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SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF NUCLEOSIDE ANALOGS BASED ON L-ASCORBIC ACID AND BASES

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Abstract:
5,6-*O*- isopropylidene-2,3- di-*O*- methyl ascorbic acid (**2**), obtained by reaction of acetone with ascorbic acid (**1**) followed by methylation with methyl iodide, on DBU catalysed elimination of acetone moiety led to the formation of respective 2,3-di-*O*-methyl didehydro-L-ascorbic acid (**4**) in good yield. The latter on methanesulphonylation with methanesulphonyl chloride and subsequent reaction of the crude methanesulphonyloxy derivative with imidazole, benzimidazole and adenine resulted in respective tetronolactonyl nucleoside analogs **5**, **6** and **7**. Compound **5** on reaction with benzyl amine led to N- benzylated teramyl nucleoside analog, while compounds **6** and **7** did not react under similar condition. All the synthesized compounds were evaluated for their antitubercular activity against *M. tuberculosis H37Ra* and *H37Rv* exhibiting MIC >12.5 μg/mL.

Introduction

Ascorbic acid, commonly known as Vitamin C has served as an excellent scaffold for the synthesis of many biologically active compounds. [1-2] Its antioxidant and immunomodulatory activities of many of its *O*- alkyl derivatives has extensively been studied.[3] Ascorbic acid is basically a tetranolactone and the two enols at 2- and 3-positions play very crucial role both in organic synthesis and in eliciting the biological response. Replacement of the ring oxygen atom with sulphur in the tetranolactone results in thionotetranolactone, a pharmacophore in compounds exhibiting antimalarial and antitubercular activities via FAS-II inhibition. One of such compounds possessing thiotetranolactone moiety, thiolactomycin is under preclinical development as new antitubercular drug. [4a,b] However, replacement of ring oxygen with nitrogen atom results in tetramates, another moiety found in a variety of antibiotics, [5-9] anti HIV, [10] anticancer and antitumor agents. Very recently few reports came into light where ascorbic based nucleosides were prepared as antiviral and antitumor agents. [11]

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Nucleoside analogs themselves are known for diverse range of biological activities.[12] Ascorbic acid has also been used in the synthesis of pyrano[3,4-b]indoles and a variety of other heterocycles by Preobrzhenskaya's group.[13]

In our continuing programme towards the development of new antitubercular agents we were curious to synthesise certain teramic acid based nucleosides and see their antitubercular activity. Our curiosity arose due to the fact that many purines, nucleoside analogs and thiolactomycins have displayed significant antitubercular activity.[4, 14] Thus the present manuscript describes the methodology to synthesise tetranolactonyl and tetramyl nucleoside analogs. The compounds synthesized were evaluated for their antitubercular activities.

The synthetic strategy involves the reaction of ascorbic acid (**1)** with acetone in presence of catalytic amount of acetyl chloride leading to selective protection of 5, 6-diol to give 5,6- *O*- isopropylidene-L- ascorbic acid (**2**) in quantitative yield. The above compound **2** was methylated with methyl iodide in DMSO/ acetone (1:4) as solvent in presence K_2CO_3 and tetrabutylammonium bromide (TBAB) to give 2, 3-*O*-*bis*-methyl-5,6-O-isopropylidene ascorbic acid (**3**).[15] The latter was reacted with DBU (50 mol %) using THF as solvent leading to an elimination of a molecule of acetone to give 3,4-*bis*-methyl-5-(2-hydroxyethylidene)-5*H*-furan-2-one (**4**).[16] The compound **4** was treated with methanesulphonyl chloride in dry CH_2Cl_2 in the presence of catalytic compound of triethylamine resulting in the intermediate methanesulphonyloxy derivative (**5**) which was used as such in the subsequent steps of the desired synthesis.

The intermediate methanesulphonyloxy derivative (**4**) was reacted with imidazole and catalytic amount of DBU (25 mol %) in dry toluene at 120^0C to give (*Z*)-5-[2-(imidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**5**) in 65.7 % yield.

Similar reaction of compound **5** with benzimidazole under gave the corresponding give (*Z*)-5-[2-(benzimidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**6**) in good yield.

To prepare adeninyl thiotetranolactone we prepared persilylated adenine by refluxing it in anh. toluene with HMDS in presence of catalytic amount of $(NH_4)_2SO_4$ in argon atmosphere for 3-4h. The silyated adenine, thus obtained was reacted with methanesulphonyloxy tetranolactone derivative **5** as above using DBU (25 mol% in

refluxing toluene to give (*Z*)-5-[2-(adenin-9-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**7**) in 10 % yield. (**Scheme1**).

The above compounds imidazolyl tetranolactone **5** was reacted with benzyl amine in ethanol to give the corresponding tetramate 1-benzyl-5-hydroxy-5-(2-imidazol-1-yl-ethyl)-3,4-dimethoxy-1,5-dihydro-pyrrol-2-one (**8**) in very good yield. Similar reaction of compounds **6** and **7** did not afford the desired tetramates. The possible reason for the inertness of (*Z*)-5-[2-(benzimidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**6**) and (*Z*)-5-[2-(adenin-9-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**7**) towards benzyl amine may be due to steric hinderance by the bulky benzimidazole and adenine moieties.

