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# Design, synthesis and antibacterial activity of novel 1, 3-thiazolidine purine nucleosides

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## ABSTRACT

*We have described a facile synthesis of novel 1,3-thiazolidine purine nucleosides. All these analogues are derived from the key intermediate N-tert-butoxycarbonyl-1,3-thiazolidine-2-ol, which was obtained from L-cysteine methylester hydrochloride. The tetrabutylammonium fluoride (TBAF) induced coupling of the 1,3-thiazolidine moiety with suitably protected purines afforded highly regioselective N-9 substituted purine nucleosides. All the newly synthesized products were characterized by $^1$H NMR, ES-MS and elemental analyses. Their antibacterial activity is reported.*

**Key words**: Modified nucleosides, 1,3-thiazolidines, purines, TBAF, antibacterial activity.

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## INTRODUCTION

With the advent of new modified nucleosides, scientists from all over the world have invested tremendous efforts towards the synthesis of purine libraries which will serve as a pathway for both drug leads and probes for analyzing complex cellular processes [1, 2]. Branched chain sugars and particularly branched chain nucleosides have received much attention as potential chemotherapeutic agent as well as in antisense oligonucleotide research. Some of these analogues have been shown to display a very interesting spectrum of antiviral and anticancer activities [3]. The purine nucleosides have established their importance in curbing human immunodeficiency virus (HIV) infection, by interfering in different steps of HIV life cycle (reverse transcriptase and protease inhibitors). Good results were obtained by using a combination of agents and by adopting this strategy, considerable delay in progression of the disease and prolonged survival in patients with advanced HIV [4] have been observed.

In the last two decades, modified nucleosides (AZT, ddC, etc) have shown very good antiviral properties and have become a major area of research in medicinal chemistry. The structural modifications of natural derivatives have enrouted to important synthetic strategy for the preparation of new bioactive nucleoside analogues [5]. Extensive modifications have been

performed on both the heterocyclic base as well as on the sugar moiety [6]. In this context, nucleoside analogues in which the furanose has been replaced by acyclic [7, 8], carbocyclic [9] and heterocyclic moieties [10, 11] have attracted special interest by virtue of their biological action as potent antiviral and anticancer agents.

The typical procedure adopted for the synthesis of N-9 alkylated purines is by employing Mitsunobu condition with a variety of alcohols [12] or by using strong basic conditions with a variety of alkyl halides or benzyl halides [13]. These reactions suffer from drawbacks like long reaction time, low temperature for a Mitsunobu condition or elevated temperature for the basic conditions and an inert atmosphere due to the sensitivity of the reagents in order to offer high yielding and highly regioselective N-9 alkylated isomer.

Considerable evidences have been accumulated to demonstrate the efficacy of 1,3-thiazolidines as anticancer, antithyroid, antiinflammatory, cardiovascular and antiviral agents. Due to their various biological activities, they can be used as powerful building blocks for the heterocyclic combinatorial rows and structure modeling of the potential biological active compounds [14]. Significantly to the best of our knowledge and extensive literature search, we are the first to introduce 1,3-thiazolidine ring into purine bases at N-9 position which will serve as a platform in the drug discovery as both drug leads and probes for analyzing complex cellular activities. Herein we have reported a simple, efficient and mild approach for coupling of protected purine bases with 1,3-thiazolidine ring by employing a suitable base, tetrabutylammonium fluoride (TBAF) at room temperature, which has resulted in high yield and highly regioselective alkylation at N-9 position.

## MATERIALS AND METHODS

The $^1$H NMR spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-$d_6$ as a solvent and TMS as internal standard (chemical shift δ in ppm). All the solvents and reagents used were of AR grade and used as such without purification. Melting points were determined using Thomas Hoover melting point apparatus and are uncorrected. The IR spectra ($\nu_{max}$) were recorded on Perkin Elmer FT-IR spectrophotometer using KBr pellet method. Elemental (C, H, N) analyses were obtained on Vario EL III Elementar. Silica gel column chromatography was performed using Merck silica gel (100-200 mesh) and Merck made TLC plates were used for reaction monitoring.

**9-(N-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-yl)-2,6-dichloropurine**(**7**): To a stirred solution of compound **6** (0.50g, 2.64 mmol) and compound **5** (1.37g, 2.64 mmol) in 10 ml of anhydrous THF, 1.0 M TBAF solution in THF (5.3 ml, 5.28 mmol) was added dropwise at 0˚C under $N_2$ atmosphere. The reaction mixture was stirred at rt for 2h. Upon completion of the reaction, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc) to give compound **7** as a white solid (1.20g, 70%).

