Synthesis and Characterization of Model Ultimate Carcinogens/Metabolites Derived from Lead Tetraacetate Oxidation of Arylnitrones: 2’-Deoxyguanosine Adducts

Honnaiah Mallesha, Kodagahally R. Ravi Kumar, Kempegowda Mantelingu, Kanchugarakoppal S. Rangappa*

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570006, India
Fax +91(821)518835; E-mail: rangappak@yahoo.com

Received 9 March 2001; revised 23 April 2001

Abstract: The synthesis of model reactive metabolites 4a–c by lead tetraacetate (LTA) oxidation of aryl nitrones 3a–c is described. Compounds 4a–c react with deoxyguanosine (dG) to give N-benzoylated C8-adducts 5a–c. Following debenzoylation with the heterogeneous system (sodium carbonate/methanol) leads to the corresponding C8-adducts 6a–c.

Key words: model ultimate carcinogens, reactive metabolites, LTA oxidation, aryl nitrones, debenzoylation, 2’-deoxyguanosine adducts

The chemistry of aromatic amines, amides and nitro compounds is of considerable interest due to their carcinogenic activity.1 Nitrones have figured prominently in both synthetic2–6 and biomedical science.4,6,7 So far, most of the work on nitrene utility has concentrated on the construction of heterocyclic rings,2 however, several examples of biologically interesting molecules have not been noted. Though nitrones and their reaction products have been identified and extensively studied in carcinogenesis by various workers,3,8 the literature survey has indicated further scope for identification and other studies. In the interest of the biological3,5,8 and physical properties9 of compounds 4a–c, 5a–c and 6a–c, it is necessary to obtain sufficient quantities by chemical synthesis. All the above, prompted us to design a new route to synthesize adducts 5a–c and 6a–c via aryl nitrones 3a–c (Scheme 1).

The N-hydroxyarylamines 2a–c, precursors for the synthesis of 3a–c were prepared by the reduction of nitro compounds 1a–c. Condensation of N-hydroxyarylamines 2a–c with benzaldehyde in ethanol gave 3a–c. The nitrones 3a–c were found to be light sensitive,10 decomposing to aldehyde, amine, azobenzene, imine etc. Hence they were stored in the dark until further use.

N-Acetoxy compounds 4a–c were prepared by the lead tetraacetate (LTA) oxidation11 of 3a–c in benzene. The reaction appears to proceed through the intramolecular 1,4-acetyl transfer, leads to the formation of products, 4a–c (Scheme 2).

Most reactions are temperature dependent and maintained between –5 °C to 0 °C. Two of the three, 3a and 3c were obtained as solids after purification, while 3b was an oil11 after distillation under reduced pressure. Compounds 4a–c were stable in a freezer for several days. IR and 1H NMR spectral data have confirmed the structures of 4a–c. IR absorption in the region ν = 1762–1788 cm⁻¹ was assigned for ester C=O stretching frequency and the absorption at comparatively lower region ν = 1708–1714 cm⁻¹ was assigned for amide C=O stretching frequency. The 1H NMR peak in the region δ = 2.16–2.25 was assigned for OCOCH₃ protons. Other spectral data were consistent with structures of 4a–c.

The reaction of 4a–c with deoxyguanosine (dG) in sodium citrate buffer of pH 6.9 gave 5a–c. They were confirmed by the 1H NMR spectral studies. The absence of the very important peak at δ = 8.01, which was attributed to the C8-H proton in dG12 indicated that substitution had taken place.
place at C8 position of the dG moiety. 1H NMR spectra showed peaks in the region δ = 10.38–10.72 and 6.38–6.55 to NH and NH2 protons, respectively, of deoxyguanosine. This indicates that there was no substituent on NH and NH2 in the dG moiety. Absence of one NH proton in 5a–c in the region δ = 8.48–8.81 assigned for 6a–c confirmed the presence of a substituent on nitrogen atom of 5a–c. The disappearance of all active protons in NH and OH upon the addition of D2O indicates that, NH and OH in 5a–c are free from substituent. Other spectral data were consistent with structures of 5a–c.