Of all the compounds described above only (*Z*)-5-[2-(imidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**5**) undergos a ring- opening reaction with the benzyl amine to give the enol-keto-amide.

Biological activity

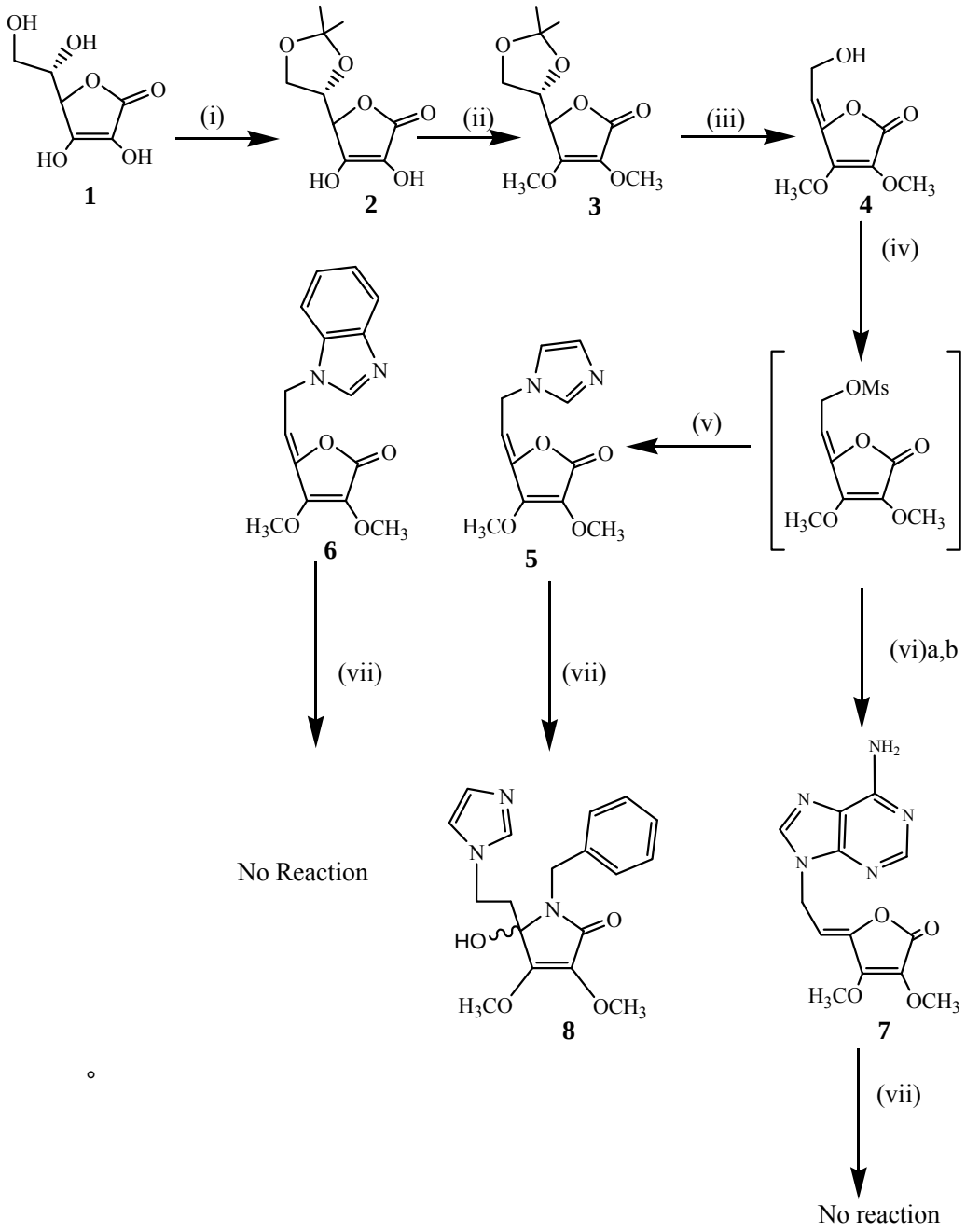
All compounds synthesized were evaluated for their antitubercular activity against *M. Tuberculosis* $H_{37}R_a$ by MABA technique[17] while agar micro dilution method was used against $H_{37}R_v$.[18]

As evident from the table none of the compounds showed significant activity against *M. tuberculosis* $H_{37}R_A$ and virulent $H_{37}R_A$ strains since the MIC values for them are >25μg/mL.

Table 1: Antimycobacterial activity of synthesized compounds (**5**-**8**)

S. No	Comp. No.	MIC (μg ml^{-1}) $H_{37}R_a$	MIC (μg ml^{-1}) $H_{37}Rv$
1	**5**	>12.5	>25
2	**6**	>12.5	>25
3	**7**	>12.5	>25
4	**8**	>12.5	>25

MIC = defined as minimum inhibitory concentration



(i)Acetone, Acetylchloride,30 °C, 4 h (ii) MeI, TBAB, DMSO:Acetone (1:4), K_2CO_3, 30 °C, 18h (iii) THF , DBU, 30 °C, 7 h (iv) Methanesulphonyl chloride, CH_2Cl_2, Et_3N, 0-30 °C, 5h (v) imidazole or benzimidazole,Toluene, DBU, 120°C, 3-6 h.(vi) *a*. adenine, HMDS, $(NH_4)_2SO_4$, reflux, 3h; *b.* DBU, 120°C, 3-4h (vii) Ethanol, benzyl amine 3-4 h

Scheme 1

In summary, we have developed a simple method for the synthesis of tetranolctonyl nucleosides which can be manipulated to tetramyl nucleoside analogs. The compounds have been screened against M. tuberculosis and they exhibited MIC >25 μg/mL.

