenyl), 8.31-8.36(d, J=8.9Hz, 2H, phenyl), 8.34-8.36(m, 1H, pyridine), 8.70-8.72(m, 1H, pyridine), 9.25-9.26(m, 1H, pyridine). 13CNMR(100MHzCDCl3):δ116.57, 123.28, 124.28, 127.07, 129.30, 133.84, 139.64, 147.78, 151.23, 154.33, 156.77, 165.35.

4-(2-(Pyridin-3-yl) Thiazol-4-yl) Benzonitrile (3h)
Yield 75%, White, M.P. 260oC 1H NMR (400MHz, CDCl3):δ7.40(m, 1H, pyridine), 7.73 (s, 1H, thiazole), 8.02-8.04(d, J=8.10Hz, 2H, phenyl), 8.10-8.20(d, J=8.2Hz, 2H, phenyl), 8.32-8.52(m, 1H, pyridine), 8.50(m, 1H, pyridine), 9.25(m, 1H, pyridin).
13CNMR(100MHz, CDCl3):δ116.57, 118.10(CN), 122.87, 123.90, 127.00, 129.00, 132.50, 139.84, 147.10, 152.00, 154.00, 156.05, 166.35.

4-Phenyl-2-(Pyridin-4-yl) Thiazole (4a)
Yield 87%, Yellowish green, M.P.198oC 1HMNR (400MHz, CDCl3): δ 7.36-7.40(m, 1H, phenyl), 7.45-7.48(d, 8.8Hz, 2H, phenyl), 7.60(s, 1H, thiazole), 7.89-7.90(d, 5Hz, 2H, pyridine), 7.97-8.00(d, 8.8Hz, 2H, phenyl), 8.72-8.73(d, 5Hz, 2H, pyridine).
13CNMR(100MHz, CDCl3):δ110.81, 121.32, 127.13, 128.17, 129.42, 133.40, 143.75, 149.81, 152.35, 154.92.

4-(4-Fluorophenyl)-2-(Pyridin-4-yl) Thiazole (4b)
Yield 89%, Green, M.P. 114oC 1HMNR (400MHz, CDCl3): δ 7.11-7.15(d, J=8Hz, 2H, phenyl), 7.52(s, 1H, thiazole), 7.85-7.86(d, 2H, 4Hz, pyridine), 7.87-7.95(dd, J=4Hz, J=8Hz, 2H, phenyl), 8.70-8.71(d, 2H, 4Hz, pyridine).
13CNMR(100MHz, CDCl3):δ110.85, 116.36, 121.34, 128.67, 130.61, 143.74, 149.83, 152.34, 154.94, 162.98.

4-(4-Chlorophenyl)-2-(Pyridin-4-yl) Thiazole (4c)
Yield 81%, Light Brown, M.P. 136oC, 1HMNR (400MHz, CDCl3): δ 7.41-7.45(d, J=8.5Hz, 2H phenyl), 7.58(s, 1H, thiazole), 7.87-7.88(d, J=6.12Hz, 2H, pyridine), 7.90-7.94(d, J=8.5Hz, 2H, phenyl), 8.72-8.74(d, J=6.12Hz, 2H pyridine).
13CNMR(100MHz, CDCl3):δ110.85, 121.30, 128.95, 129.37, 131.15, 134.34, 143.76, 149.86, 152.37, 154.94

4-(4-Bromophenyl)-2-(Pyridin-4-yl) Thiazole (4d)
Yield 52%, Cream, M.P.132oC, 1HMNR (400MHz, CDCl3): δ 7.57-7.58(d, J=9Hz, 2H, phenyl), 7.59(s, 1H, thiazole), 7.85-7.86(d, J=6.16Hz, 2H, pyridine), 7.87-7.89(d, J=9Hz, 2H, phenyl), 8.72-8.74(d, J=6.16Hz, 2H, pyridine).
13CNMR(100MHz, CDCl3):δ110.81, 121.20, 123.15, 128.34, 132.08, 132.15, 143.74, 149.87, 152.34, 154.56.

2-(Pyridin-4-yl)4-(p-Tolyl)-Thiazole (4e)
Yield 73%, Yellow, M.P. 110oC, 1HMNR (400MHz, CDCl3): δ 7.25-7.27(d, J=5.8Hz, 2H, pyridine), 7.52(s, 1H, Thiazole), 7.86-7.87(d, J=8.16Hz, 2H, phenyl), 7.87-7.88(d, J=8.16Hz, 2H, phenyl), 8.70-8.72(d, J=6.16Hz, 2H, pyridine).
13CNMR(CDCl3) δ 110.86, 121.00, 123.02, 128.22, 131.08, 132.05, 143.44, 149.57, 152.44, 154.38, 21.33(-CH3). m/z (70 eV): 253.0790 [M+1].

