

SYNTHESIS AND BIOLOGICAL EVALUATION OF METHYLATED DERIVATIVES OF THE COOKED  
FOOD MUTAGEN METABOLITE 2-AMINO-3,6-DIHYDRO-3-METHYL-7H-IMIDAZO[4,5-f]-  
QUINOLIN-7-ONE (7-OH-IQ)

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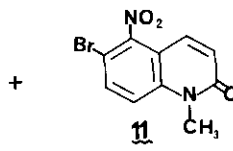
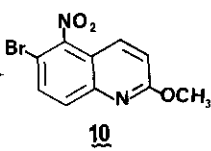
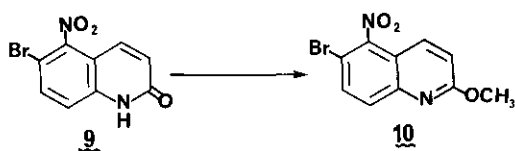
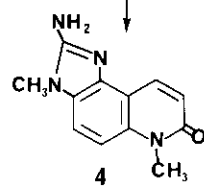
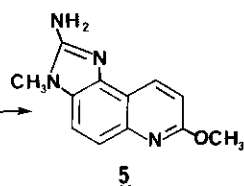
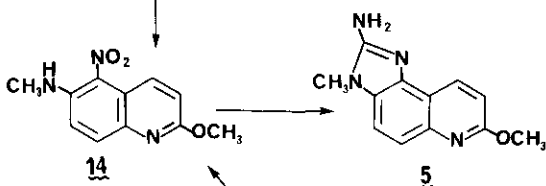
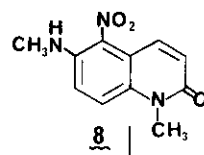
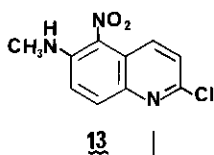
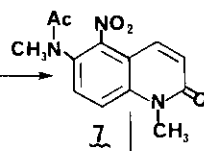
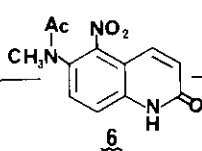
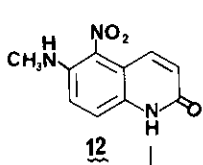
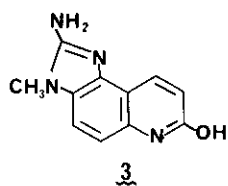
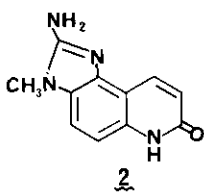
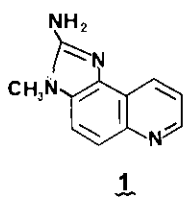
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Abstract--The major anaerobic metabolite of the potent cooked food mutacarcinogen IQ is the oxidised product 7-OH-IQ, which is itself a powerful direct-acting mutagen. The O-methyl and N-methyl derivatives of 7-OH-IQ have been prepared to determine whether the tautomeric form of 7-OH-IQ plays any role in its bioactivity. Both N-methyl 7-OH-IQ and O-methyl 7-OH-IQ show comparable mutagenicity when tested directly against the T98 strain of *S. typhimurium*, indicating that the quinolone structure does not play a major role in the mutagenicity of 7-OH-IQ. Neither 7-OH-IQ nor the methylated derivatives cleaved DNA in the presence of metal cations.

The cooked-food mutagen 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ, 1) has been shown to be a carcinogen in rats<sup>1</sup> and may thus play a significant role in the etiology of colon cancer. In the presence of human feces and also in the human intestine it is metabolized to the oxidized derivative 2-amino-3,6-dihydro-3-methyl-7H-imidazo[4,5-f]quinolin-7-one (7-OH-IQ, 2).<sup>2,3</sup> Significantly, although IQ requires metabolic activation for its mutagenicity, 7-OH-IQ is a powerful direct-acting mutagen.<sup>4</sup> The possibility thus exists that the carcinogenicity of IQ is mediated in part through its conversion to 7-OH-IQ in the human colon, followed by cancer induction by 7-OH-IQ. For this reason, it is essential to understand the origin of the direct acting mutagenicity of 7-OH-IQ. Direct acting mutagens are normally presumed to be alkylating agents,<sup>5</sup> following the initial proposal that active carcinogen metabolites act as electrophilic reagents.<sup>6</sup> We thus initially elected to test the hypothesis that 7-OH-IQ (2) functions as an alkylating agent. The structure of 7-OH-IQ is predominantly the quinolin-2-one form 2 rather