Experimental

5, 6-*O*-isopropylidene-L-ascorbic acid (2)

To a magnetically stirred solution of ascorbic acid (30g, 170.4 mmol) in acetone (120 mL), acetyl chloride (3 ml, 42.6 mmol) was added and reaction mixture stirred for 2-3 hrs at ambient temperature. The reaction mixture was kept for 7-8 hrs in freeze, the solid separated was filtered and washed with cold acetone and was dried to give compound **2** as colourless granules (27g, 73.7 %), m.p. 195-198^{0}C. lit mp

2, 3-Dimethoxy-5, 6-*O*-isopropylidene-L-ascorbic acid (3)

A mixture of the above compound **2** (25g, 115.7 mmol) and anhy. K_2CO_3 (32 g, 231.4 mmol) in acetone:dimethyl sulphoxide (4:1, 250mL) was magnetically stirred for 25 min. A solution of methyl iodide (14.9 mL, 231.4 mmol) dissolved in acetone (30 mL) was slowly during 30 min. followed by addition of tetrabutyl ammonium bromide (2.0 g) and the reaction mixture stirred overnight at ambient temperature. It was filtered and the filtrate, thus obtained, was evaporated under reduced pressure to give the crude mass, which was dissolved in ethyl acetate and washed with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude solid, which was chromatographed over silica gel (230-400 mesh) using a gradient of hexane-EtOAc (17:3) as eluent to give the compound **3** as colorless solid (22g, 78 %).

(*Z*) – 3, 4-Dimethoxy-5-(2-hydroxyethylidene)-5*H*-furan-2-one (4)

To a magnetically stirred solution of compound **3** (20 g, 81.96 mmol) in THF (80 mL), DBU (6.2 mL, 50 mol %) was slowly added and the reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure and the residue, thus obtained, was dissolved in ethyl acetate (100mL), washed with water (25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude mass, which was chromatographed over silica gel (230-400 mesh) using a hexane-ethyl acetate (9:1) as eluent to give the above compound **4** as colorless solid (

10g, 65.7%), m.p 60^{0}C, IR (Neat) 3391, 1688 cm^{-1}, MS (FAB) 187(M+H)$^+$ ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.39 (bs, 1H), 3.92 (s, 3H, OCH_3), 4.16 (s, 3H, -OCH_3), 4.41 (d, *J* = 7.0, 2H, OCH_2), 5.50 (t, *J* = 7.0 Hz, 1H, =CH-); ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.6, 59.9, 60.6, 108.1, 125.0, 142.3, 149.1, 164.7.

Preparation of the intermediate 5-(2-methanesulfonyloxy ethylidene)-3,4-dimethoxy-5H-furan-2-one: To a stirring solution of the above compound **4** (2 g, 10.65 mmol) and triethylamine (1mL) in anh. dichloromethane (10 mL) a solution of methanesulphonyl chloride (1.11mL, 10.65 mmol) in CH_2Cl_2 (2 ml) was slowly added at 0 °C during 5 min. The reaction mixture was brought to 30 °C and stirring continued for further 1 hr. The solvent was evaporated under reduced pressure and the residue, thus obtained, was dissolved in ethyl acetate (100 mL), washed with water (25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a crude mass of the title compound which was used as such in the subsequent reactions.

(*Z*)-5-[2-(Imidazol-1-yl) ethylidene]-3, 4-dimethoxy-5*H*-furan-2-one (5)

A mixture of the above crude methanesulphonyloxy derivative (1 g, 3.78 mmol) imidazole (0.257 g, 3.78 mmol) and DBU (0.285 mL, 25 mol %) in anh. toluene was stirred at 120°C for 5h. The solvent was evaporated under reduced pressure and the residue, thus obtained, was dissolved in ethyl acetate (100 mL) and washed with water (25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude mass, which was chromatographed over silica gel (230-400 mesh) using a gradient of hexane:ethylacetate (3:2) as eluent to give the above compounds **6** as light yellow foam (1 g, 65.7 %), as foam IR (KBr) υ_{max} 3210, 1775 ; FABMS 237(M+H)$^+$; ^{1}H NMR (200Mz, $CDCl_3$) *δ* 3.95 (s, 3H, OCH_3), 4.14 (s, 3H, OCH_3), 4.81 (d, *J* = 7.4 Hz, 2H, CH_2), 5.73 (t, *J* = 7.4 Hz, 1H, =CH), 6.93, 7.07 (2s, 2H, Imidazole CH), 7.51 (s, 1H, imidazolyl CH); Anal. Calcd for $C_{11}H_{12}N_2O_4$ C, 55.92; H, 5.12, N, 11.85 ;. Found: C, 55.87; H, 5.08; N, 11.93.