4-(4-Methoxyphenyl)-2-(Pyridin-4-yl) Thiazole (4f)
Yield90%, Dark yellow, M.P. 108oC, 1HMNR (400MHz, CDCl3): δ 6.97-6.99(d, J=8.84Hz, 2H phenyl), 7.45(s, 1H, thiazole), 7.87-7.88(d, J=6.12Hz, 2H, pyridine), 7.90-7.93(d, J=8.84Hz, 2H, phenyl), 8.70-8.72(d, J=6.12Hz, 2H, pyridine)
13CNMR(100MHz, CDCl3):δ55.37(OMe), 112.61, 114.21, 120.34, 126.93, 127.82, 140.47, 150.64, 157.00, 159.97, 164.67. m/z (70 eV): 269.0733 [M+1].

4-(4-Nitrophenyl)-2-(Pyridin-4-yl) Thiazole (4g)
Yield92%, M.P.178oC, 1HMNR(400MHz, CDCl3):δ7.82(s, 1H, thiazole), 7.907.91(d, J=6.16Hz, 2H, pyridine), 8.16-8.19(d, J=9.2Hz, 2H, phenyl), 8.32-8.35(d, J=9.2Hz, 2H, phenyl), 8.76-8.77(d, J=6.16Hz, 2H, pyridine).
13CNMR(100MHz, CDCl3):δ110.47, 121.33, 124.11, 126.23, 139.03, 143.11, 147.57, 149.48, 152.63, 154.45.

4-(2-(Pyridin-4yl) Thiazole-4-yl)-Benzonitrile (4h)
Yield93%, White, M.P.1680C, 1HMNR(400MHz, CDCl3):δ7.74-7.77(d, J=8.4Hz, 2H, phenyl), 7.76(s, 1H, thiazole), 7.88-7.90(d, J=6.12Hz, 2H, pyridine), 8.10-8.12(d, J=8.4Hz, 2H, phenyl), 8.75-8.76(d, J=6.12Hz, 2H pyridine).
13CNMR(100MHz, CDCl3):δ110.11, 112.66, 118.66(CN), 121.23, 126.05, 132.41, 137.63, 143.47, 149.58, 152.43, 154.95.

2.2. Biological assays

2.2.1. Culture Collection and Maintenance

Standard cultures: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were obtained from BAC-TEST laboratory, Nasik.
Clinical isolates of *Candida albicans, Escherichia coli, Staphylococcus aureus*, *Pseudomonas Aeruginosa, Klebsiella pneumoniae* were obtained from the BAC-TEST laboratory, Nasik. These cultures were grown on nutrient agar (NA) and Yeast was grown Sabourauds Dextrose Agar (SDA) and incubated at 37°C for 24 hours. *Mycobacterium smegmatis* NCIM 5138 was procured from NCIM, National Chemical Laboratory, Pune and maintained on Mycobacterium phlei (MP) medium and incubated at 37ºC for 72hours.
Antibiotics and Test Compound used:
Standard antibiotic disc impregnated with Gentamicin (10 mg), Imipenem (10 mg), Penicillin (10 mg) were obtained from Hi-Media, Mumbai for testing the antibiogram against standard cultures. Itraconazole, Combutol-microzide and 6 Pyridyl Thiazole Variants viz. 2b, 3b, 4b and 2f, 3f, 4f.

2.2.2. Solubility Testing

Solvents: Water, Ethyl acetate (SDFCL, Mumbai), Hexane (Hi-Media, Mumbai), Benzene (Research Lab, Mumbai) and Dimethyl sulfoxide (Hi-Media, Mumbai) were screened for testing the solubility of4-pyridyl compound variants (NO 2, CN, Cl, F, Br, OMe, Me).The solubility of 6 Pyridyl Thiazole Variants test were assessed in variant solvents: Ethyl acetate(HiMedia, Mumbai), Methanol (Rankem, Thane), Ethanol (Merck Mumbai), Diethyl ether (HiMedia, Mumbai), DMSO, Petrolium ether (HiMedia, Mumbai), Cyclohexane (Ranbaxy), Chloroform(HiMedia, Mumbai), Acetone (HiMedia, Mumbai), Dichloromethane (HiMedia, Mumbai), Toluene(Merck, Mumbai) and Dimethylformamide (DMF) (Hi-Media, Mumbai). A pinch of test compound was dissolved in 1ml of solvent.

2.2.3. Preparation of 0.5 McFarland Standard

A volume of 0.5ml of 0.048mol/L $BaCl_2$ was added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain suspensions. Absorbance at 625nm should be 0.008 to 0.10 for 0.5McFarland standard (CLSI Document, 7th ed, M07-09).

