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Evaluation of Thiazole Derivatives for *M. tuberculosis* and *d*TDP-rhamnose Inhibitors

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Abstract: To determine antimycobacterium and dTDP rhamnose inhibitor activity of the synthesized azetidinone, thiazolidinone derivatives of thiazole, we studied different derivatives for the activity. One pot synthesis of 2-amino-4-methylthiazole-5-carboxylic acid ethyl ester has been carried out and synthesized different derivative compounds. Compounds were tested for antimicrobial activity against different strains of microorganism and antitubercular activity against *M. tuberculosis* H37Rv. Compounds 7c, 7d, 7i, 8d, 8e, 8g and 8h, were showed antimicrobial activity against S*taphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhosa* using Gentamycin as standard, while 7b, 7e, 7f, 7i, 8b, 8e, 8f and 8i showed very strong antimycobacterial activity using rifampicine as a standard. Thiazole derivatives especially 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.

Keywords: Thiazole, thiazolidinone derivatives, azetidinone derivative, well diffusion method, broth microdilution assay, antitubercular activity, antimicrobial activity.

INTRODUCTION

Microbial infections 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 [2, 14]. 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 *de novo* structural design has demonstrated that the thiazole derivatives especially with carbonyl group scaffold inhibit an enzyme *RmlC*, which is an essential component for the biosynthesis of *d*TDP-rhamnose [3]. While reports [7, 8] are available stating emergence of thiazole as potent antibacterial agent. β-Lactams are the most successful antimicrobials [1, 6, 9, 15]. Till recent days, unless microorganisms targeted, are not resistant. (By production of β-lactamase) Appreciation of these finding towards the development of novel antimicrobial agents, coupled with our program of drug design [12, 13]. It was thought to club together two or three nuclei having different sites or mechanism of action. This initiated us constructing compounds containing both the thiazole, azetidinone & thiazolidinone ring systems in the same matrix to serve as a new scaffold.

MATERIALS AND METHODS

The various chemicals used in the synthesis of the titled compounds were purchased from, sigma-aldrich pvt ltd, spectrochem pvt ltd and s.d. fine chem pvt ltd. The nucleus and its derivatives were analyzed by different ways. The melting points were recorded on electrothermal apparatus and are uncorrected. ^{1}H 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. Signal multiplicities were represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and bs (broad singlet). The purity of the compounds was checked on silica gel coated Al plates (Merck).

Synthesis of Ethyl-2-amino–4-methylthiazol–5-carboxylate [3]

Thiourea (1) (1 mmol, 15.2 g), ethylacetoacetate **(**2**)** (1 mmol, 26.24 mL) & N-bromo succinamide (1 mmol, 26.2 g) were mixed properly. Benzene (60 mL) was added and mixture was stirred for 5 min. A pinch of benzoyl peroxide was added to above mixture, which was then, refluxed for 5 h. The reaction mixture was cooled and benzene was decanted. The solid obtained was washed with benzene and again decanted. This solid, then was dissolved in water, neutralized with K_2CO_3 to yield white precipitate of ethyl-2-amino-4-methylthiazol-5-carboxylate (3). The solid thus separated was filtered, washed thoroughly with water and recrystallized from aq. ethanol.

Synthesis of Ethyl-2-substitutedamido-4-methylthiazol-5-carboxylate [4]

Ethyl-2-amino–4-methylthiazol–5-carboxylate (3) (1 mmol, 2 g) was taken in a 50 mL round bottom flask. Acetic anhydride or benzoyl chloride (1 mmol) was added to the above solution slowly with constant stirring. The mixture was then refluxed for 1 h. The solution was poured in ice-cold water with vigorous stirring, to yield precipitate. The suspension was then heated to boiling and cooled under tap water. The product was filtered, dried and recrystallized by ethanol to get (4);

Synthesis of 2-Substitutedamido-4-methylthiazol-5-carboxylic acid hydrazide [5]

Ethyl-2-substitutedamido-4-methylthiazol-5-carboxylate (4) (6 mmol) and 98% hydrazine hydrate (1.2 mmol) was taken in a round bottom flask and heated for five minutes. Ethanol was added to the above solution till the mixture becomes clear solution. Then the mixture was refluxed for 4 h on water bath maintaining the temperature between 70-75 ºC. The excess of alcohol was removed by distillation. On cooling, fine white precipitate of 2-substitutedamido-4-methylthiazol-5-carboxylic acid hydrazide (**5**) was obtained. The product was washed with water, filtered and dried and recrystallized from water.