(*Z*)-5-[2-(Adenin-9-yl) ethylidene]-3, 4-dimethoxy-5*H*-furan-2-one (7): Adenine(1.5 g, mmol) was refluxed in anh. toluene with HMDS in presence of catalytic amount of $(NH_4)_2SO_4$ under N_2 atmosphere for 3-4 h and the excess of reagent and solvent were removed under reduced pressure to give silylated adenine derivative. A mixture of this silylated adenine derivative, the above intermediate methanesulphonyloxy derivative (1

g, 3.78 mmol) and DBU (0.285 mL, 50 mol %) in anh. toluene (5 mL) was stirred at 120 °C for 5h. The solvent evaporated and the residue thus obtained was dissolved in chloroform and washed with water and the organic layer dried (Na_2SO_4) and solvent evaporated under reduced pressure to afford a crude mass. The latter was chromatographed over SiO_2 column using CH_2Cl_2: MeOH (9:1) as eluent to give the title compound **7** as colourless granules (0.32g) in 10% yield: mp 138-140°C; IR (KBr) υ_{max} 3210, 1775 ; FABMS 304$(M+H)^+$; ^{1}H NMR (200Mz, DMSO) δ 3.82 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 5.15 (d, J = 7.0 Hz, 2H, CH_2), 5.73 (t, J= 7.0 Hz, 1H, =CH), 7.76 (s, 1H, CH), 7.95 (br, 2H, NH_2), 8.14 (s, 1H, CH); Anal. Calcd for $C_{13}H_{13}N_5O_4$ C, 51.48; H, 4.29, N, 23.10 ;. Found: C, 51.40; H, 4.32; N, 23.18.

1-Benzyl-5-hydroxy-5-(2-imidazol-1-yl-ethyl)-3, 4-dimethoxy-1,5-dihydro-pyrrol-one (8)

A solution of the above compound **5** (0.2g, 0**.**847mmol) and benzylamine (0.09mL 0.847mmol) in ethanol (1 mL) at was stirred at 40˚C for five hours. The solvent was evaporated to a crude product which was chromatographed over silica gel (230-400 mesh) using a gradient of hexane:ethylacetate (3:2) as eluent to give compounds **8** as light yellow foam. Yield (174 mg, 60%); IR (KBr) υ_{max} 3210, 1775 cm^{-1}; FABMS 343$(M+H)^+$; ^{1}H NMR (200Mz, $CDCl_3$) δ 2.00- 2.24 (m, 2H, H-6), 3.22- 3.40 (m, 2H, H-7), 3.85 (s, 3H, OCH_3), 4.00(s, 3 H, OCH_3), 4.21 (d, J= 15.2 Hz, 1 H, NCH_{2A}), 4.90 (d, J= 15.2 Hz, 1 H, NCH_{2B}), 6.30 (s, 1H, Imidazole H), 6.85(m, 2H, Imidazole CH), 7.33-7.43(m, 5H, Ar-H); Anal. Calcd for $C_{18}H_{21}N_3O_4$: C, 62.93; H, 6.16, N, 12.24 ;. Found: C, 62.90; H, 6.16; N, 12.24.

Antitubercular Screening:

Activity against *M. tuberculosis* H_{37}Ra Strain[17]

All the compounds synthesized were evaluated for their efficacy against *M. tuberculosis* H_{37}Ra at concentration ranging from 100 μg ml^{-1} to 1.56 μg ml^{-1} using twofold dilutions in the initial screen. Log phase culture of *M. tuberculosis*H_{37} Ra is diluted so as to give final $OD_{550\,nm}$ of 0.05 in Sauton's medium. In 96 well white plate 190 μl of culture is

dispensed in each well. A dimethyl sulfoxide (DMSO) solution of test compounds is dispensed to 96 well plates so as to make final test concentration 25 μg ml^{-1} (5 μg test compound is dispensed in 10 μl of DMSO). Then the plate is incubated at 37 °C/5% CO_2 for 5 days. On 5th day 15 μl Alamar blue solution is added to the each well of plate. The plate is again incubated overnight at 37 °C/5% CO_2 incubator. The fluorescence is read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found active (>90% inhibition as compared with control) at this concentration are then tested at 6 serial dilutions starting from 50 to 1.56 μg ml^{-1}

Activity against *M. tuberculosis* H_{37}Rv strain[18]

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H_{37}Rv was performed by agar microdilution method where twofold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H_{37}Rv growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 μg ml^{-1} concentration of extracts/compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentration of compound keeping the volume constant i.e. 0.1 ml. Medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H_{37}Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H_{37}Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

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Revised

Synthesis and antitubercular activity of nucleoside analogs based on L-ascorbic acid and bases

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Abstract:
5,6-*O*- isopropylidene-2,3- di-*O*- methyl ascorbic acid (**2**), obtained by reaction of acetone with ascorbic acid (**1**) followed by methylation with methyl iodide, on DBU catalysed elimination of acetone moiety led to the formation of respective 2,3-di-*O*-methyl didehydro-L-ascorbic acid (**4**) in good yield. The latter on methanesulphonylation with methanesulphonyl chloride and subsequent reaction of the crude methanesulphonyloxy derivative with imidazole, benzimidazole and adenine resulted in respective tetronolactonyl nucleoside analogs **5**, **6** and **7**. Compound **5** on reaction with benzyl amine led to N- benzylated teramyl nucleoside analog, while compounds **6** and **7** did not react under similar condition. All the synthesized compounds were evaluated for their antitubercular activity against *M. tuberculosis H37Ra* and *H37Rv* exhibiting MIC >12.5 μg/mL.