Anti-bacterial screening: The newly synthesized and pure 4-pyridyl compound variants were evaluated for their antibacterial activity against Escherichia coli ATCC 215922 and Staphylococcus aureus ATCC 25923.Two fold serial dilutions (1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16µg/ml, 8 µg/ml) of 4-pyridyl variants (NO, CN, Cl, OMe, Me, Br, F) were made in sterile Muller-Hinton broth (MHB) inoculated with 15 µl of log phase culture of Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 culture adjusted to 0.5McFarland standard that is 1.5×10 8CFU/ml incubated at 37°C for 24 hours. Next day, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was recorded as the Minimum Inhibitory Concentration (MIC). A loopful from the highest dilution was streaked on sterile Muller-Hinton agar (MHA) plates which did not show growth after incubation was recorded as Minimum Bactericidal Concentration (MBC). The concentration which did not inhibit the growth was recorded as Sub-Inhibitory Concentration (SIC) (Wahab *et al*., 2011).Standard method of Bauer *et al*., 1996 by agar well diffusion method was done for determining antibiotic sensitivity. Antibacterial activity of test compound was assessed against two standard cultures (one Gram positive-Staphylococcus aureus ATCC 25923 and one Gram negative-*Escherichia coli* ATCC 25922). Tubes containing sterile 20 ml of MHA medium were seeded with 0.1 ml logphase culture adjusted to 0.5 McFarland standard when the temperature of the medium reached 40-50°C and were plate out in sterile empty petri plates. The media was allowed to solidify and wells of 6mm diameter were punched with the help of sterile cork borer. Each well was filled with (50 µl) test compounds. The solutions of the test compound with concentration of 10 mg/ml were prepared in Ethyl acetate and DMSO respectively. The disks of Penicillin (10 mg/ml) and Gentamicin (10 mg/ml) were also placed on agar medium for comparison. The plates were kept in the refrigerator for 15 minutes for prediffusion and then incubated at 37° C for 24 hours. Solvent without test compound served as a control. The sensitivities of the microorganisms were determined by measuring the zone of inhibition (including the diameter of the well) on the agar surface around the wells (Bauer *et al*., 1996).

2.2.4. Inoculums Development and Growth Curve of Candida Albicans

Single colony of Candida albicans culture grown on SDA plate for 24 hours at 37ºC was

Inoculated in 25ml SD broth and incubated at 37ºC for 32 hours. At an interval of 1 hour, the aliquots were withdrawn and Optical Density (OD) was determined at 530nm and the total viable cell count was estimated using saline by serial dilution method. From each dilution, 0.1ml was spread onto SDA plates and incubated at 37ºC for 24 hours. The total number of colonies was enumerated in each plate. (Pfaller *et al*., 1988).

3. Pharmacological Evaluation

3.1. Anti-Candidal Activity

The newly synthesized and pure 4-pyridyl compound variants were evaluated for their anticandidal activity against Candida albicans. Two fold serial dilutions (1024 µg/ml, 512 µg/ml, 256µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml) of 4-pyridyl variants (NO, CN, Cl, OMe, Me, Br, F) were made in sterile Sabouraud Dextrose Broth (SDB) inoculated with 15 µl of log phase culture adjusted to 0.5 McFarland standard that is 1.5×10 6 CFU/ml (Leite *et al*., 2014) incubated at37°C for 24 hours. Next day, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was recorded as the Minimum Inhibitory Concentration (MIC). A loopful from the highest dilution was streaked on sterile Sabourauds Dextrose Agar (SDA) plates which did not show growth after incubation was recorded as Minimum Fungicidal Concentration (MFC). The concentration which did not inhibit the growth was recorded as Sub-Inhibitory Concentration (SIC) (Carradori *et al*., 2013).Standard method of Bauer *et al*., 1996 by agar well diffusion method was carried out for determining antibiotic sensitivity. Anticandidal activity of test compound was assessed against Candida albicans. Tubes containing sterile 20 ml of sterile SDA medium were seeded with 0.1 ml log phase culture adjusted to 0.5 McFarland standards when the temperature of the medium reached 40-50°C and were platted out in sterile empty Petri plates. The media was allowed to solidify and wells of 6 mm diameter were punched with the help of sterile cork borer. Each well was filled with (50 µl) test compounds. The solutions of the test compound with concentration of 10 mg/ml were prepared in Ethyl acetate and DMSO respectively. The disks of Itraconazole were also placed on agar medium for comparison. The plates were kept in the refrigerator for 15 minutes for prediffusion and then incubated at 37° C for 24 hours. Solvent without test compound served as a control. The sensitivities of the microorganisms were determined by measuring the zone of inhibition (including the diameter of the well) on the agar surface around the wells (Bauer *et al*., 1996).