The compounds 5a–c were debenzoylated13 to form products14 6a–c. Appearance of a peak in the region δ = 8.48–8.81, attributed to the NH proton, and its disappearance upon addition of D2O confirmed the structures of products 6a–c.

In conclusion, the LTA oxidation of 3a–c to give 4a–c, and their reaction with dG to form C8 adducts 5a–c can be summarized as follows: (i) 4a–c are also reactive metabolites like other N-acetoxyarylamines and amides reported earlier,14,15 (ii) nitrones 3a–c are precarcinogens i.e., they essentially require activation14 to produce the reactive metabolites 4a–c. LTA is used as an excellent oxidant1 to activate 3a–c into reactive metabolites 4a–c. This is a simple activation pathway to obtain 4a–c via aryl nitrones 3a–c.

TLC was performed with 0.2 mm silica gel GF254 (E-Merck) with fluorescent indication. The mobile phases used for TLC: CHCl3, benzene, CHCl3–hexane (7:2), EtO–H2O (2:1), MeOH–H2O (7:3) and (9:1), EtOAc–hexane (2:1), MeOH, MeCN–MeOH (4:3), benzene–EtO (1:2). All HPLC analyses were performed with one of the mobile phases: H2O–MeCN (6:1, 7:3 and 8:3). Compounds were obtained from the commercial sources as indicated: zinc dust (E-Merck), NH4Cl (s.d. fine), hydrazine (s.d. fine), Pd 5% on activated carbon (E-Merck), dG (Aldrich), nitrobenzene (E-Merck), 4-nitrotoluene (E-Merck), 1-chloro-4-nitrobenzene (E-Merck), LTA (Aldrich), benzaldehyde (E-Merck), silica gel-923 (Aldrich), Al (E-Merck). Melting points were recorded on SELACO 605 melting point apparatus and were uncorrected. 1H NMR Spectra were recorded on Bruker AMX-400, 400Mz, NMR spectrophotometer using CDCl3 and DMSO-d6 as solvent with TMS as an internal standard. IR spectra were recorded on a Bio-Rad Win-IR spectrometer. Elemental analyses were obtained on a Vario-EL instrument. Low temperature reactions were carried out using cryostat model MRP 700. All HPLC analysis were performed with Lachrom-2000 Merck-Hitachi L7100 pump with RP18.250-4 mm column and UV Detector-UV-VIS L7400.

Nitrone 3a–c: General Procedure

Equimolar solutions of aryl hydroxylamines17 2a–c and benzaldehyde in a minimum volume of EtOH or aq EtOH were kept at r.t. in the dark for 5–7 h. When no precipitate had formed, the solution was diluted with H2O until milky, warmed slightly to give a clear solution and stored overnight at 0 °C. Needle like crystals obtained were recrystallized to constant mp from EtOH or aq EtOH. The analytical data of 3a,18 3b,19 and 3c20 were consistent with those reported in the literature.

N-Acetoxy-N-benzoyl Derivatives 4a–c; N-Acetoxy-N-benzoylaniiline (4b)11 Typical Procedure

A solution of 3b (500 mg, 2.54 mmol) in anhyd benzene (10 mL) was kept between −5 °C to 0 °C. Exothermic reaction took place immediately on portion wise addition of LTA (1.60 g, 3.61 mmol). The mixture was stirred for 15 min at r.t. Filtration of the white lead diacetate precipitate and evaporation of the solvent at reduced pressure yielded the crude product. Distillation of resulting oil under reduced pressure gave 4b.

1H NMR (400 MHz, CDCl3): δ = 2.20 (s, 3 H, OCOCH3), 7.06–7.38 (m, 5 H, NC6H5), 7.6–7.80 (m, 5 H, COC6H5). IR (KBr): ν = 1710 (PhC=O), 1786 (CH3C=O), 1482, 1502 (C–N), 1118 cm−1 (O–COCH3). Anal. Calcd for C16H15NO3: C, 71.37; H, 5.58; N, 5.20. Found: C, 70.65; H, 5.02; N, 5.44.