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Key words: Ascorbic acid. Vitamin C. Nucleosides. Adenine. Tetramic acid

Introduction

Ascorbic acid, commonly known as Vitamin C has served as an excellent scaffold for the synthesis of many biologically active compounds (Cinatl et al., 1995); (Bram et al.

1980); (Grdisa et al., 1995). Its antioxidant activity and immunomodulatory activities of many of its *O*- alkyl derivatives have extensively been studied (Olabisi, Wimalasena, 2004). Ascorbic acid is basically a tetranolactone and the two enols at 2- and 3- positions play very crucial role both in organic synthesis and in eliciting the biological response. Replacement of the ring oxygen atom with sulphur in the tetranolactone results in thionotetranolactone, a pharmacophore in compounds exhibiting antimalarial and antitubercular activities via FAS-II inhibition. One of such compounds possessing thiotetranolactone moiety, thiolactomycin is under preclinical development as new antitubercular drug (Miyakawa et al., 1982); (Kremer et al., 2000); However, replacement of ring oxygen with nitrogen atom results in tetramates, another moiety found in a variety

of antibiotics, (Royles BJL 1995); (Ley et al., 1992); (Wang et al., 2003); (Elbe et.al.,1956); anti HIV, (Roggoet. al.,1994) anticancer and antitumor agents (Holtzel et.al., 2000); (Iwata et. al., 2005);. Very recently few reports came into light where ascorbic based nucleosides were prepared as antiviral and antitumor agents (Malic et. al., 2000). Nucleoside analogs themselves are known for diverse range of biological activities (Mishra et. al. 2005); Ascorbic acid has also been used in the synthesis of pyrano[3,4-b]indoles and a variety of other heterocycles by Preobrzhenskaya's group (Larvrenov et. al., 2005); (Preobrzhenskaya, Korolev 2000).

In our continuing programme towards the development of new antitubercular agents we were curious to synthesise certain teramic acid based nucleosides and see their antitubercular activity. Our curiosity arose due to the fact that many purines, nucleoside analogs and thiolactomycins have displayed significant antitubercular activity (Miyakawa et. al., 1982); (Kremer et. al., 2000); (Somu 2006); Thus the present manuscript describes the methodology to synthesise tetranolactonyl and tetramyl nucleoside analogs. The compounds synthesized were evaluated for their antitubercular activities.

Results and Discussion:

The synthetic strategy involves the reaction of ascorbic acid (**1)** with acetone in presence of catalytic amount of acetyl chloride leading to selective protection of 5, 6-diol to give 5,6- *O*- isopropylidene-L- ascorbic acid (**2**) in quantitative yield. The above compound **2** was methylated with methyl iodide in DMSO/ acetone (1:4) as solvent in presence K_2CO_3 and tetrabutylammonium bromide (TBAB) to give 2, 3-*O*-*bis*-methyl-5,6-O-isopropylidene ascorbic acid (**3**) (Khan, Adams, 1999); The latter was reacted with DBU (50 mol %) using THF as solvent leading to an elimination of a molecule of acetone to give 3,4-*bis*-methyl-5-(2-hydroxyethylidene)-5*H*-furan-2-one (**4**) (Singh, et.al., 2006); The compound **4** was treated with methanesulphonyl chloride in dry CH_2Cl_2 in the presence of catalytic compound of triethylamine resulting in the intermediate methanesulphonyloxy derivative (**5**) which was used as such in the subsequent steps of the desired synthesis.

The intermediate methanesulphonyloxy derivative (**4**) was reacted with imidazole and catalytic amount of DBU (25 mol %) in dry toluene at 120^0C to give (*Z*)-5-[2-(imidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**5**) in 65.7 % yield.
Similar reaction of compound **5** with benzimidazole under gave the corresponding (*Z*)-5-[2-(benzimidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**6**) in good yield.

To prepare adenine derivative of thiotetranolactone, the adenine was persilylated by refluxing it with HMDS in anhydrous toluene in presence of catalytic amount of $(NH_4)_2SO_4$ under nitrogen atmosphere for 3-4h. The silyated adenine, thus obtained was reacted with methanesulphonyloxy tetranolactone derivative **5** as above using DBU (25 mol% in refluxing toluene to give (*Z*)-5-[2-(adenin-9-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**7**) in 10 % yield. (**Scheme1**).

The reaction of the above imidazolyl tetranolactone **6** with benzyl amine in ethanol gave the corresponding tetramate, 1-benzyl-5-hydroxy-5-(2-imidazol-1-yl-ethyl)-3,4-dimethoxy-1,5-dihydro-pyrrol-2-one (**8**) in very good yield. Similar reaction of compounds **6** and **7** did not afford the desired tetramates. The possible reason for the inertness of (*Z*)-5-[2-(benzimidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**6**) and (*Z*)-5-[2-(adenin-9-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**7**) towards benzyl amine may be due the steric hindrance offered during reaction by the bulky benzimidazole and adenine moieties. Thus among compounds **5**, **6** and **7**

described above only (*Z*)-5-[2-(imidazol-1-yl) ethylidene]-3, 4-dimethoxy-5H-furan-2-one (**5**) undergoes a ring- opening reaction with the benzyl amine to give the enol-keto-amide leading to compound **8**. The structures of all the products synthesized were established on the basis of their spectroscopic data and analysis (experimental).