Synthesis of N-[5-(arylidene-hydrazinocarbonyl)-4-methyl-thiazol-2-yl]-substituted-amide [6]

2-substitutedamido-4-methylthiazol-5-carboxylic acid hydrazide (5) (1 mmol) and aryl aldehyde (1 mmol) was taken in ethanol (15 mL), 3-4 drops of concentrated H_2SO_4 was added and refluxed for 2 h. The reaction mixture was cooled and the solid separated was filtered, washed with cold ethanol, and recrystallized from ethanol to obtained *N*-[5-(arylidene-hydrazinocarbonyl)-4-methyl-thiazol-2-yl]-substituted-amide (6a-j).

Synthesis of 2-Substituted-amino-4-methyl-thiazol-5-carboxalic acid (3-chloro-2-oxo-4-aryl-azetidin-1-yl)-amide [7]

N-[5-(arylidene-hydrazinocarbonyl)-4-methyl-thiazol-2-yl]-substituted-amide (**6**) (1 mmol) was dissolved in 25 mL 1, 4-dioxane. Triethylamine (1 mmol, 1 mL) was added drop wise with constant stirring to the solution, followed by similar addition of chloroacetyl chloride (1 mmol, 2 mL). The mixture was then stirred for 30 min followed by refluxing for 5 h. It was then cooled and filtered to remove insoluble salts. Excess of solvent was then distilled off. The concentrated solution was poured on crushed ice with vigorous stirring. The precipitate formed was filtered, washed with water and recrystallized by ethanol and water.

Synthesis of 2-Substituted-amino-4-methyl-thiazol-5-carboxalic acid (4-oxo-2-aryl-thiazolidin-3-yl)-amide [8]

N-[5-(arylidene-hydrazinocarbonyl)-4-methyl-thiazol-2-yl]-substituted-amide (6) (1 mmol) was dissolved in 20 mL anhydrous 1, 4-dioxane. Thioglycollic acid (1.5 mmol, 1.38 g) was added slowly drop wise with stirring. A pinch of aluminium chloride was added and the mixture was then stirred for 30 min followed by refluxing for 6 h. It was then cooled and filtered to remove insoluble salt. The concentrated solution was poured into aqueous saturated solution of $NaHCO_3$ with stirring. The precipitate formed was filtered, washed with water and recrystallized by alcohol and water.

Antitubercular activity

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) [5]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [4]. 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 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.

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 [10, 11], 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 hours. 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 hour (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 hours 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

During the preliminary screening 20 compounds 7a-j and 8a-j were tested (Table-1) at 6.25 µg/mL concentration for their antimycobacterial activity, eight compounds 7b, 7e, 7f, 7i, 8b, 8e, 8f and 8i 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 7i, 7e and 8i 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.

Scheme for synthesis of 2-amino-4-methylthiazole-5-carboxylic acid ethyl ester (3) and its derivatives (4-8).

Table-1: MIC and growth inhibition of the compounds against M. tuberculosis H37Rv