The newly synthesized Pyridyl Thiazole compound variants were evaluated for their anti-candidal activity against Candida albicans. The Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and Sub-Inhibitory Concentration (SIC) of the Pyridyl Thiazole compound were determined. Two fold serial dilutions (4096 µg/ml, 2048 µg/ml, 1024 µg/ml, 512µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml and0.5 µg/ml) of Pyridyl Thiazole variants were made in sterile Sabouraud Dextrose (SD) Broth were inoculated with 100 µl of log phase culture adjusted to approximately 1×10 6 CFU/ml by optical density method (Guinet *et al*., 1988) and incubated at 37°C for 24 hours. Next day, the tubes were examined visually for turbidity indicating growth and no turbidity indicating no growth. The highest dilution inhibiting the growth was recorded as the Minimum Inhibitory Concentration (MIC). A loopful from the higher dilutions was streaked on sterile Sabouraud Dextrose Agar (SDA) plates and the one which did not exhibit growth after incubation was recorded as Minimum Fungicidal Concentration (MFC). The highest concentration which did not inhibit the growth prior to MIC was recorded as Sub-Inhibitory Concentration (SIC) (Cordeiro *et al*., 2013).

For determining the anti-candidal activity of the test compound against Candida albicans, Agar well diffusion method was performed. Tubes containing 20 ml of sterile SDA medium (at 40°C) were seeded with 0.1 ml log phase culture and poured in sterile empty petri plates. The media was allowed to solidify and wells of 7 mm diameter were punched with the help of sterile cork borer. Each well was filled with 50 µl test compounds as solutions with their MFC concentration in Acetone, DMF and Ethanol respectively. The plates were kept in the refrigerator for 15 minutes for pre-diffusion and then incubated at 37°C for 24 hours. Solvent without test compound served as a control. The sensitivities of the microorganisms were determined by measuring the zone of inhibition on the agar surface around the wells (Bauer *et al*., 1966).

3.2. Inoculum Development and Growth Curve of Mycobacterium Smegmatis

Single colony of Mycobacterium smegmatis culture grown on Mycobacterium Phlei agar (MPA) for 72 hours at 37 0 C was inoculated in 25ml MP broth and incubated at 37ºC for 81 hours. At an interval of 3 hour, the aliquots were withdrawn for the total viable cell count by spreading 0.1mlculture onto MP Agar plates in triplicate and incubated at 37ºC for 72 hours. The total number of colonies was enumerated in each plate (Merchand *et al*., 2012).

3.3. Anti-Mycobacterial Activity

The newly synthesized and pure 4-pyridyl compound variants were evaluated for their antimycobacterial activity against Mycobacterium smegmatis NCIM 5138. Two fold serial dilutions(1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml) of 4-pyridylvariants (NO, CN, Cl, OMe, Me, Br, F) were made in sterile Mycobacterium phlei medium inoculated with 15 µl of log phase culture 5×10 3 CFU/ml (Balouiri *et al*., 2016) incubated at 37°C for 24 hours. Next day, the tubes were examined visually for growth (turbidity) and no growth (no turbidity). The highest dilution inhibiting the growth was recorded as the Minimum Inhibitory Concentration (MIC). A loopful from the highest dilution was streaked on sterile Mycobacterium phlei agar (MPA) plates which did not show growth after incubation was recorded as Minimum Bactericidal Concentration (MBC). The concentration which did not inhibit the growth was recorded as Sub-Inhibitory Concentration (SIC).Standard method of Bauer *et al*., 1996 by agar well diffusion method was done for determining antibiotic sensitivity. Antimycobacterial activity of test compound was assessed against Mycobacterium smegmatis NCIM 5138. Culture 0.1 ml was spread on sterile MPA. Wells of 6 mm diameter were punched with the help of sterile cork borer. Each well was filled with test compounds (50 µl). The solutions of the test compound with concentration of 10 mg/ml were prepared in Ethyl acetate and DMSO respectively. The disks of combutol-microzide were also placed on agar medium for comparison. The plates were kept in the refrigerator for 15 minutes for prediffusion and then incubated at 37°C for 48 hours. Solvent without test compound served as a control. The sensitivities of the microorganisms were determined by measuring the zone of inhibition (including the diameter of the well) on the agar surface around the wells (Bauer *et al*., 1996). The newly synthesized Pyridyl Thiazole variants were evaluated for their anti-mycobacterial activity using Mycobacterium smegmatis NCIM 5138. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Sub-Inhibitory Concentration (SIC) of the Pyridyl Thiazole compound were determined. Two fold serial dilutions (4096 µg/ml, 2048 µg/ml, 1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml and 0.5 µg/ml) of Pyridyl Thiazole variants were made in sterile Mycobacterium phlei medium inoculated with 100 µl of log phase culture 1×10 3 to 5×10 3 CFU/ml (Balouiri *et al*., 2016) and incubated at 37°C for 72 hours. After incubation, the tubes were examined visually for turbidity indicating growth and no turbidity indicating no growth. The highest dilution inhibiting the growth was recorded as the Minimum Inhibitory Concentration (MIC). A loopful from the higher dilutions was streaked on sterile Mycobacterium phlei agar (MPA) plates and the one which did not exhibit growth after incubation was recorded as Minimum Bactericidal Concentration (MBC). The highest concentration which did not inhibit the growth prior to MIC was recorded as Sub-Inhibitory Concentration (SIC). For determining the anti-mycobacterial activity of the test compound against *Mycobacterium smegmatis* NCIM 5138, Agar well diffusion method was performed. Tubes containing 20 ml of sterile MPA medium (at 40°C) were seeded with 0.1 ml log phase culture and poured in sterile empty petri plates. The media was allowed to solidify and wells of 7 mm diameter were punched with the help of sterile cork borer. Each well was filled with 50 µl test compounds as solutions with their MBC concentration in Acetone, DMF and Ethanol respectively. The plates were kept in the refrigerator for 15 minutes for pre-diffusion and then incubated at 37° C for 72 hours. Solvent without test compound served as a control. The sensitivities of the microorganisms were determined by measuring the zone of inhibition on the agar surface around the wells (Bauer *et al*., 1966).