**9-(4-Hydroxymethyl-1,3-thiazolidine-2-yl)-2,6-dichloropurine** (**8**): To a stirred solution of compound **7** (0.50g, 0.77 mmol) in 10 ml of anhydrous dichloromethane, 4ml of trifluoroacetic acid was added dropwise at 0˚C. The reaction mixture was stirred at rt until TLC showed no starting material. A few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to obtain compound **8** as a white solid (0.19g, 80%).

M. P: 150-152˚C: IR (KBr): 3302, 3155, 1683 $cm^{-1}$: $^1H$ NMR (DMSO-$d_6$) (ppm): δ 3.85-3.86 (m, 1H), 3.86-3.87 (m, 1H), 3.87-3.88 (m, 2H), 4.00-4.13 (m, 2H), 4.15-4.17 (m, 1H), 4.83 (s, 1H), 8.00 (s, 1H, 8-CH): ES-MS (m/z): 306 (M+): Anal. Calcd. For $C_9H_9Cl_2N_5OS$: C, 35.31; H, 2.96; N, 22.87; Found: C, 35.26; H, 2.90; N, 22.81.

**9-((4-Hydroxymethyl-1,3-thiazolidine-2-yl)-2-chloro-6-hydroxy)purine** (**10**): To a stirred solution of compound **7** (0.50g, 0.77 mmol) in 15ml of methanol, NaOH (0.06g, 1.54 mmol) was added and stirred at rt for 12h. After completion of the reaction, the reaction mixture was evaporated under reduced pressure, cooled and 10 ml of water was added followed by 1N HCl solution dropwise at 0˚C, till the solution was slightly acidic. The residue obtained was filtered, washed with 20 ml of ice cold water and dissolved in 20 ml of EtOAc. The organic layer was washed with 20 ml of water, 10 ml of brine solution, dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The crude product was purified by column chromatography (hexane/EtOAc) to afford compound **9** as a white solid (0.43g, 90%).

To a stirred suspension of compound **9** (0.43g, 0.69 mmol) in 10 ml of dichloromethane, 4 ml of trifluoroacetic acid was added dropwise at 0˚C. The reaction mixture was stirred at rt until TLC showed no starting material. A few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) resulted in compound **10** as a white solid (0.16g, 85%).

M.P: 180-182˚C: IR (KBr): 3302, 3255, 1693 $cm^{-1}$: $^1H$ NMR (DMSO-$d_6$) (ppm): δ 3.84-3.85 (m, 1H), 3.86-3.88 (m, 1H), 3.88-4.04 (m, 1H), 4.08-4.14 (m, 1H), 4.83 (s, 4H), 5.90 (s, 1H, 8-CH), 8.01 (s, 1H, 1-NH): ES-MS (m/z): 288 (M+): Anal. Calcd. For $C_9H_{10}ClN_5O_2S$: C, 37.57; H, 3.50; N, 24.34: Found: C, 37. 51; H, 3.46; N, 24.31.

**9-(4-Hydroxymethyl-1,3-thiazolidine-2-yl)-2,6-dihydroxypurine (12)**: To a stirred solution of compound **7** (0.50g, 0.77 mmol) in 15ml of methanol, NaOH (0.16g, 3.85 mmol) was added and refluxed to 60˚C for 8h. The reaction mixture was evaporated under reduced pressure, cooled and 10ml of water was added followed by 1N HCl solution dropwise at 0˚C, till the solution was slightly acidic. The residue obtained was filtered, washed with 20ml of ice cold water and dissolved in 20ml of EtOAc. The organic layer was washed with 20ml of water, 10ml of brine solution, dried over anhydrous $Na_2SO_4$ and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to give compound **11** as a white solid (0.42g, 90%).

To a stirred solution of compound **11** (0.42g, 0.69 mmol) in 10 ml of dichloromethane, 4ml of trifluoroacetic acid was added dropwise at 0˚C. After completion of the reaction, a few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to give compound **12** as a white solid (0.13g, 70%).

M.P: >250˚C: IR (KBr): 3312, 3255, 1690 $cm^{-1}$: $^1H$ NMR (DMSO-$d_6$) (ppm): δ 3.84-3.85 (m, 1H), 3.87-3.88 (m, 1H), 3.90-4.04 (m, 1H), 4.19-4.30 (m, 1H), 4.33-4.35 (m, 1H), 4.43-4.45 (m, 2 H), 5.15 (s, 1H), 8.01 (s, 1H, 8-CH), 8.10 (s, 1H, 1-NH), 8.10 (s, 1H, 3-NH): ES-MS (m/z): 269 (M+): Anal. Calcd. For $C_9H_{11}N_5O_3S$: C, 40.14; H, 4.12; N, 26.01: Found: C, 40.11; H, 4.09; N, 26.00.

