THE SYNTHESIS OF HAPTENIC DERIVATIVES OF AMINOIMIDAZOAZAARENE COOKED-FOOD MUTAGENS

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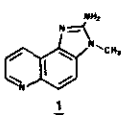
Abstract — 2-N-Aminopropyl derivatives of the food mutagens IQ, MeIQx and PhIP were synthesized along with 3-(2-amino-3-methylimidazo[4,5-f]quinoxalin-8-yl) propionic acid. These were used as haptens for the production of monoclonal antibodies.

World-wide epidemiological studies show that dietary considerations are one of the most important factors in the induction of human cancers[1-3]. Many researchers[4-6] have demonstrated the presence of high levels of mutagenic activity in foods as a result of the cooking process. The major mutagens present appear to be the aminoimidazoazaarenes (AIA's), some of which are among the most mutagenic compounds ever tested on the Ames/*Salmonella* test[7].

In order to assess the risk that these compounds present, accurate determinations must be made of the amounts present in the diet. Currently it requires about one man-month of labor to prepare a sample of cooked meat for analysis by liquid chromatography and subsequent mass spectrometry[8]. Quantitation is uncertain because of the many steps involved and many mutagenic AIA's are present which have yet to be identified. An immunoassay could reduce the time necessary for each analysis by reducing the sample clean-up prior to analysis and thus offer more accurate quantitation of known mutagens. Immunoassays have been shown to be useful in monitoring human tissue samples and the environment for trace chemicals[9]. Also, an immuno-affinity column which specifically bound AIA mutagens would facilitate the purification of the unidentified mutagens.

We, therefore, chose to develop two different groups of monoclonal antibodies 1) a class specific antibody that would bind with equal affinity all of the AIA's which possess 2-amino-N-methylimidazole functionality, and 2) compound specific antibodies, each of which would recognize a single mutagen with a high degree of specificity. Representative compounds from this group of mutagens were selected to be 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 1; 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx), 2; and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 3.



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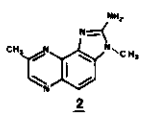

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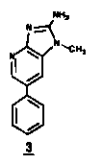

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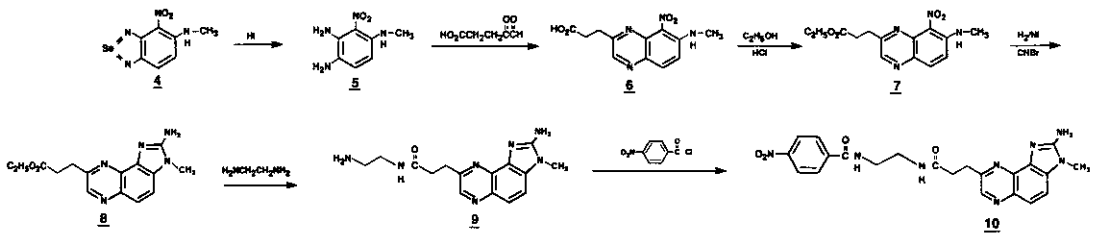
Low molecular weight compounds such as these do not elicit an immune response in their own right and must first be conjugated to a high molecular weight carrier. These compounds do not have a functionality which would allow for straight foward conjugation and therefore derivatives were synthesized. The hapten to generate the class specific (anti-AIA) antibody was MeIQx linked through the 8-methyl group. Grivas[10] described a synthesis of MeIQx using the benzoselenadiazole 4 as an intermediate. This strategy (Scheme 1) conveniently allowed the incorporation of additional functionality late in the synthesis.

Thus, compound 4 (7.20 g, 27.9 mmole) was demetalated by the action of concentrated hydriodic acid[11]. The crude triamine 5 was suspended in 300 ml of water, 4,5-dioxopentanoic acid[12] (from 11.0 g (53.9 mmole) of 5-benzylidinelevulinic acid) was added and the mixture was heated for 3 h at 70°C. The suspension was cooled to 0°C and the resulting quinoxaline was collected by filtration and recrystalized from water/ethanol to give 3.90 g (14.1 mmole, 51% yield) of 6[13]. This condensation produces a 95/5 mixture of a 3 and 2-substituted quinoxaline. The structure of the major isomer was confirmed by the downfield chemical shift of the 2-proton relative to the 3-proton in the nmr spectra[14] and was easily purified by fractional crystalization.