3.2. Determination of MIC and FIC Index (FICI)

The combined effect of Pyridyl Thiazole variants (2PT-OMe, 4PT-OMe, 2PT-F and 4PT-F) were assessed using two fold serial dilution method. Using these 4 variants, 4 combinations were made. The combinations are 2PT-F with 2PT-OMe, 2PT-F with 4PT-OMe, 4PT-F with 2PT-OMe and 4PT-F with 4PT-OMe. These 4 combinations were assessed for the activity against Candida albicans. Two fold serial dilutions (4096 µg/ml, 2048 µg/ml, 1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml and 0.5 µg/ml) of combinations were made in sterile Sabourauds Dextrose Broth (SDB) inoculated with 100 µl of log phase culture 1×10 6 CFU/ml incubated at 37°C for 24 hours for Candida albicans (Fratini *et al*., 2016 and Krol *et al*., 2018). For each combination FICI values were calculated using the following formula:

$$\text{FIC index (FICI)} = \frac{\text{MIC drug 1 combined with drug 2}}{\text{MIC drug 1 alone}} + \frac{\text{MIC drug 2 combined with drug 1}}{\text{MIC drug 2 alone}}$$

Interactions were determined by assigning values calculated in the following way:
Synergism-FIC index < 0.5,
Indifference-FIC index 0.5-4,
Antagonism-FIC index > 4

4. Results and Discussion

4.1. Chemistry

The detailed synthetic strategy for the target compounds is illustrated in Scheme 1 which was previously reported

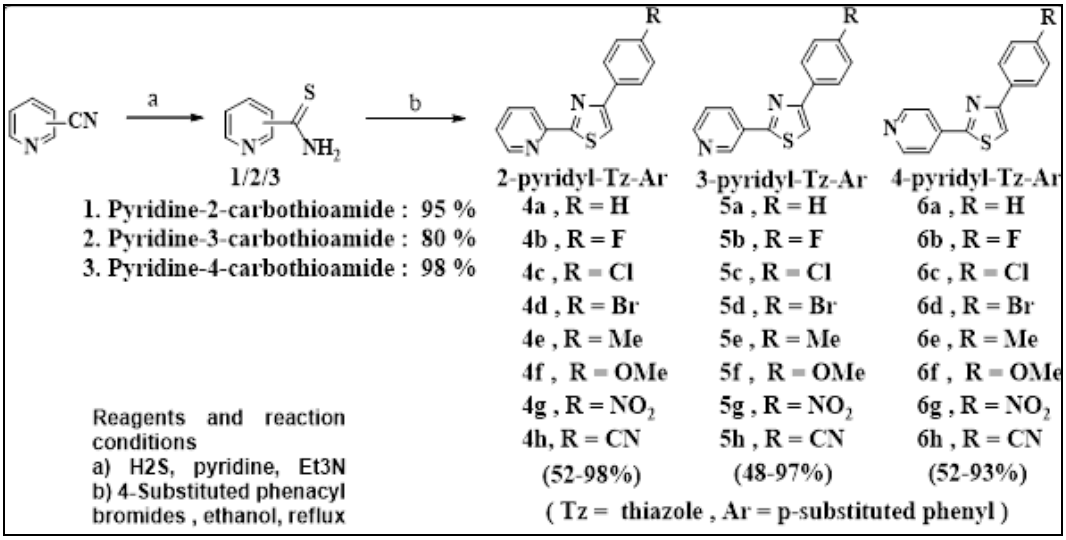

Fig 2: Synthesis of 2-pyridyl 4-phenyl-thiazoles

The lead structure can be split into three parts: the central thiazole, the 2-pyridyl/3-pyridyl and 4-pyridyl substituent at C-2, and the aryl substituent at C-2 (Scheme 1). The desired target molecules 2a-2g, 3a-3g and 4a-4g were synthesized via the classic Hantzsch thiazole synthesis.