**9-(N-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-yl)-6-chloropurine** (**14**): To a stirred solution of compound **13** (0.50g, 3.23 mmol) and compound **5** (1.68g, 3.23 mmol) in 10 ml of anhydrous THF, 1.0 M TBAF solution in THF (6.5ml, 6.46 mmol) was added dropwise at 0˚C under $N_2$ atmosphere. The reaction mixture was stirred at rt for 2h. After completion of the reaction, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc) to give compound **14** as a white solid (1.58g, 80%).

**9-(4-Hydroxymethyl-1,3-thiazolidine-2-yl)-6-chloropurine (15)**: To a stirred solution of compound **14** (0.50 g, 0.81 mmol) in 10 ml of anhydrous dichloromethane, 4ml of trifluoroacetic acid was added dropwise at 0˚C. The reaction mixture was stirred at rt until TLC showed no starting material. A few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to give compound **15** as a white solid (0.19g, 90%).

M.P: 153-155˚C: IR (KBr): 3202, 3100, 1690 $cm^{-1}$: $^1$H NMR (DMSO-$d_6$) (ppm): δ 3.84-3.86 (m, 1H), 3.86-3.88 (m, 1H), 3.89-4.00 (m, 2H), 4.00-4.13 (m, 2H), 4.15-4.17 (m, 1H), 4.83 (s, 1H), 8.00 (s, 1H, 8-CH) 8.12 (s, 1H, 2-CH): ES-MS (m/z): 272 (M+): Anal. Calcd. For $C_9H_{10}ClN_5OS$: C, 39.78; H, 3.71; N, 25.77: Found: C, 39.78; H, 3.66; N, 25.72.

**9-(N-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-yl)-6-hydroxypurine(16)**: To a stirred solution of compound **14** (0.50 g, 0.81 mmol) in 15ml of methanol, NaOH (0.067g, 1.62 mmol) was added and stirred at rt for 12h. The reaction mixture was evaporated under reduced pressure, cooled and 10 ml of water was added followed by 1N HCl solution dropwise at 0˚C until the solution was slightly acidic. The residue obtained was filtered, and washed with 20 ml of ice cold water and dissolved in 20ml of EtOAc. The organic layer was washed with 20 ml of water followed by10 ml of brine solution, dried over anhydrous $Na_2SO_4$ and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to give compound **16** as a white solid (0.43g, 90%).

**9-(4-Hydroxymethyl-1,3-thiazolidine-2-yl)-6-hydroxypurine (17)**: To a stirred solution of compound **16** (0.43 g, 0.73 mmol) in 5 ml of anhydrous dichloromethane, 4ml of trifluoroacetic acid was added dropwise at 0˚C. After completion of the reaction, a few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to give compound **17** as a white solid (0.14g, 80%).

M.P: 170-172˚C: IR (KBr): 3222, 3110, 1690 $cm^{-1}$: $^1$H NMR (DMSO-$d_6$) (ppm): δ 3.84-3.86 (m, 1H), 3.86-3.88 (m, 1H), 3.89-4.00 (m, 2H), 4.00-4.13 (m, 2H), 4.15-4.17 (m, 1H), 4.83 (s, 1H), 8.00 (s, 1H, 8-CH) 8.12 (s, 1H, 2-CH), 8.15(s, 1H, 1-NH): ES-MS (m/z): 253 (M+): Anal. Calcd. For $C_9H_{11}N_5O_2S$: C, 42.68; H, 4.38; N, 27.65: Found: C, 42.61; H, 4.36; N, 27.60.

**N,N-di-tert-butoxycarbonyl-6-amino-9H-purine (19)**: To a stirred solution of compound **18** (1g, 7.4mmol) in 20 ml of dry THF, $(BOC)_2O$ (5ml, 22.2 mmol) and a pinch of DMAP were added. The reaction mixture was stirred at rt for 24h. Upon completion of the reaction, 10 ml of $NaHCO_3$ solution and 15 ml of methanol were added. The reaction mixture was heated to 50˚C for 1h, cooled and poured into 50 ml of ice cold water and stirred for 30mins. The precipitate was filtered to obtain compound **19** as a white solid (1.73g, 70%).

