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Antimicrobial screening and synthesis of some novel benzo[*a*]phenothiazines and ribofuranosides

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Synthesis of 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*]phenothiazin-5-thione,12*H*-benzo[*a*] phenothiazin-5-ols, 5-acetoxy-12*H*-benzo[*a*] phenothiazines and 5-methoxy-12*H*-benzo[*a*]phenothiazines have been carried out from 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*] phenothiazin-5-ones; which have been synthesized by condensing zinc mercaptide of 2-amino-5-chloro-benzenethiol and 2-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-3-chloro-1,4-naphthoquinone. The ribofuranosides *viz*, N-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5-acetoxy/5-methoxy-12*H*-benzo[*a*]phenothiazines have been synthesized by condensing synthesized heterocycles bases with (+)-β-D-ribofuranose-1-acetate-2,3,5-tribenzoate. Structural assignments have been done on the basis of elemental analysis, IR, and ^{1}H NMR. All the synthesized compounds have been screened for their antimicrobial activity.

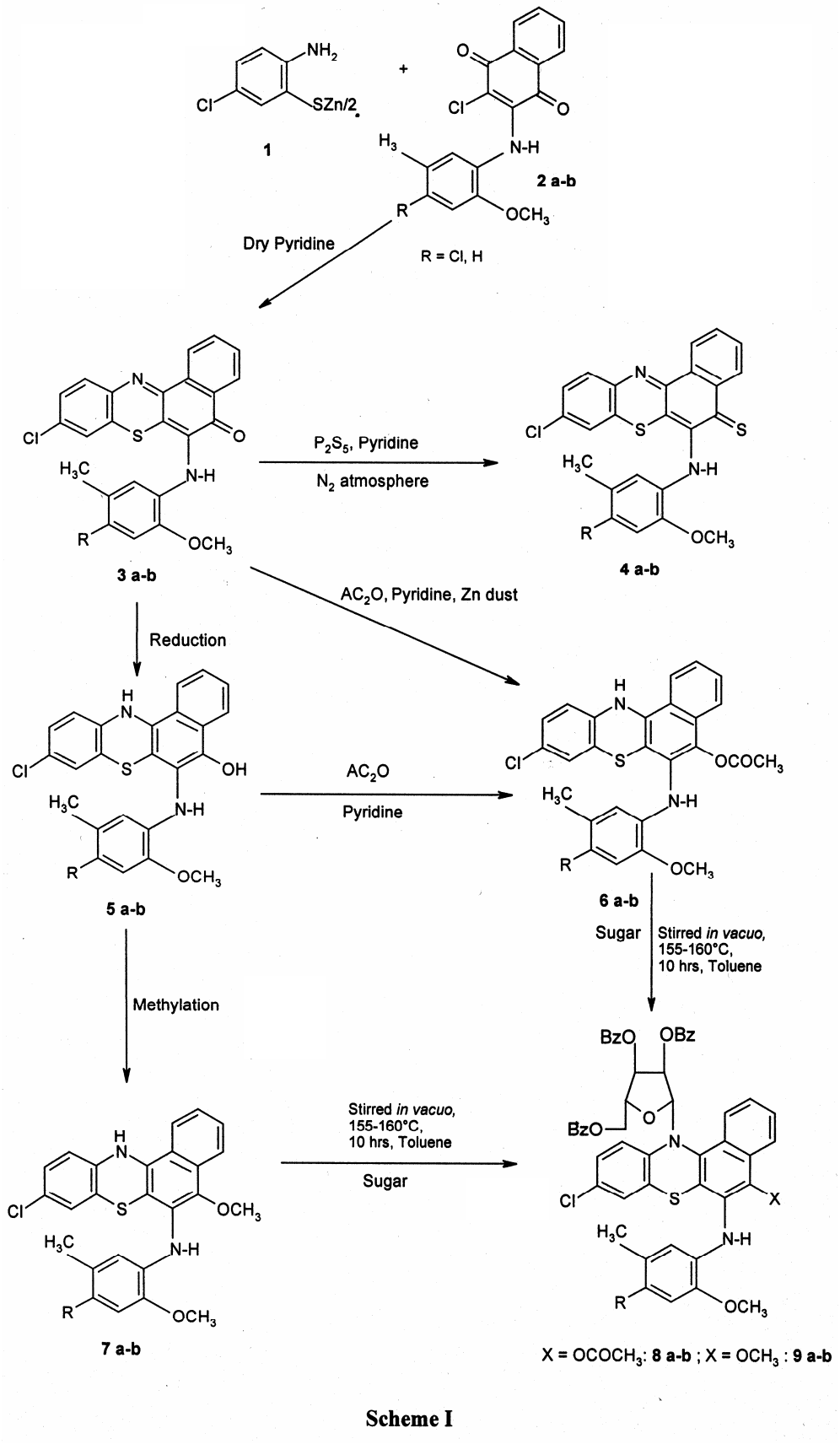
Keywords: 5*H*-benzo[*a*]phenothiazines, 12*H*-benzo[*a*]phenothiazine thiones, ribofuranosides, spectral data, antimicrobial activity