Three new series of 2-pyridyl 4-phenyl-thiazole were synthesised by the reaction of thioamides and 4-substituted phenacyl bromides as shown in the Scheme I. Three series resulted to the formation of 24 derivatives 2a-h, 3a-h and 4a-h. The structures of the title compounds were confirmed by analytical ^{1}H NMR, ^{13}C NMR spectroscopy and HRMS.

The 1H NMR of compound 2b shows singlet at δ 7.53 for thiazole proton, while the other protons of phenyl ring and pyridine ring showed multiplet at δ 7.12-8.63. The 12 carbons of the 2b showed 12lines for 12 carbons at δ 114.86-164.12.The structure is further confirmed by HRMS 257.06(m/z). The 1HNMR of compound 2f shows singlet at 8.71δ for thiazole proton, while the other protons of phenyl ring and pyridine ring showed multiplet at δ 7.40-9.25. The 12 carbons of the 2f showed 12 lines at 116.57-166.00 and the carbon of methoxy group showed a line at 55.10 in the 13C NMR. The molecular ion peak is at 269(m/z) in the ESI-MS. The 1HNMR of compound 3d shows singlet at δ 8.27 for thiazole proton, while the other protons of phenyl ring and pyridine ring showed multiplet at δ7.60-9.51. The 12 carbons of the 3d showed 12 lines for 12 carbons at δ 114.86-164.12.The molecular ion peak is at 316 and M^{+2} peaks is at 318 for the molecular formula C14H9N2SBr. The 1HNMR of compound 3g shows singlet at δ 7.76 for thiazole proton, while the other protons of phenyl ring and pyridine ring showed multiplet at δ 7.43-9.26. The 12 carbons of the 3g showed 12 lines in the 13CNMR.The molecular ion peak is at 284 in the ESI-MS. The 4e compound is having singlet for the thiazole proton at δ7.52.The pyridine ortho protons are deshielded at δ8.70-8.72 showed doublet with coupling constant value 6.16Hz.The phenyl ring ortho protons appeared as doublet at δ7.86-7.88 and meta protons appeared as doublet at δ7.25-7.27 with coupling constant 8Hz.The 13CNMR clearly showed a line at δ21.33 for methyl carbon and the other 12 lines for the rest sp2 carbons at δ112-155. The ESI-MS showed line at 253.Similarly the compound 4f showed a singlet at δ7.45.The pyridine ortho protons deshielded and appeared as doublet at δ 8.70-8.72 with coupling constant 6.12Hz and the coupling partner meta protons appeared as doublet at δ7.87-7.88.The phenyl protons coupled with each other with value 8.84Hz and the doublet signal appeared at δ7.90-7.93 and δ 6.97-6.99. The 13CNMR clearly showed a line at δ 55.37 for methoxy carbon and the other 12 lines for the rest sp2 carbons at δ112.61-164. The HRMS showed molecular ion peak at 269.

4.2. Anti-bacterial Activity

4.2.1. Agar Well Diffusion

Agar well diffusion method was performed for studying the potential activities of these variants as shown in graph no.1. The antibacterial effects of test variants was evaluated by measuring the zone diameters and their results were compare with those of well-known drugs (standard). Amongst the tested variants, Cl substituted variant (2c, 3c and 4c) exhibited activity against both S. aureus and E. coli although; it was found to be greater against the test. On the other hand, F-variant (2b, 3b and 4b) exhibited more inhibitory activity against *E. coli* than those of other variants which showed intermediate inhibitory activity.

Despite the inhibition of bacteria by the test variants it was less when compared to standards.