**9-((N-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-yl)-N,N-di-tert-butoxycarbonyl-6-amino)purine (20)**: To a stirred solution of 19 (1.73g, 5.15 mmol) and compound **5** (2.68g, 5.15mmol) in 10 ml of anhydrous THF, 1.0 M TBAF solution in THF

(10.3ml, 10.3 mmol) was added dropwise at 0˚C under $N_2$ atmosphere. The reaction mixture was stirred at rt for 2h. Upon completion of the reaction, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc) to give compound **20** as a white solid (3.28g, 80%).

**9-(4-Hydroxymethyl-1,3-thiazolidine-2-yl)-6-aminopurine (21)**: To a stirred solution of compound **20** (0.5g, 0.62 mmol) in 5 ml of anhydrous dichloromethane, 4ml of trifluoroacetic acid was added dropwise at 0˚C. After completion of the reaction, a few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to afford compound **21** as a white solid (0.11g, 70%).

M.P: 190-192˚C; IR (KBr): 3222, 3110, 1700 $cm^{-1}$: $^1H$ NMR (DMSO-$d_6$) (ppm): δ 3.85-3.86 (m, 1H), 3.86-3.88 (m, 1H), 3.89-4.00 (m, 2H), 4.10-4.14 (m, 2H), 4.15-4.19 (m, 1H), 5.23 (s, 1H), 8.00 (s, 1H, 8-CH) 8.12 (s, 1H, 2-CH), 9.00 (bs, 2H, 6-$NH_2$): ES-MS (m/z): 253 (M+): Anal. Calcd. For $C_9H_{12}N_6OS$: C, 42.85; H, 4.79; N, 33.31: Found: C, 42.78; H, 4.74; N, 33.26.

**2-Acetamido-6-(N,N-diphenylcarbamoyloxy)-9H-purine (23)**: To a stirred solution of compound **22** (1g, 6.62 mmol) in 8ml of N,N-dimethylacetamide, acetic anhydride (2ml, 21.16 mmol) was added and refluxed to 160˚C under nitrogen atmosphere. After completion of the reaction, the mass was cooled and the white solid obtained was filtered and washed with EtOH to obtain 2, 9-diacetyl guanine (1.4g, 90%).

To the stirred suspension of 2, 9-diacetyl guanine (1g, 4.25 mmol) in 20 ml of pyridine, 1.5ml of N, N-diisopropyl ethylamine (DIPEA) and diphenylcarbamoyl chloride (1.08g, 4.69 mmol) were added. The mixture was stirred at rt for 4h, 5ml of water was added and stirred at rt for 10 mins. The solvents were removed under reduced pressure and the obtained residue was placed in a solution of EtOH/$H_2O$ (1:1 v/v) and heated to 60˚C for 3h. The suspension was cooled and the obtained solid was filtered to obtain compound **23** as a white solid (1.15g, 70%).

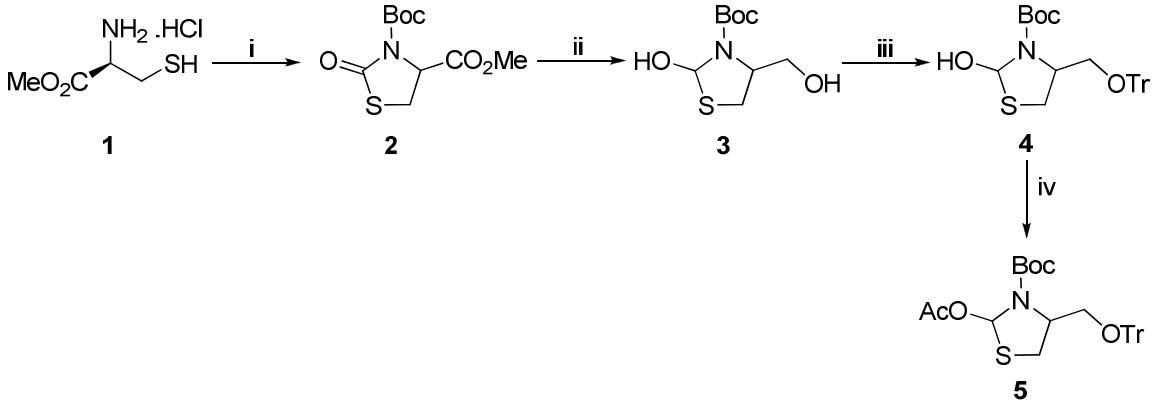
**9-((N-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-yl)-2-acetamido-6-(N,N-diphenylcarbamoyloxy))purine (24)**: To a stirred solution of compound **23** (1g, 2.57 mmol) and compound **5** (1.33g, 2.57mmol) in 10 ml of anhydrous THF, 1.0 M TBAF solution in THF (5.2ml, 5.14mmol) were added dropwise at 0˚C under $N_2$ atmosphere. The reaction mixture was stirred at rt for 2h. After completion of the reaction, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc) to give compound **24** as a white solid (1.31g, 60%).