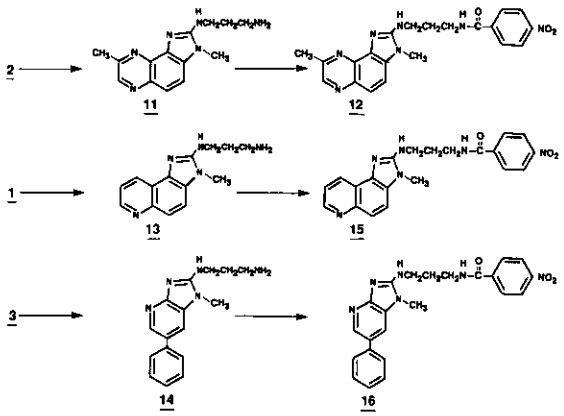
Scheme 1



Quinoxaline 6 was then esterified in hydrogen chloride saturated ethanol to give 7[15] in 90% yield after an acid/base workup. The ester 7 (3.00 g, 9.9 mmole) was reduced to the diamine and cyclized with cyanogen bromide as was MeIQx[14]. The reaction mixture was evaporated in vacuo and the residue crystalized from chloroform/ethyl ether to give 8[16] (2.3 g, 6.9 mmole, 70% yield). The ester 8 (300 mg, 1.0 mmole) was dissolved in 1.0 ml of ethylenediamine and 5.0 ml of ethanol and heated at 70°C for 12 h. The solvent was evaporated and the residue was nearly pure 9[17] of which a small amount was purified by HPLC[18] for characterization. The crude amine 9 (250 mg, 0.80 mmole) was suspended in 10 ml of pyridine and 200 mg (1.0 mmole) of 4-nitrobenzoyl chloride was added and the mixture stirred for 2 h at room temperature. The reaction was quenched with water and evaporated. The residue was triturated with chloroform to give the nitrobenzamide 10[19] (200 mg, 0.47 mmole, 54% yield) which was further purified by HPLC[18].

The hapten used to derive antibodies specific for MeIQx was synthesized by attaching a linker group to the 2-amino group of MeIQx[10] by a straightfoward exchange reaction. MeIQx(2, 200 mg, 0.94 mmole) was heated in a teflon, pressure vessel with 4.4 g of propylenediamine at 175°C for 7 days. The solvent was removed *in vacuo* and the residue purified by flash chromatography[20] on silica gel (chloroform/methanol, 3:1) to give 2-N-aminopropyl-MeIQx 11[21] (172 mg, 0.64 mmole) in 68% yield. Some MeIQx (15 mg) was also recovered. The propylamino-MeIQx, 11, was acylated with 4-nitrobenzoyl chloride in pyridine to give after chromatography the nitrobenzamide 12[22]. IQ[23] and PhIP[24] were converted to their corresponding propylamino derivatives 13[25] and 14[26] and nitrobenzamides 15[27] and 16[28] respectively, and were used to generate the anti-IQ and anti-PhIP specific antibodies.

Scheme 2



The nitro groups of compounds 10,12, 15, and 16 have been reduced to the corresponding amines (H_2,10% Pd/C, ethanol), the amines were converted to the diazonium salts by the action of nitrous acid and then allowed to react with bovine serum albumin and keyhole limpet hemocyanin[29]. The diazatization of the 2-amino group of compound 10 was not a competing reaction due to it's low reactivity with nitrous acid[8]. We have immunized mice with these protein conjugates and have produced a set of monoclonal antibodies with the desired range of specificities[30,31].

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15. Ms, m/z (rel. int.): 304.1172(23), calculated for $C_{14}H_{16}N_4O_4$, 304.1171, 259(19), 231(100), 197(29); ^{1}H nmr (DMSO-d_6): δ8.60 (2-H,s), 8.01 (8-H,d,J=9.5 Hz), 7.48 (7-H,d,J=9.5 Hz), 7.29 (N-H,q,J=4.7 Hz), 4.04 (O-CH_2,q,J=7.1 Hz), 3.20 (OC-CH_2,t,J=6.8 Hz), 2.97 (N-CH_3,d,J=4.7 Hz)), 2.81 (3-CH_2,t,J=6.8 Hz), 1.61 (C-CH_3,t,J=7.1 Hz); mp 137-138 °C.

16. Ms, m/z (rel. int.): 299.1392(23), calculated for $C_{15}H_{17}N_5O_2$ 299.1382, 254(10), 226(100); ^{1}H nmr (DMSO-d_6): δ8.66 (7-H,s), 7.74 (5-H,d,J=8.7 Hz), 7.56 (4-H,d,J=8.7 Hz), 6.57 (NH_2,s), 4.04 (O-CH_2,q,J=7.1 Hz), 3.66 (N-CH_3,s), 3.24 (CO-CH_2,t,J=7.2 Hz), 2.94 (8-CH_2,t, J=7.2 Hz), 1.14 (C-CH_3,t,J=7.1 Hz); mp 221-222°C(decomp.).