IPC: **Int.Cl.[7] C 07 D**

A survey of the pertinent literature reveals that benzo[*a*]phenothiazines have been found to possess a wide spectrum of biological activity such as antifungal[1], antibacterial[2], antiplasmid[3], anticarcinogenic[4,5], cytotoxic[6], antileukemic[7], antitumor[8,9], antimutagenic[10], antiinflammatory[11], antiherpes[12], *etc*. Besides pharmaceutical applications, these derivatives have also been used as stablizers[13], light sensitive copying material[14], antioxidants[15], dyes[16], *etc*. The enormous potentialities of these compounds[17] led to the exploration of some new derivatives of benzo[*a*]phenothiazines *viz*, 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*] phenothiazin-5-ones, 5*H*-benzo[*a*]-phenothiazine-5-thiones, 12*H*-benzo[*a*]phenothiazin-5-ols, 5-acetoxy-12*H*-benzo[*a*]phenothiazines and 5-methoxy-12*H*-benzo[*a*]phenothiazines and their ribofuranosides, *viz*, N-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5-acetoxy/5-methoxy-12*H*-benzo[*a*]phenothiazines. These compounds have been characterized by elemental analysis, spectroscopic studies *viz*, IR and ^{1}H NMR.

Results and Discussion

2,3-Dichloro-1,4-naphthoquinone was refluxed with 4-chloro-2-methoxy-5-methylaniline/2-methoxy-5-methylaniline to give 2-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-3-chloro-1,4-naphthoquinone **2**. Compound **2** on treatment with zinc mercaptide of 2-amino-5-chloro-benzenethiol **1** in dry pyridine afforded 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*]phenothiazin-5-ones **3**. Compound **3** on refluxing with P_2S_5 in pyridine gave 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*]phenothiazine-5-thiones **4**. The compound 6-(4-chloro-2-methoxy -5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-12*H*-benzo[*a*]phenothiazin-5-ols **5** was prepared by reduction of compound **3** with sodium dithionite. Reductive acetylation of compound **3** with Zn dust-Ac_2O and alternatively acetylation of compound **5** with Ac_2O in pyridine afforded 5-acetoxy-12*H*-benzo[*a*]phenothiazines **6**. Compound **5** on treatment with dimethylsulphate in 10% ethanolic KOH, yielded 5-methoxy-12*H*-benzo[*a*]phenothiazines **7** (**Scheme I**). A pasty mixture of


NH2
Cl
SZn/2
1
+
O
Cl
O
N-H
H3
R
OCH3
2 a-b
R = Cl, H
Dry Pyridine
P2S5, Pyridine
N2 atmosphere
3 a-b
4 a-b
AC2O, Pyridine, Zn dust
Reduction
OH
5 a-b
AC2O
Pyridine
OCOCH3
6 a-b
Sugar
Stirred in vacuo,
155-160°C,
10 hrs, Toluene
Methylation
7 a-b
BzO
OBz
X
X = OCOCH3: 8 a-b ; X = OCH3 : 9 a-b