**9-(4-Hydroxymethyl-1,3-thiazolidine-2-yl)-2-amino-6-hydroxypurine (26)**: To the stirred solution of compound **24** (1g, 1.18 mmol) in 20ml of methanol, ammonia gas was purged at 0˚C for 30 mins. The reaction mixture was warmed to 60˚C for 6h, cooled and evaporated to dryness which gave compound **25**. To the stirred solution of compound **25** in 10ml of dichloromethane, 4ml of trifluoroacetic acid was added and stirred at rt. After completion of the reaction, a few drops of $Et_3N$ were added and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography (hexane/EtOAc) to afford compound **26** as a white solid (0.28g, 40%).

M.P: 210-212˚C: IR (KBr): 3225, 3120, 1700 $cm^{-1}$: $^1H$ NMR (DMSO-$d_6$) (ppm): δ 3.85-3.86 (m, 1H), 3.86-3.88 (m, 1H), 3.89-4.00 (m, 2H), 4.10-4.12 (m, 2H), 4.17-4.19 (m, 1H), 5.28 (s, 1H), 8.00 (s, 1H, 8-CH) 8.12 (s, 1H, 1-NH), 6.00 (bs, 2H, 2-$NH_2$): ES-MS m/z: 268 (M+): Anal. Calcd. For $C_9H_{12}N_6O_2S$: C, 40.29; H, 4.51; N, 31.32: Found: C, 40.24; H, 4.49; N, 31.27.

## RESULTS AND DISCUSSION

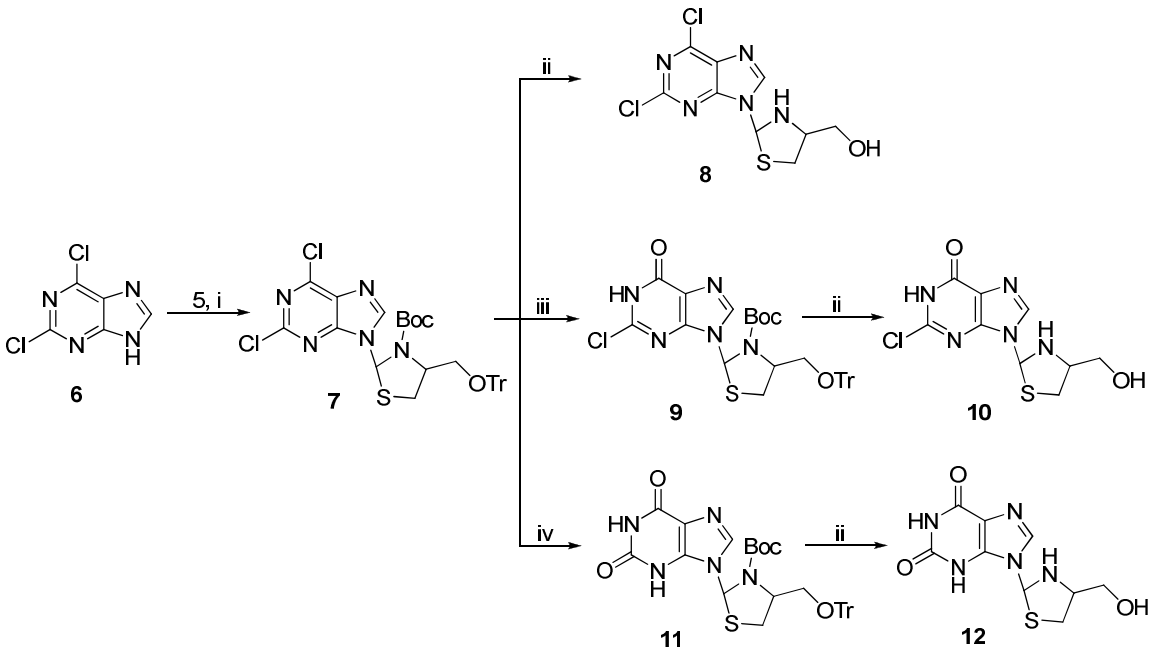
High yielding and highly regioselective alkylation of purines base at N-9 is obtained by utilizing a suitable purine precursor in their protected form and employing proper reaction conditions. Substitution of organic halides at N-7 or N-9 is confirmed from the $^{1}H$ & $^{13}C$ NMR. The $^{1}H$ NMR signal of H-8 for the N-9 isomer is shifted upfield relative to the corresponding H-8 signal for the N-7 isomer. The $^{13}C$ NMR signals of C-8 and C-1 for the N-9 isomer are shifted upfield relative to the corresponding signals of the N-7 isomer. On the contrary, the signals of C-5 are deshielded relative to those of the N-7 alkylated compound, but the difference in shift is most pronounced for the C-5 signal. The formation of the regioisomer can also be easily differentiated from $^{15}N$ NMR [15].