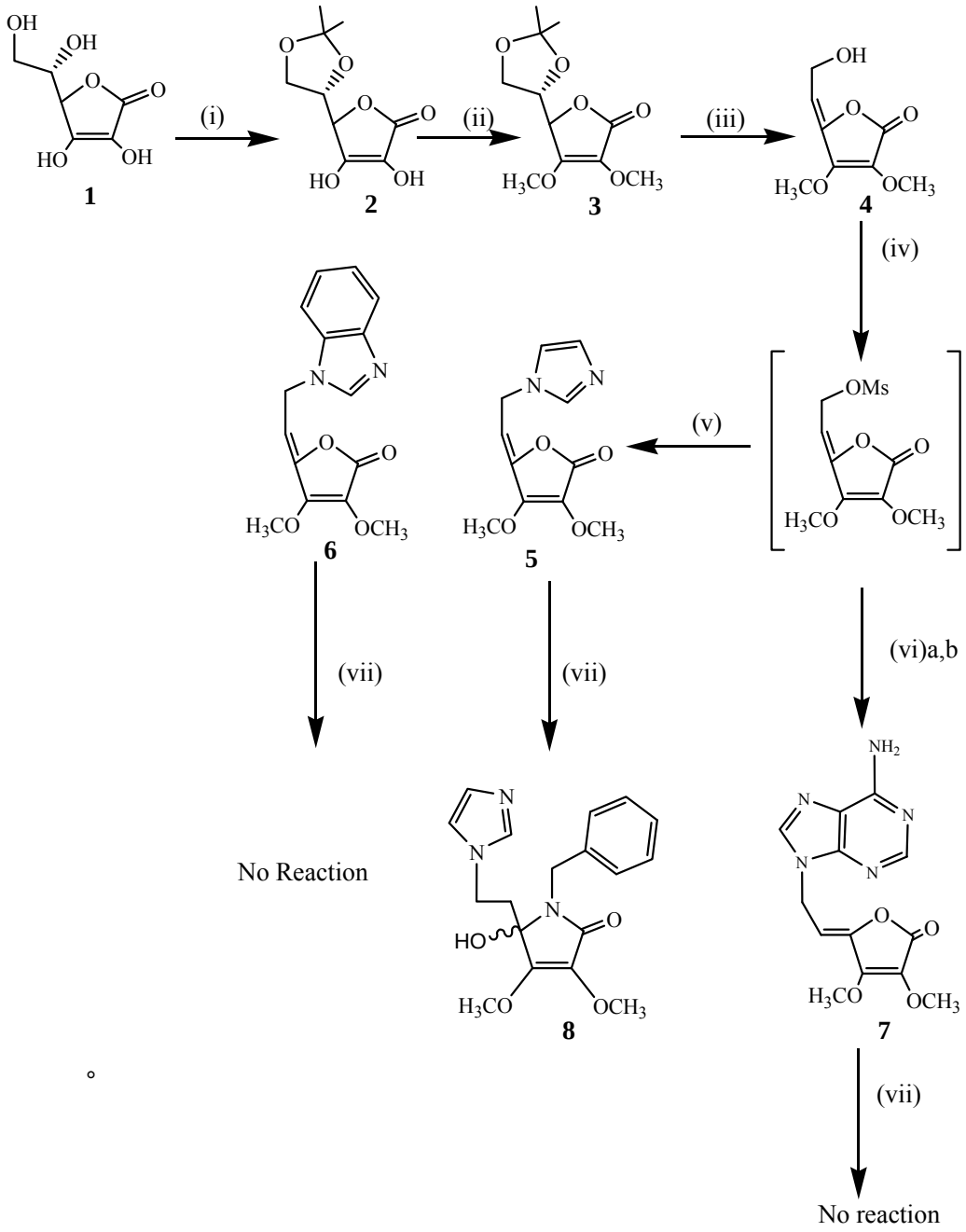
Biological activity

The above ascorbic acid based nucleosides **5**, **6**, **7** and **8** were evaluated for their antitubercular activity against *M. Tuberculosis* $H_{37}R_a$ by MABA technique (Collins, Franzblan, 1997); while agar micro dilution method (Saito et.al., 1991); was used against $H_{37}R_v$. As evident from the table none of the compounds showed significant activity against *M. tuberculosis* $H_{37}R_A$ and virulent $H_{37}R_A$ strains since the MIC values for them are >25μg/mL as compared to the standard drug INH. One of the possible reasons for their inactivity against mycobacterium may be their transport or instability in the cellular medium.