Scheme I

Table I — Antimicrobial activity of the synthesized benzo[*a*]phenothiazine and their ribofuranosides: zone of growth inhibition (mm) (Activity index)*

Bacteria/Fungi	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**	**6a**	**6b**	**7a**	**7b**	**8a**	**8b**	**9a**	**9b**
Escherichia coli (gram negative)	7.6 (0.92)	7.1 (0.86)	8.4 (1.01)	8.0 (0.96)	8.1 (0.98)	8.5 (1.02)	8.1 (0.98)	8.0 (0.96)	8.2 (0.99)	8.0 (0.96)	8.6 (1.04)	8.3 (1.00)	8.4 (1.01)	8.7 (1.05)
Staphylococcus aureus (gram positive)	8.7 (0.99)	8.4 (0.95)	8.9 (1.01)	8.5 (0.97)	7.9 (0.90)	8.3 (0.94)	8.7 (0.99)	8.5 (0.97)	8.9 (1.01)	8.5 (0.97)	8.2 (1.05)	8.9 (1.01)	8.9 (1.01)	9.0 (1.02)
Aspergillus niger	9.3 (0.98)	9.1 (0.96)	9.5 (1.00)	9.2 (0.97)	9.3 (0.98)	9.2 (0.97)	9.8 (1.03)	9.5 (1.00)	9.3 (0.98)	9.0 (0.95)	9.5 (1.00)	9.9 (1.04)	10.1 (1.06)	9.6 (1.01)
Aspergillus flavus	9.0 (0.97)	9.4 (1.01)	9.5 (1.02)	9.1 (0.98)	9.2 (0.99)	9.1 (0.98)	9.5 (1.02)	9.0 (0.97)	9.0 (0.97)	9.2 (0.99)	10.0 (1.08)	9.3 (1.00)	9.4 (1.01)	9.7 (1.04)
Fusarium oxysporium	9.6 (0.95)	9.8 (0.97)	10.2 (1.02)	9.9 (0.98)	9.5 (0.94)	10.1 (1.00)	9.9 (0.98)	9.8 (0.97)	9.8 (0.97)	9.5 (0.94)	10.3 (1.02)	10.1 (1.00)	10.7 (1.86)	10.2 (1.01)

*(Activity index) = Inhibition area of the sample / inhibition area of the standard

compound **6**/**7** in toluene, on stirring with sugar *viz* (+)-β-D-ribofuranose-1-acetate-2,3,5-tribenzoate, gave the corresponding ribofuranosides **8**/**9** (**Scheme I**).

The IR spectra of compound **3** showed >NH stretching vibration in the region 3125-3090 cm^{-1} as a weak band and the >C=O group as strong band in the region 1680-1665 cm^{-1}. Bands between 1520-1510 cm^{-1} and 1365-1345 cm^{-1} were assigned to C=N and C-N stretching vibrations in compounds **3** and **4**, respectively. The disappearance of a band due to >C=O and appearance of a new band due to >C=S in compound **4** in the region 1145-1130 cm^{-1} indicated the formation of compound **4** from compound **3**. Reduction of compound **3** to compound **5** was confirmed from the absence of >C=O group band and appearance of a band due to –OH group. >NH stretching vibrations were observed in the region 3345-3265 cm^{-1} in compounds **5**, **6** and **7**. The >C=O group and C-O-C linkage in compounds **8** and **9** showed absorption bands in the region 1735-1710 cm^{-1} and 1190-1025 cm^{-1}, respectively. Band for C-Cl group was observed between 780-770 cm^{-1}.