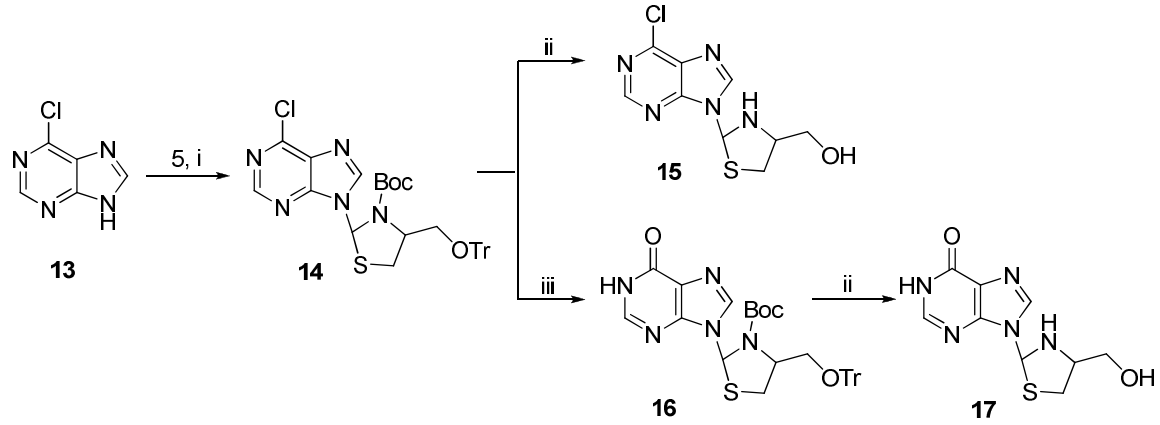

**Scheme-1: Reagents and conditions: (i): $(Boc)_2O$, DMAP, $(Et)_3N$, rt; (ii): $Li(Et)_3BH$, THF, 0˚C; (iii): Trityl chloride, pyridine, reflux; (iv): $(CH_3CO)_2O$, DMAP, $(Et)_3N$, rt.**

Our synthetic strategy was based on the preparation of N-tert-butoxycarbonyl-4-methoxy carbonyl -1,3-thiazolidine-2-one (**2**), followed by the reduction and acylation gave N-tert-butoxycarbonyl-2-acetoxy-4-trityloxymethyl-1,3-thiazolidine (**5**). The compound **2** was prepared by treating $(BOC)_2O$, with L-Cysteine methyl ester hydrochloride (**1**), in presence of DMAP and TEA [16]. The ester and the thiolactam functional groups were reduced to corresponding alcohols at one pot using lithium triethylborohydride $Li(Et)_3BH$ at 0˚C to yield N-tert-butoxycarbonyl-4-hydroxymethyl-1,3-thiazolidine-2-ol (**3**). The protection of primary alcoholic group in **3** was achieved using trityl chloride and pyridine as a solvent to give N-tert-butoxycarbonyl-4-trityloxymethyl-1,3-thiazolidine-2-ol (**4**). The acylation of compound **4** using acetic anhydride in presence of DMAP and triethyl amine afforded N-tert-butoxycarbonyl-2-acetoxy-4-trityloxymethyl-1,3-thiazolidine (**5**) (scheme-1).

The suitable precursors for compounds **10**, **12** (scheme-2) and **17** (scheme-3) were their respective chloro substituted compounds because of their greater solubility in non-polar solvents and the chloro groups were easily transformed to OH groups by treating with NaOH in methanol. The OH protecting diphenyl carbamoyl and N-acetyl groups were simultaneously removed by heating with $NH_3$ in methanol at 60˚C. The NH-protecting (Boc) and the primary aliphatic OH-protecting (trityl) groups were easily removed by treating the requisite compound with TFA in dichloromethane.