Comp	R	Ar	MIC (µg/mL^{-1})[a]	GI (%)[b]
7a	$NHCOCH_3$	$-C_6H_5$	<6.25	----
7b	$NHCOCH_3$	$-4-F-C_6H_4$	<6.25	100
7c	$NHCOCH_3$	$-3,4,5-CH_3O-C_6H_2$	<6.25	----
7d	$NHCOCH_3$	$-4-(CH_3)_2N-C_6H_4$	<6.25	----
7e	$NHCOCH_3$	$-2-F-C_6H_4$	<6.25	100
7f	$NHCOC_6H_5$	$-4-Cl-C_6H_4$	<6.25	96
7g	$NHCOC_6H_5$	$-3-NO_2-C_6H_4$	<6.25	----
7h	$NHCOC_6H_5$	$-4-OH-C_6H_4$	<6.25	----
7i	$NHCOC_6H_5$	$-2-Cl-C_6H_4$	<6.25	98
7j	$NHCOC_6H_5$	$-2-NO_2-C_6H_4$	<6.25	----
8a	$NHCOCH_3$	$-C_6H_5$	<6.25	----
8b	$NHCOCH_3$	$-4-F-C_6H_4$	<6.25	98
8c	$NHCOCH_3$	$-3,4,5-CH_3O-C_6H_2$	<6.25	----
8d	$NHCOCH_3$	$-4-(CH_3)_2N-C_6H_4$	<6.25	----
8e	$NHCOCH_3$	$-2-F-C_6H_4$	<6.25	100
8f	$NHCOC_6H_5$	$-4-Cl-C_6H_4$	<6.25	97
8g	$NHCOC_6H_5$	$-3-NO_2-C_6H_4$	<6.25	----
8h	$NHCOC_6H_5$	$-4-OH-C_6H_4$	<6.25	----
8i	$NHCOC_6H_5$	$-2-Cl-C_6H_4$	<6.25	97
8j	$NHCOC_6H_5$	$-2-NO_2-C_6H_4$	<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.