In the ^{1}H NMR spectra of compounds **3-9**, the multiplet for aromatic protons appeared in the region δ 6.53-8.31. The >NH proton signal of anilino group in all these compounds was found to be merged with the aromatic proton signals, whereas, the >NH proton signal of phenothiazine ring in compounds **5-7** appeared between δ 8.81-8.97. The -OCH_3 protons and -CH_3 protons in these compounds showed a singlet in the region δ 3.97-3.95 and δ 2.18-2.22, respectively. A peak due to >NH proton of the phenothiazine ring in compounds **8** and **9** was found to be absent, indicating the site of attachment of the sugar. C′$_4$-H and C′$_5$-H protons of the sugar moiety gave a multiplet in the region δ 4.33-4.85 and protons of C′$_2$-H and C′$_3$-H resonate in the region δ 5.70-5.97. C′$_1$-H proton gave a singlet at δ 6.43, confirming the β configuration of the sugar.

Antimicrobial Activity

All the synthesized compounds were screened for their antimicrobial activity at concentration 100 μg/disc in agar media following the method of Bauer *et al.*[18] using Streptomycin, in antibacterial and Mycostatin, in antifungal activity as reference compounds. All the compounds were found to be moderately active against *Escherichia coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive bacteria) and *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium* (fungi). A close look at the activity indices reveals that the ribofuranosides are better antimicrobial agents than their parent bases. These results have been tabulated in the form of inhibition zone and activity indices (**Table I**).

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. The IR spectra were recorded on a Nicolet-Megna FT-IR 550 spectrometer in KBr pellets. The ^{1}H NMR spectra were scanned on a Jeol FX 90Q spectrometer using TMS as internal standard (chemical shift in δ). The homogeneity of the compounds was checked by TLC using silica gel "G" as adsorbent and visualization was accomplished by UV light/iodine vapour.

Synthesis of 2-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-3-chloro-1,4-naphthoquinone 2. These compounds were prepared by reported methods[19].

Synthesis of 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*]phenothiazin-5-ones 3. A mixture of **2** (0.01 mole) and zinc mercaptide of 2-amino-5-chlorobenzenethiol (**1**; 0.005 mole) in dry pyridine (50 mL) was refluxed for 3 hr. The reaction mass was cooled and an equal volume of methanol was added, followed by crushed ice to afford compound **3**. The solid thus obtained was filtered, washed with 5% hydrochloric acid and ethanol. Then, it was dried and purified by recrystallization from benzene. **3a**: R=Cl; m.p.=138°C; yield 65%; IR (KBr): 3120 (>NH str), 1680 (>C=O), 1510 (>C=N), 650(C-S-C), 1250, 1050 (C-O-C), 775 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.59-8.29 (Ar-H & >NH anilino), 3.90 (-OCH_3), 2.25 (-CH_3). **3b**: R=H; m.p.=130°C; yield 61%; IR(KBr): 3125 (>NH str), 1665 (>C=O), 1515 (>C=N), 660 (C-S-C), 1300, 1060 (C-O-C), 770 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.61-8.31 (Ar-H & >NH anilino), 3.82 (–OCH_3), 2.22 (–CH_3).

Synthesis of 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-5*H*-benzo[*a*]phenothiazine-5-thiones 4. Compound **3** (0.01 mole) and P_2S_5 (0.01 mole) in dry pyridine (25 mL) were stirred at 130°C, under nitrogen atmosphere for 12 hr. After completion of the reaction, the contents were cooled and poured onto a cold solution of NaCl (15 g) in water (60 mL). The suspension thus obtained was stirred for 1 hr more and the precipitate thus obtained was filtered, washed with water and purified by recrystallization from benzene. **4a**: R = Cl; m.p.=118°C; yield 70%; IR (KBr): 3310 (>NH str), 1130 (>C=S), 1520 (>C=N), 660 (C-S-C), 1260, 1020 (C–O–C), 775 cm^{-1} (C–Cl); ^1H NMR ($CDCl_3$): δ 6.53-8.13 (Ar-H & >NH anilino), 3.80 (–OCH_3), 2.14 (–CH_3). **4b**: R=H; m.p.=132°C; yield 77%; IR (KBr): 3315 (>NH str), 1145 (>C=S), 1515 (>C=N), 650 (C-S-C), 1270, 1015 (C-O-C), 780 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.56-8.27 (Ar-H & >NH anilino), 3.81 (–OCH_3), 2.17 (-CH_3).