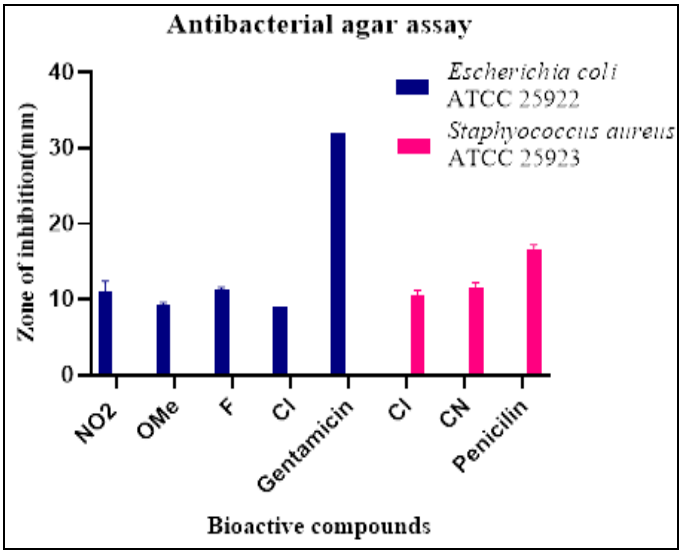

Fig 3: Antibacterial agar assay

Table 2: Anti-bacterial activity by agar well diffusion

4-pyridyl variants	Name of the bacteria			
	Escherichia coli ATCC 25922		Staphylococcus aureus ATCC 23923	
	Zone of inhibition (mm)	S/I/R	Zone of inhibition (mm)	S/I/R
4a (H)	0	R	0	R
4b (F)	11.25±0.35	R	0	R
4c (Cl)	9	R	10.5±0.7	R
4d (Br)	0	R	0	R
4e (Me)	0	R	0	R
4f (OMe)	9.25±0.35	R	0	R
4g (NO2)	11±1.41	R	0	R
4h (CN)	0	R	11.5±0.7	R
Standard	31.3±1.15	S	16.5±0.7	R

4.2.2. Broth Dilution

The investigation of antibacterial screening data revealed that the variant 4b(F) exhibited the highest inhibitory activity followed by (4g) NO2, (4f) OMe, (4h) CN and (4c)Cl while least activity was observed in (4d) Br and (4e) Me(Table 2). The MBC for most of the compounds were observed to be same as MIC. Although variant (4b)F showed its highest inhibition at MIC value ranging between 512-1024 µg/ml it was less when compared to the standard.

Table 3: Anti-bacterial activity using broth dilution

4-pyridyl variants	Escherichia coli ATCC 25922			Staphylococcus aureus ATCC 25923		
	MBC (µg/ml)	MIC (µg/ml)	SIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	SIC (µg/ml)
4a (H)	R	R	R	R	R	R
4b (F)	1024	512	256	>1024	1024	512
4c (Cl)	>1024	1024	512	>1024	1024	512
4d (Br)	R	R	R	R	R	R
4e (Me)	R	R	R	R	R	R
4f (OMe)	>1024	1024	512	>1024	1024	512
4g(NO_2)	>1024	1024	512	>1024	1024	512
4h (CN)	>1024	1024	512	>1024	1024	512
Standard antibiotic	8	<4	2	0.5	>0.25	0.125

4.2.3. Anti-Candidal Activity

4.2.3.1. Agar Well Diffusion

Amongst all the variants methoxy and fluoride containing variants exhibited promising anticandidal activity. Also it was found that the variant 4f (OMe) was found to be almost equally potent as the reference drug Itraconazole. Candida albicans was observed to be resistant for the rest variants. Derivative 3b (F) also showing similar effects as Itraconazole.

Photoplate No.3:

(A) Anticandidal activity of test compounds against Candida albicans

(B) Anti-Candidal activity of standard drug

(C) Anti-Candidal activity of Pyridyl Thiazole variants against Candida albicans

Table 4: Anticandidal activity using agar well diffusion

Derivative	Candida Albicans/Zone of inhibition (mm)
p-Fluoride substituent	
2b	8.5 ± 0.5
3b	15.34± 1.53
4b	8.67± 0.58
p-Methoxy substituent	
2f	10.34± 0.58
3f	9.17± 0.29
4f	8.5 ± 0.5
Standard	11.5 ± 0.5
Acetone	7
DMF	11.5 ± 0.71
Ethanol	8± 0.71

4.2.3.2. Broth Dilution

The MBC for most of the compounds were observed to be same as MIC. Although variant F showed its highest inhibition at MIC value ranging between 512-1024 µg/ml it was less when compared to the standard. And all variants of OMe and F showed Anti-Candidal activity (Table No.4). Amongst the variants 3b (F) was found to be most active against test organism. The variant 2f (OMe) and 3b (F) were found to be almost equally potent as the reference drugs Itraconazole.

Table 5: Table No.3: Anti-bacterial activity using broth dilution

Derivative	*Candida Albicans*		
	MBC (µg/ml)	MIC (µg/ml)	SIC (µg/ml)
p-Fluoride substituent			
2b	1024	512	256
3b	512	256	128
4b	1024	512	256
p-Methoxy substituent			
2f	4096	2048	1024
3f	2048	1024	512
4f	2048	1024	512
Standard antibiotic	2	>1	0.5
Itraconazole	2	>1	0.5

4.2.3.3. Inoculum Development and Growth Curve of Mycobacterium Smegmatis

Determination of growth curve requires inoculation of viable cells into broth medium and incubated at optimum conditions. Results show Candida albicans, lag phase was approximately for 10 hours. Log phase of Candida albicans was from 16 to 30 hours. From 16 to 20 hours the OD was 1 to 1.5 and number of cells was 1× 106 to 5 × 106. Thus, this time of incubation and number of cells.