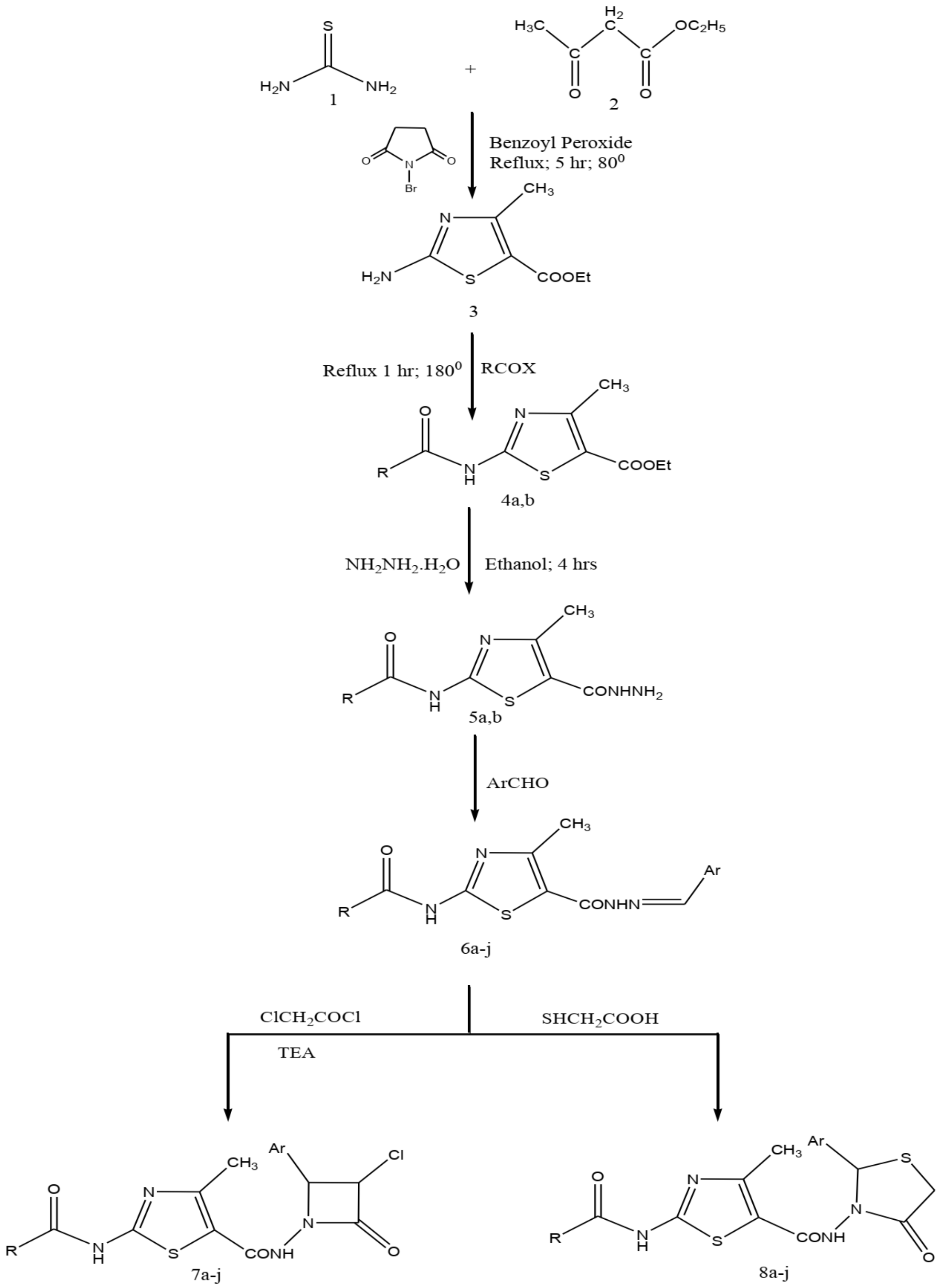

Table-2: Second level Actual minimum inhibitory concentration of the compounds

SN	MIC (μM)[a]	SN	MIC (μM)[a]
7b	6.25	8b	3.13
7e	1.56	8e	6.25
7f	3.13	8f	6.25
7i	0.39	8i	0.78

[a]Actual minimum inhibitory concentration (MABA assay)

Antimicrobial activity

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 7i, 8i and 8e whereas 7c, 7d, 8d, 8f, 8g and 8h have shown moderate activity and others showed least activity (Table-3).

Table-3: Antibacterial activity of the synthesized compounds

Comp.	Organisms				Comp.	Organisms			
	Sa	Pa	Ec	St		Sa	Pa	Ec	St
7a	18	17	14	12	8a	18	16	10	12
7b	18	16	15	14	8b	20	16	10	10
7c	22	20	18	14	8c	22	22	20	16
7d	25	22	20	16	8d	26	24	22	18
7e	20	20	18	14	8e	36	34	30	30
7f	16	16	20	16	8f	24	22	20	18
7g	16	10	10	18	8g	22	11	24	24
7h	11	17	15	22	8h	22	22	20	20
7i	32	32	30	36	8i	36	38	32	32
7j	16	16	12	12	8j	16	16	12	14
Gent	34	35	31	30	Gent	34	35	31	30

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

DISCUSSION

2-Amino-4-methyl-thiazole-5-carboxylic acid ethyl ester (3) was synthesized by cyclization of 2-bromo-ethylacetoacetate with thiourea. The internal bromination of ethylacetoacetate was achieved by treatment with N-bromosuccinamide. Free radical reaction generated by benzoyl peroxide was used initiator. The reactive amino group was then protected, either by acetylation or benzoylation process to yield 2-substituted-amino-4-methyl-thiazole-5-carboxylic acid ethyl ester (4a,b). Chemical transformation of compound (4a,b) to hydrazide derivative (5a,b) was achieved. The free amino group of hydrazide was condensed to schiff's base (6a-j), by reacting with arylaldehydes in ethanol, which on treatment with mercaptoacetic acid and chloroacetyl chloride gave thiazolidinone (8a-j) and azetidinone (7a-j) derivatives respectively. Compounds 7c, 7d, 7i, 8d, 8e, 8g and 8h have shown antimicrobial activity against microorganism, while 7b, 7e, 7f, 7i, 8b, 8e, 8f and 8i have shown very strong antimycobacterial activity. 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 and all four enzymes therefore represent potential therapeutic targets. dTDP-D-glucose 4,6-dehydratase (RmlB; EC 4.2.1.46) is the second enzyme in the dTDP-L-rhamnose biosynthetic pathway. The immediate source of rhamnose in carbohydrate polymers is dTDP-L-rhamnose. The other enantiomer, D-rhamnose, is primarily utilised by *Pseudomonas aeruginosa.* A *de novo* structural design has demonstrated that the thiazole derivatives especially with carbonyl group scaffold inhibit an enzyme *RmlC*, which is an essential component for the biosynthesis of *d*TDP-rhamnose. While, reports are available stating emergence of thiazole as potent antibacterial agent. The structure has been refined to a crystallographic R-factor of 20.4% and an R-free value of 24.9% with good stereochemistry. (By production of β-lactamase) Appreciation of these finding towards the development of novel antimicrobial agents, coupled with our program of drug design, it was thought to club 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.

CONCLUSION

Thiazole derivatives especially 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.

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