Synthesis of 6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino) - 9-chloro-12*H*-benzo[*a*]phenothiazin-5-ols 5. Compound **3** (0.005 mole) and sodium dithionite (0.01 mole) in water (5 mL) and acetone (50 mL) were refluxed for 2 hr. The colourless solution thus obtained was allowed to cool and poured onto a very dilute solution of sodium dithionite (0.5g) in ice cold water (1 L). The precipitate thus obtained was extracted with ether and purified by recrystallization from benzene-petroleum ether (60-80°C). **5a**: R = Cl; m.p.=164°C; yield 64%; IR (KBr): 3345 (>NH str), 2855 (-OH), 650 (C-S-C), 1260, 1040 (C-O-C), 780 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.63-8.24 (Ar-H & >NH anilino), 8.97 (>NH ring), 3.95 (-OCH_3), 2.22 (-CH_3), 9.84 (-OH). **5b**: R=H; m.p.=142°C; yield 68%; IR (KBr): 3330 (>NH str), 2840 (-OH), 670 (C-S-C), 1245, 1030 (C-O-C), 775 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.65-8.21 (Ar-H & >NH anilino), 8.94 (>NH ring), 3.83 (–OCH_3), 2.18 (-CH_3), 9.73 (-OH).

Synthesis of 5-acetoxy-6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino)-9-chloro-12*H*-benzo[*a*]phenothiazines 6. Method A: By reductive acetylation of 3. A mixture of **3** (0.001 mole) and zinc dust (0.001 mole) in acetic anhydride (1.5 mL) and pyridine (0.2 mL) was stirred for 15 min at rt and then warmed on water-bath for the next 15 min. The excess of zinc dust was removed by filtration. The filtrate was poured into crushed ice, to yield a brown precipitate of compound **6**. It was then extracted by chloroform. The chloroform layer was washed with saturated aqueous solution of sodium bicarbonate, water and then dried over anhydrous sodium sulphate. It was filtered and the solvent was distilled off. The crude product thus obtained was purified by recrystallization from benzene-petroleum ether (60-80°C).

Method B: By acetylation of 5. Acetic anhydride (1.5 mL) was added to compound **5** (0.001 mole) in pyridine (0.2 mL). The reaction mixture was refluxed for 6 hr. Upon cooling, crude **6** was obtained. It was filtered, washed with water, dried and purified by recrystallization from benzene-petroleum ether (60-80°C). **6a**: R=Cl; m.p.=138°C; yield 69%; IR (KBr): 3325 (>NH str), 1760 (>C=O), 675 (C-S-C), 1290,1030 (C-O-C), 750 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.72-8.24 (Ar-H & >NH anilino), 8.81 (>NH ring), 3.89 (-OCH_3), 2.25 (-CH_3). **6b**: R=H; m.p.=122°C; yield 62%; IR (KBr): 3320 (>NH str), 1770 (>C=O), 680 (C-S-C) 1280, 1070 (C-O-C), 775 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.86-8.23 (Ar-H & >NH anilino), 8.93 (>NH ring), 3.82 (-OCH_3), 2.19 (-CH_3).