4.3. Anti-Mycobacterial Assay

4.3.1. Broth Dilution

Amongst the variants 3f (OMe) was found to be most active against test organism. Fluoride containing derivatives has similar MIC concentration. None of the drug has MIC equal or less than reference drug.

Table 6: Anti-Mycobacterial Activity Using Broth Dilution

Derivative	Mycobacterium smegmatis NCIM 5138		
	MBC (µg/ml)	MIC (µg/ml)	SIC (µg/ml)
p-Fluoride substituent			
2b	1024	512	256
3b	1024	512	256
4b	1024	512	256
p-Methoxy substituent			
2f	2048	1024	512
3f	256	128	4
4f	2048	1024	512
Standard antibiotic	1024	512	256
Combutol	8	4	2

4.3.2. Agar Well Diffusion

The study of antimycobacterial screening revealed OMe was the highest amongst all the variants to exhibit inhibition, followed by Me and Br. Although they demonstrated the ability to inhibit the growth of test organism it was far less as compared to the standard.

Anti-Mycobacterial activity by agar well diffusion

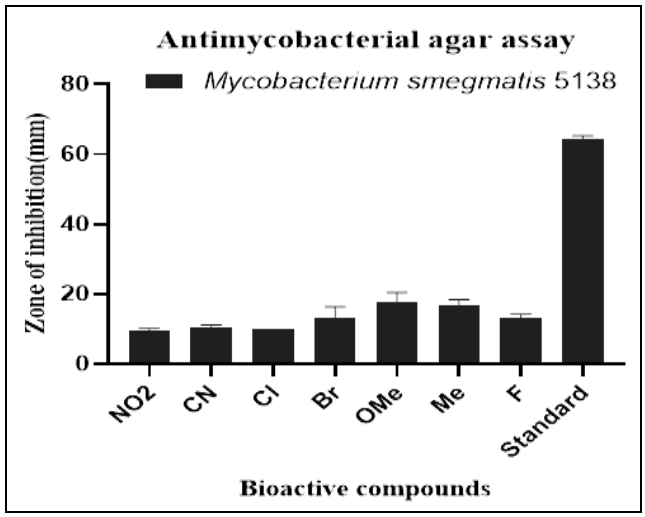

Fig 4: Antimycobacterial Agar Assay

Table 7: Antimycobacterial activity using agar assay

4-pyridyl variants	Mycobacterium smegmatis NCIM 5138	
	Zone of inhibition(mm)	S/I/R
4b (F)	13.25±0.75	R
4c (Cl)	10±0	R
4d (Br)	13.25±2.25	R
4e (Me)	17±1	R
4f (OMe)	17.6±2.1	R
4g (NO_2)	9.5±0.5	R
4h (CN)	10.5±0.5	R
Standard	64.25±0.75	S

Table 8: Anti-bacterial activity using broth dilution.

4-pyridyl variants	Mycobacterium smegmatis NCIM 5138					
	MBC (µg/ml)	MIC (µg/ml)	SIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	SIC (µg/ml)
4a (H)						
4b (F)	1024	512	256	>1024	1024	512
4c (Cl)	>1024	1024	512	>1024	1024	512
4d (Br)	R	R	R	R	R	R
4e (Me)	R	R	R	R	R	R
4f (OMe)	>1024	1024	512	>1024	1024	512
4g(NO2)	>1024	1024	512	>1024	1024	512
4h (CN)	>1024	1024	512	>1024	1024	512
Standard antibiotic	8	<4	2	0.5	>0.25	0.125

4.4. Determination of MIC and FIC index (FICI)

For Candida albicans only one combination i.e. 4PT-F (4b) + 4PT-OMe (4f) showing synergistic interaction and other 3 combinations are showing indifference interaction.

Table 9: Fractional Inhibitory Concentration Index (FICI) for combinations of Pyridyl Thiazole variants

Combinations (Drug 1+ Drug 2)	Candida albicans			
	FICdrug1	FICdrug2	FICI	Interaction
4b + 2f	0.5	0.125	0.625	IND
4b + 4f	0.25	0.125	0.375	SYN
2b + 2f	0.5	0.125	0.625	IND
2b + 4f	1	0.5	1.5	IND

(ANT: antagonism; SYN: synergism; IND: indifference)

Conclusion

Three series of isomeric pyridyl-thiazole derivatives were successfully synthesized by a simple method. Anti-bacterial, Anti-candidal and Anti-mycobacterial activity was checked using agar well diffusion and broth dilution method. Amongst the three isomeric series 4-pyridyl variants with (4b)F, (4c)Cl, (4f)OMe, (4g)NO_2 and (4h)CN was found to have moderate activities. Pyridyl-variants with fluorine and methoxy containing substituents was then further focussed for anticandidal and antimycobacterial activities. In general 4-position of the pyridine is more active than 2 and 3-position. Compound 4b and 4f can serve as lead molecules for further studies to ascertain the trend described in this work.

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