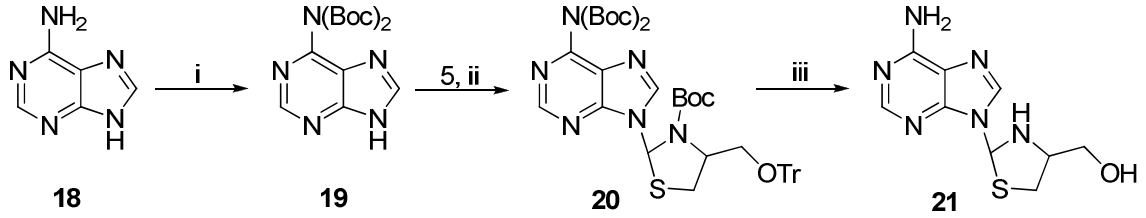


**Scheme-2: Reagents and conditions: (i): TBAF, THF, rt; (ii): TFA, $CH_2Cl_2$, rt, 2h; (iii): NaOH/MeOH; rt; (iv): NaOH/MeOH, 60˚C.**



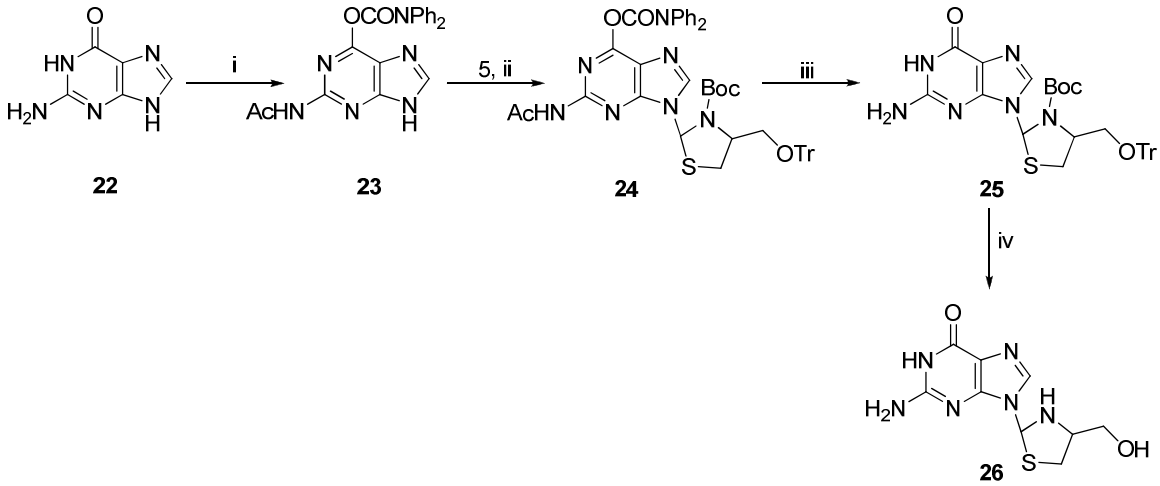
**Scheme-3: Reagents and conditions: (i): TBAF, THF, rt; (ii): TFA, $CH_2Cl_2$, rt, 2h; (iii) NaOH/MeOH, rt.**

Coupling of compound **5** with protected purine bases in presence of tetrabutyl ammonium fluoride (TBAF) followed by deprotection resulted in highly regioselective N-9 alkylated isomer of modified nucleoside analogues. The compound **18** was treated with $(BOC)_2O$ to obtain compound **19**, which served as a suitable precursor [17] to react with compound **5**, followed by deprotection afforded highly regioselective N-9 isomer of modified nucleoside analogue **21** (scheme-4). The requisite precursor compound **19** was synthesized from compound **18** in one pot tris-Boc protection and subsequent selective cleavage of one of the Boc groups with basic methanol at elevated temperature. The reaction was monitored closely as removal of the second Boc group slowly occurred under these conditions. The compound **18** was not a good precursor to start with because of their notoriously low solubility in most of the organic solvents [18] and due to the formation of regioisomers N-9 and N-7 in the ratio 1:1[19-21] under usual conditions.




**Scheme-4: Reagents and conditions: (i): (a) $(Boc)_2O$, cat. 4-DMAP, THF, rt, 10h; (b) saturated $NaHCO_3$, MeOH, 50°C, 1h; (ii): TBAF, THF, rt; (iii): TFA, $CH_2Cl_2$, rt, 2h.**

In continuation of our effort to search for the suitable guanine precursor, we ended up with 2-N-acetyl-6-O-diphenyl carbamoyl guanine (**23**), which was very much successful in affording highly regioselective coupling with acetylated pentafuranose and α-halo esters [22]. We were delighted to use the same substrate for coupling with modified sugar moiety **5** because of its high regioselectivity at N-9 and high yield, by treating them with TBAF at room temperature to give compound **26** (scheme-5).