Table 1: Antimycobacterial activity of synthesized compounds (**5-8**)

S. No	Comp. No.	MIC (μg ml^{-1}) $H_{37}R_a$	MIC (μg ml^{-1}) $H_{37}Rv$
1	**5**	>12.5	>25
2	**6**	>12.5	>25
3	**7**	>12.5	>25
4	**8**	>12.5	>25
INH	-	-	0.65

MIC = defined as minimum inhibitory concentration



(i)Acetone, Acetylchloride,30 °C, 4 h (ii) MeI, TBAB, DMSO:Acetone (1:4), K_2CO_3, 30 °C, 18h (iii) THF , DBU, 30 °C, 7 h (iv) Methanesulphonyl chloride, CH_2Cl_2, Et_3N, 0-30 °C, 5h (v) imidazole or benzimidazole,Toluene, DBU, 120°C, 3-6 h.(vi) *a*. adenine, HMDS, $(NH_4)_2SO_4$, reflux, 3h; *b.* DBU, 120°C, 3-4h (vii) Ethanol, benzyl amine 3-4 h

Scheme 1

In summary, we have developed a simple method for the synthesis of tetranolctonyl nucleosides which can be manipulated to tetramyl nucleoside analogs. The compounds have been screened against M. tuberculosis and they exhibited MIC >25 μg/mL.

Experimental

5, 6-*O*-isopropylidene-L-ascorbic acid (2)

To a magnetically stirred solution of ascorbic acid (30g, 170.4 mmol) in acetone (120 mL), acetyl chloride (3 ml, 42.6 mmol) was added and reaction mixture stirred for 2-3 hrs at ambient temperature. The reaction mixture was kept for 7-8 hrs in freeze, the solid separated was filtered and washed with cold acetone and was dried to give compound **2** as colourless granules (27g, 73.7 %), m.p. 195-198^{0}C. lit mp

2, 3-Dimethoxy-5, 6-*O*-isopropylidene-L-ascorbic acid (3)

A mixture of the above compound **2** (25g, 115.7 mmol) and anhy. K_2CO_3 (32 g, 231.4 mmol) in acetone:dimethyl sulphoxide (4:1, 250mL) was magnetically stirred for 25 min. A solution of methyl iodide (14.9 mL, 231.4 mmol) dissolved in acetone (30 mL) was slowly during 30 min. followed by addition of tetrabutyl ammonium bromide (2.0 g) and the reaction mixture stirred overnight at ambient temperature. It was filtered and the filtrate, thus obtained, was evaporated under reduced pressure to give the crude mass, which was dissolved in ethyl acetate and washed with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to afford a crude solid, which was chromatographed over silica gel (230-400 mesh) using a gradient of hexane-EtOAc (17:3) as eluent to give the compound **3** as colorless solid (22g, 78 %).

(*Z*) – 3, 4-Dimethoxy-5-(2-hydroxyethylidene)-5*H*-furan-2-one (4)

To a magnetically stirred solution of compound **3** (20 g, 81.96 mmol) in THF (80 mL), DBU (6.2 mL, 50 mol %) was slowly added and the reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure and the residue, thus obtained, was dissolved in ethyl acetate (100mL), washed with water (25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude mass, which was chromatographed over silica gel (230-400 mesh) using a hexane-ethyl acetate (9:1) as eluent to give the above compound **4** as colorless solid (

10g, 65.7%), m.p 60^{0}C, IR (Neat) 3391, 1688 cm^{-1}, MS (FAB) 187(M+H)$^+$ ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.39 (bs, 1H), 3.92 (s, 3H, OCH_3), 4.16 (s, 3H, -OCH_3), 4.41 (d, *J* = 7.0, 2H, OCH_2), 5.50 (t, *J* = 7.0 Hz, 1H, =CH-); ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.6, 59.9, 60.6, 108.1, 125.0, 142.3, 149.1, 164.7.

Preparation of the intermediate 5-(2-methanesulfonyloxy ethylidene)-3,4-dimethoxy-5H-furan-2-one: To a stirring solution of the above compound **4** (2 g, 10.65 mmol) and triethylamine (1mL) in anh. dichloromethane (10 mL) a solution of methanesulphonyl chloride (1.11mL, 10.65 mmol) in CH_2Cl_2 (2 ml) was slowly added at 0 °C during 5 min. The reaction mixture was brought to 30 °C and stirring continued for further 1 hr. The solvent was evaporated under reduced pressure and the residue, thus obtained, was dissolved in ethyl acetate (100 mL), washed with water (25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a crude mass of the title compound which was used as such in the subsequent reactions.

(*Z*)-5-[2-(Imidazol-1-yl) ethylidene]-3, 4-dimethoxy-5*H*-furan-2-one (5)

A mixture of the above crude methanesulphonyloxy derivative (1 g, 3.78 mmol) imidazole (0.257 g, 3.78 mmol) and DBU (0.285 mL, 25 mol %) in anh. toluene was stirred at 120°C for 5h. The solvent was evaporated under reduced pressure and the residue, thus obtained, was dissolved in ethyl acetate (100 mL) and washed with water (25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford a crude mass, which was chromatographed over silica gel (230-400 mesh) using a gradient of hexane:ethylacetate (3:2) as eluent to give the above compounds **6** as light yellow foam (1 g, 65.7 %), as foam IR (KBr) υ_{max} 3210, 1775 ; FABMS 237(M+H)$^+$; ^{1}H NMR (200Mz, $CDCl_3$) *δ* 3.95 (s, 3H, OCH_3), 4.14 (s, 3H, OCH_3), 4.81 (d, *J* = 7.4 Hz, 2H, CH_2), 5.73 (t, *J* = 7.4 Hz, 1H, =CH), 6.93, 7.07 (2s, 2H, Imidazole CH), 7.51 (s, 1H, imidazolyl CH); Anal. Calcd for $C_{11}H_{12}N_2O_4$ C, 55.92; H, 5.12, N, 11.85 ;. Found: C, 55.87; H, 5.08; N, 11.93.