N-Acetoxy-N-benzoyl-4-methylaniline (4a)

Obtained from 3a (500 mg, 2.37 mmol) and LTA (1.50 g, 3.38 mmol), as solid (592 mg, 93%); mp 131–132 °C.

1H NMR (400 MHz, CDCl3): δ = 2.16 (s, 3 H, OCOCH3), 7.02–7.18 (m, 4 H, NC6H5), 7.4–7.45 (m, 5 H, COC6H5). IR (KBr): ν = 1708 (PhC=O), 1762 (CH3C=O), 1486, 1508 (C–N), 1120 cm−1 (O–COCH3). Anal. Calcd for C16H15NO3: C, 71.37; H, 5.58; N, 5.20. Found: C, 71.08; H, 5.52; N, 5.28.

N-Acetoxy-N-benzoyl-4-chloroaniline (4c)

Obtained from 3c (500 mg, 2.61 mmol) and LTA (1.70 g, 3.83 mmol), as solid (592 mg, 93%); mp 131–132 °C.

1H NMR (400 MHz, CDCl3): δ = 2.25 (s, 3 H, OCOCH3), 7.12–7.60 (m, 9 H, Ar–CH3). IR (KBr): ν = 1714 (PhC=O), 1788 (CH3C=O), 1488, 1502 (C–N), 1112 cm−1 (O–OCOCH3). Anal. Calcd for C16H13ClNO3: Cl, C, 62.18; H, 4.14; N, 4.84. Found: C, 61.09; H, 4.24; N, 4.92.

dG Adducts 5a–c; N-(Benzoyl)-N-(deoxyguanosin-8-yl)-4-methylaniline (5a); Typical Procedure

Significant modifications were made to the procedure used by Kriek et al.20 in the synthesis of Gu-adducts.

Compound 4a (183 mg, 0.68 mmol) in 95% EtOH (15 mL) was added to dG (49 mg, 0.17 mmol) in a 2 mL sodium citrate buffer of pH 6.9 (30 mL) at 40–45 °C over 2 h and the mixture was stirred further.
8 h at 60–65 °C. The reaction mixture was diluted with H2O (50 mL) and the EtOH was evaporated. The aqueous phase was extracted successively with Et2O (2×10 mL), butanol (3×10 mL) and EtOAc (2×10 mL). The combined extracts of Et2O, butanol and EtOAc were dried (Na2SO4). The crude product obtained on removal of solvent was first purified over a silica gel column with MeOH–CHCl3 (2:7) and then chromatographed on sephadex G-15 with EtOH–CHCl3 (7:3) to give the product 5a (6 mg, 9%). Analysis of the aqueous solution by HPLC with H2O–MeCN (6:1) showed that the product 5a was 99% pure.

1H NMR (400 MHz, DMSO-d6); δ = 1.82 (m, 1 H, H1'), 2.14 (s, 3 H, Ar-CH3), 2.60 (m, 1 H, H1'), 3.61 (m, 2 H, H5'), 3.82 (d, 1 H, H1'), 4.36 (m, 1 H, H1'), 5.28 (s, 1 H, H1'-OH), 5.82 (s, 1 H, H1'-OH), 6.24 (dd, 1 H, H1'), 6.38 (s, 2 H, Gu-NH2), 6.92 (d, 2 H, Ar-H), 7.55 (d, 2 H, Ar-H), 7.59–7.82 (m, 5 H, Ar-H), 10.72 (br s, 1 H, Gu-NH).

IR (KBr): v = 3390, 2925, 1684, 1638, 1516, 1455, 1102, 1050, 1024, 1002, 830 cm–1.

These compounds were prepared by the method of Underwood et al.13 using heterogeneous system (Na2CO3/MeOH) for the debenzoylation of 5a–c. Spectral data are in good agreement with an authentic sample.14,16

Acknowledgement
We are grateful to the Department of Science and Technology (DST), Government of India, New Delhi, for financial support under project No. SP/S1/G05/97.

References


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