**Scheme-5: Reagents and conditions: (i): $(Ac)_2O$, $Ph_2NCOCl$, pyridine; (ii): TBAF, THF, rt; (iii): $NH_3$, MeOH; (iv): TFA, $CH_2Cl_2$, rt, 2h.**

We carried out the coupling reaction of nucleobases with compound **5** by varying the solvent polarity from high polar aprotic solvent to low polar aprotic solvents and ended up with good result for THF. Apparently THF has favoured the thermodynamic product N-9 isomer over the N-7 isomer in presence of TBAF at room temperature as described previously for the synthesis of guanosine [23].

## Antibacterial Studies

The synthesized compounds were dissolved to prepare a stock solution of 1mg/ml using N, N-dimethyl formamide (DMF). All the chemical compounds were tested for antibacterial activity against human and phytopathogenic Gram-positive bacteria (*Staphylococcus aureus*; MTCC 7443, *Staphylococcus epidermidis*; MTCC 435, *Bacillus subtilis*; MTCC 121) and Gram-negative (*E.coli*; MTCC 7410, *Salmonella typhi*; MTCC 733, *Xanthomonas campestris*; MTCC 7908) and gentamicin (1mg/ml) was used as positive control. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately $1.5x10^8$ CFU/ml. 20 ml of nutrient agar media was poured into each petri plate allowed to solidify under aseptic condition and plates were swabbed with 100µl inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 6 mm

diameter, wells were bored into the seeded agar plates and these were loaded with a 50 µl volume with concentration of 1.0 mg/ml of each compound reconstituted in the N, N-dimethylformamide (DMF). All the plates were incubated at 37°C for 24 h. Antimicrobial activity of all the synthesized compounds was evaluated by measuring the zone of inhibition against the test organisms. The medium with DMF as solvent was used as a negative control whereas media with gentamicin (standard antibiotic) used as positive control. The experiments were performed in triplicates [24].

**Determination of minimum inhibitory concentration**

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001). All tests were performed in a Mueller-Hinton broth for the bacterial strains. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to $2\times10^6$ CFU/ ml. Compounds were dissolved in 10% dimethylformamide (DMF) and then diluted to the highest concentration. Two-fold serial concentrations of the compounds were prepared (over the range 1000–0.19 µg/ml) in a 96-well microtiter plate. In the tests, triphenyltetrazolium chloride (TTC) (Aldrich Chemical Company Inc., USA) was also added to the culture medium as a growth indicator. The final concentration of TTC after inoculation was 0.05%. The microbial growth was determined by the absorbance at 600 nm using a universal microplate reader after incubation at 37°C for 24 h. The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth [25].

**Table-1: *In-vitro* antibacterial activity data (Minimum inhibitory concentration (MIC) in µg/ml)**

| Zone of inhibition in mm (MIC in mg/mL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Compound No.** | **5** | **8** | **10** | **12** | **15** | **17** | **21** | **26** | **Gentamicin (1mg/ml)** | **Streptocycline (1mg/ml)** |
| *Staphylococcus epidermidis* | 20(25) | 23(25) | 21(25) | 17(25) | 20(25) | 18(25) | 15(25) | 14(25) | 30(4) | - |
| *Staphylococcus aureus* | - | 19(12.5) | - | 21(50) | 13(25) | 20(25) | - | 19(25) | 30(2) | - |
| *Bacillus subtilis* | - | 15(12.5) | - | 15(12.5) | 22(12.5) | 17(12.5) | 13(25) | 16(25) | 35(2) | - |
| *Xanthomonas campestris* | - | 29(12.5) | - | 14(12.5) | - | - | 19(25) | - | - | 31(4) |
| *Pseudomonas aeruginosa* | - | 26(12.5) | - | - | 20(12.5) | 23(12.5) | - | 21(12.5) | 24(8) | - |
| *Salmonella typhi* | - | 23(25) | - | - | 11(50) | 20(50) | - | 19(50) | 35(4) | - |
| *E.coli* | - | 14(25) | - | - | - | - | - | - | 30(4) | - |

## CONCLUSION

In conclusion, we have reported a simple, efficient and mild approach for coupling of protected purine bases with sugar modified thiazolidine ring by employing a suitable base tetrabutylammonium fluoride (TBAF) at room temperature, which has resulted in high yield and highly regioselective N-9 substituted products. The synthesized compounds were tested against three Gram positive and four Gram negative bacterial strains. Among them, the compound **8** with two chloro groups in the purine skeleton exhibited potent activity at lower concentrations against all the tested bacteria. The other compounds have shown moderate activity compared to standard drug.

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