17. Ms, m/z (rel. int.): 313.1632(3), calculated for $C_{15}H_{19}N_7O$ 313.1652, 226(38), 213(100); ^{1}nmr (DMSO-d_6): δ8.66 (7-H,s), 7.73 (5-H,d,J=8.7 Hz), 7.56 (4-H,d,J=8.7 Hz), 3.66 (N-CH_3,s), 3.5-3.0 (CH_2,NH_2,m), 2.69 (8-CH_2,t,J=7.6).

18. High purity samples were produced by high pressure liquid chromatography on a 0.87 x 30 cm 5 μm Nucleosil-NH_2 silica column, eluted with a gradient hexane/n-propanol/acetic acid (89:10:1 to 59:40:1) at a flow rate of 2 ml/min.

19. Ms, m/z (rel. int.): 462.1741(4), calculated for $C_{22}H_{22}N_8O_4$ 462.1764, 445(4), 254(32), 241(18), 226(65), 150(100). ^{1}H nmr (DMSO-d_6): δ8.66 (7-H,s), 8.19 (2'-H,d,J=9.0 Hz), 7.94 (3'-H,d,J=9.0 Hz), 7.71 (5-H,d,J=8.8 Hz), 7.54 (4-H,d,J=8.8 Hz), 3.64 (CH_3, s), 3.3 (CH_2,m), 2.70 (8-CH_2,t,J=7.7 Hz).

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22. Ms, m/e (rel. int.): 419.1710(6), calculated for $C_{21}H_{21}N_7O_3$ 419.1705, 372(42), 150(100); ^{1}H nmr (DMSO-d_6): δ8.65 (7-H,s), 8.31 (3'-H,d,J=9.0 Hz), 8.18 (2'-H,d,J=9.0 Hz), 7.76 (5-H,d,J=8.7 Hz), 7.58 (4-H,d,J=8.7 Hz), 3.66 (N-CH_3,s) 3.56 (2-N-CH_2), t,J=6.6 Hz), 3.44 (CON-CH_2, t,J=6.6 Hz), 2.63 (8-CH_3,s), 1.94 (C-CH_2-C,m,J=6.6 Hz).

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25. Ms, m/e (rel. int.): 255.1501(92), calculated for $C_{14}H_{17}N_5$ 255.1484, 225(100), 211(72), 197(47); ^{1}H-nmr (DMSO-d_6) δ 8.72 (7-H,dd,J=4.2 Hz J=1.7 Hz), 8.61 (9-H,dd,J=8.3, 1.7 Hz), 7.73 (5-H,d,J=8.8 Hz), 7.58 (4-H,d,J=8.8 Hz), 7.4 (8-H,dd,J=8.3, 4.2 Hz), 3.64 (CH_3,s), 3.53 (2-N-CH_2,t,J=6.4 Hz), 3.28 (NH_2-CH_2,t,J=6.4 Hz), 1.84 (C-CH_2-C,m,J=6.4 Hz).

26. Ms, m/z (rel. int.): 281.1636(55), calculated for $C_{16}H_{19}N_5$ 281.1640, 251(80), 238(100), 223(60); ^{1}H-nmr (DMSO-d_6) δ 8.29 (5-H,d,J=2.0 Hz), 7.75 (7-H,d,J=2.0 Hz), 7.69 (2'-H,d,J=7.3 Hz), 7.46 (3'-H,t,J=7.3 Hz) 7.32 (4'-H,t,J=7.3 Hz), 3.56 (CH_3,s), 3.28 (N-CH_2,m), 1.85 (C-CH_2-C,m,J=6.0 Hz).

27. Ms, m/z (rel. int.): 404.1539(45), calculated for $C_{21}H_{20}N_8O_3$ 404.1597, 225(73), 211(100), 198(37), 150(55); ^{1}H nmr (DMSO-d_6): δ8.94 (CON-H,t,J=8.9 Hz), 8.72 (7-H,dd,J=4.2 Hz,J=1.7 Hz), 8.53 (9-H,dd,J=8.3 Hz,J=1.7 Hz), 8.32 (3'-H,d,J=6.0 Hz), 8.08 (2'-H,d,J=6.0 Hz), 7.74 (5-H,d,J=8.8 Hz), 7.59 (4-H,d,J=8.8 Hz), 7.34 (8-H,dd,J=8.3 Hz, J=4.2 Hz), 6.8 (2-N-H,t,J=5.7 Hz), 3.64 (N-CH_3,s), 3.55 (CON-CH_2,t,J=6.9 Hz), 3.44 (2-N-CH_2,t,J=6.9 Hz), 1.97 (C-CH_2-C,m,J=6.9 Hz).

28. Ms, m/z (rel. int.): 430.1771(7) calculated for $C_{23}H_{22}N_6O_3$ 430.1754, 261(100), 251(66), 237(63), 224(72).

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30. To be published elsewhere.

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