(*Z*)-5-[2-(Adenin-9-yl) ethylidene]-3, 4-dimethoxy-5*H*-furan-2-one (7): Adenine(1.5 g, mmol) was refluxed in anh. toluene with HMDS in presence of catalytic amount of $(NH_4)_2SO_4$ under N_2 atmosphere for 3-4 h and the excess of reagent and solvent were removed under reduced pressure to give silylated adenine derivative. A mixture of this silylated adenine derivative, the above intermediate methanesulphonyloxy derivative (1

g, 3.78 mmol) and DBU (0.285 mL, 50 mol %) in anh. toluene (5 mL) was stirred at 120 °C for 5h. The solvent evaporated and the residue thus obtained was dissolved in chloroform and washed with water and the organic layer dried (Na_2SO_4) and solvent evaporated under reduced pressure to afford a crude mass. The latter was chromatographed over SiO_2 column using CH_2Cl_2: MeOH (9:1) as eluent to give the title compound **7** as colourless granules (0.32g) in 10% yield: mp 138-140°C; IR (KBr) υ_{max} 3210, 1775 ; FABMS 304$(M+H)^+$; ^{1}H NMR (200Mz, DMSO) δ 3.82 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 5.15 (d, J = 7.0 Hz, 2H, CH_2), 5.73 (t, J= 7.0 Hz, 1H, =CH), 7.76 (s, 1H, CH), 7.95 (br, 2H, NH_2), 8.14 (s, 1H, CH); Anal. Calcd for $C_{13}H_{13}N_5O_4$ C, 51.48; H, 4.29, N, 23.10 ;. Found: C, 51.40; H, 4.32; N, 23.18.

1-Benzyl-5-hydroxy-5-(2-imidazol-1-yl-ethyl)-3, 4-dimethoxy-1,5-dihydro-pyrrol-one (8)

A solution of the above compound **5** (0.2g, 0**.**847mmol) and benzylamine (0.09mL 0.847mmol) in ethanol (1 mL) at was stirred at 40˚C for five hours. The solvent was evaporated to a crude product which was chromatographed over silica gel (230-400 mesh) using a gradient of hexane:ethylacetate (3:2) as eluent to give compounds **8** as light yellow foam. Yield (174 mg, 60%); IR (KBr) υ_{max} 3210, 1775 cm^{-1}; FABMS 343$(M+H)^+$; ^{1}H NMR (200Mz, $CDCl_3$) δ 2.00- 2.24 (m, 2H, H-6), 3.22- 3.40 (m, 2H, H-7), 3.85 (s, 3H, OCH_3), 4.00(s, 3 H, OCH_3), 4.21 (d, J= 15.2 Hz, 1 H, NCH_{2A}), 4.90 (d, J= 15.2 Hz, 1 H, NCH_{2B}), 6.30 (s, 1H, Imidazole H), 6.85(m, 2H, Imidazole CH), 7.33-7.43(m, 5H, Ar-H); Anal. Calcd for $C_{18}H_{21}N_3O_4$: C, 62.93; H, 6.16, N, 12.24 ;. Found: C, 62.90; H, 6.16; N, 12.24.

Antitubercular Screening:

Activity against *M. tuberculosis* H_{37}Ra Strain[17]

All the compounds synthesized were evaluated for their efficacy against *M. tuberculosis* H_{37}Ra at concentration ranging from 100 μg ml^{-1} to 1.56 μg ml^{-1} using twofold dilutions in the initial screen. Log phase culture of *M. tuberculosis*H_{37} Ra is diluted so as to give final $OD_{550\,nm}$ of 0.05 in Sauton's medium. In 96 well white plate 190 μl of culture is

dispensed in each well. A dimethyl sulfoxide (DMSO) solution of test compounds is dispensed to 96 well plates so as to make final test concentration 25 μg ml^{-1} (5 μg test compound is dispensed in 10 μl of DMSO). Then the plate is incubated at 37 °C/5% CO_2 for 5 days. On 5th day 15 μl Alamar blue solution is added to the each well of plate. The plate is again incubated overnight at 37 °C/5% CO_2 incubator. The fluorescence is read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found active (>90% inhibition as compared with control) at this concentration are then tested at 6 serial dilutions starting from 50 to 1.56 μg ml^{-1}

Activity against *M. tuberculosis* H_{37}Rv strain[18]

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H_{37}Rv was performed by agar microdilution method where twofold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H_{37}Rv growing on L-J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 μg ml^{-1} concentration of extracts/compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 middle brook's medium (containing 1.7 ml medium and 0.2 ml OADC supplement) at different concentration of compound keeping the volume constant i.e. 0.1 ml. Medium was allowed to cool keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H_{37}Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H_{37}Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.

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