Synthesis of 5-methoxy-6-(4-chloro-2-methoxy-5-methylanilino/2-methoxy-5-methylanilino) - 9-chloro-12*H*-benzo[*a*]phenothiazines 7. A mixture of **5** (0.001 mole) and sodium dithionite (0.002 mole) in 10% ethanolic potassium hydroxide solution (12.0 mL) was refluxed for 15 min. Dimethylsulphate (0.0015 mole) was then added to the above reaction

mixture and it was again refluxed for 6 hr. The contents of the flask were cooled and then poured into crushed ice. A brown precipitate thus obtained was filtered, washed with ethanol, dried and purified by recrystallization from benzene. **7a**: R=Cl; m.p.=205°C; yield 60%; IR (KBr): 3345 (>NH str), 680 (C-S-C), 1270, 1080 (C-O-C), 745 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.73-8.26 (Ar-H & >NH anilino), 8.93 (>NH ring), 3.92 ($-OCH_3$), 2.25 ($-CH_3$). **7b**: R=H; m.p.=178°C; yield 65%; IR (KBr): 3320 (>NH str), 670 (C-S-C), 1280, 1050 (C-O-C), 740 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.69-8.27 (Ar-H & >NH anilino), 8.90 (>NH ring), 3.96 ($-OCH_3$), 2.22 ($-CH_3$).

Synthesis of N-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-6-(4-chloro-2-methoxy-5-methylanilino-/2-methoxy-5-methylanilino)-9-chloro-5-acetoxy/5-methoxy-12*H*-benzo[*a*]phenothiazines 8/9. To a solution of **6/7** (0.0002 mole) in minimum amount of toluene, (+)-β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.0002 mole) was added and the contents were stirred *in vacuo* on an oil bath, at 155-60°C, for 15 min. The *vacuo* was broken and the reaction was protected from moisture by using a guard tube. Stirring was further continued for 10 hr, with application of vacuum for 15 min after every hour. The melt was dissolved in methanol, boiled for 10 min and cooled to rt. The precipitate was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated and kept in a refrigerator overnight to get crystalline ribofuraniosides. **8a**: R=Cl; m.p.=160°C; yield 61%; IR (KBr): 3265 (>NH anilino), 1735 (>C=O), 1190-1060 (C-O-C), 655 (C-S-C), 750 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.61-8.26 (Ar-H & >NH anilino), 3.93 ($-OCH_3$), 2.04 ($-CH_3$), 2.33 ($-OCOCH_3$), 4.33-4.72 (C-5′ and C-4′ protons of sugar), 5.70-5.82 (C-2′ & C-3′ protons), 6.40 (C-1′ proton). **8b**: R=H; m.p.=151°C; yield 66%; IR (KBr): 3250 (>NH anilino), 1720 (>C=O), 1185-1030 (C-O-C), 640 (C-S-C), 765 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.66-8.33 (Ar-H & >NH anilino), 3.87 ($-OCH_3$), 2.00 ($-CH_3$), 2.27 ($-OCOCH_3$), 4.40-4.75 (C-5′ and C-4′ protons of sugar), 5.75-5.88 (C-2′ & C-3′ protons), 6.42 (C-1′ proton). **9a**: R=Cl; m.p.=173°C; yield 70%; IR (KBr): 3255 (>NH anilino), 1710 (>C=O), 1145-1060 (C-O-C), 640 (C-S-C), 755 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.65-8.21 (Ar-H & NH anilino), 3.95 ($-OCH_3$), 2.10 ($-CH_3$), 4.44-4.80 (C-5′ and C-4′s protons of sugar), 5.78-5.90 (C-2′ & C-3′ protons), 6.44 (C-1′ proton). **9b**: R=H; m.p.=142°C; yield 64%; IR (KBr): 3240 (>NH anilino), 1730 (>C=O), 1165-1070 (C-O-C), 650 (C-S-C), 775 cm^{-1} (C-Cl); ^1H NMR ($CDCl_3$): δ 6.63-8.28 (Ar-H & >NH anilino), 3.97 ($-OCH_3$), 2.07 ($-CH_3$), 4.48-4.85 (C-5′ and C-4′ protons of sugar), 5.80-5.97 (C-2′ & C-3′ protons), 6.43 (C-1′ protons).

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