than the tautomeric 2-hydroxyquinoline form **3**; the evidence for this is based on the spectroscopic properties of the isolated metabolite<sup>2a</sup> and on the structure of quinolin-2-one itself.<sup>7</sup> It is thus possible that the  $\alpha,\beta$ -unsaturated amide function of 7-OH-IQ acts as a weakly electrophilic group by reacting as a Michael acceptor, and is thus the cause of its direct mutagenicity. The simpler  $\alpha,\beta$ -unsaturated amide acrylamide has been shown to alkylate both 2'-deoxynucleosides and calf thymus DNA at pH 7.0 and 37°C,<sup>8</sup> and it has also been shown to induce a dose-dependent cytotoxic effect in C3H/10T 1/2 and NIH/3T3 mouse fibroblast cells.<sup>9</sup> It is not, however, a direct acting mutagen against five TA strains of *Salmonella typhimurium*.<sup>10</sup> If 7-OH-IQ is mutagenic by virtue of its ability to act as a Michael acceptor, then its N-methyl derivative **4** should show comparable mutagenicity to the parent compound while its O-methyl derivative **5**, which lacks the  $\alpha,\beta$ -unsaturated amide function, should not show comparable mutagenicity. We thus prepared compounds **4** and **5** for mutagenicity testing.

#### RESULTS AND DISCUSSION

The synthesis of N-methyl 7-OH-IQ **4** was achieved in a straightforward manner from the known intermediate 6-(N-acetyl-N-methylamino)-5-nitroquinolin-2-one (**6**).<sup>2b</sup> Alkylation of **6** with methyl iodide in methanolic potassium carbonate yielded the N-methyl derivative **7** exclusively. Mild hydrolysis of **7** followed by reduction and reaction with bromocyanogen yielded **4** in an overall yield of 60% from **6**.

Preparation of O-methyl 7-OH-IQ **5** proved more difficult. Initial experiments aimed at preparing **5** by a direct alkylation procedure showed that the N-methyl derivative always predominated, even when alkylation was carried out in aprotic solvents. The best yield of O-methyl product was obtained by alkylation of 6-bromo-5-nitro-2(1H)-quinolinone (**9**) to give a 1:6 ratio of O-methyl (**10**) to N-methyl (**11**) derivatives. Treatment of the O-methyl derivative **10** with methylamine yielded the same amino nitro quinoline **14** as obtained by the route described below.

The most efficient preparation of O-methyl 7-OH-IQ **5** was found to be by displacement of a 2-chloroquinoline derivative with sodium methoxide. The known 6-methylamino-5-nitro-2(1H)-quinolinone (**12**)<sup>2b</sup> was treated with phosphorus oxychloride to give the 2-chloroquinoline **13**. Reaction of **13** with sodium methoxide gave the 2-methoxyquinoline **14**, identical with the compound prepared by the method described above. Reduction of **14** and treatment with bromocyanogen gave O-methyl 7-OH-IQ (**5**).

The structures of the alkylated 7-OH-IQ derivatives follow from their methods of preparation and their spectroscopic properties. In particular, the N-methyl derivatives consistently showed an infrared absorption for an amide carbonyl group in the 1640-1670  $\text{cm}^{-1}$  range, while the O-methyl derivatives showed only absorption for the aromatic ring in the 1600-1630  $\text{cm}^{-1}$  range. In their <sup>1</sup>H NMR spectra the signal for the O-methyl protons was consistently downfield of the signal for

the N-methyl protons of the isomeric compound by about 0.3 ppm, and the vicinal coupling constant of the 3,4-protons was slightly higher for the N-methyl derivative.

The two methylated 7-OH-IQ derivatives **4** and **5** were tested for mutagenicity against *S. typhimurium* strain TA98 by the methods described previously.<sup>4</sup> Testing was carried out without metabolic activation by arochlor-induced S9 fractions so that only direct-acting mutagenicity was determined. The results are shown below in Table 1.

Table 1

Mutagenicity of IQ Analogs against *S. typhimurium* TA98

Compound	Revertants/plate		Average net revertants
	Control	Compound	
IQ 1	28, 30, 33	25, 27, 31	0
7-OH-IQ 2	28, 30, 33	445, 472, 524	455
N-Me 7-OH-IQ <b>4</b>	28, 30, 33	362, 382, 424	362
O-Me 7-OH-IQ <b>5</b>	28, 30, 33	280, 291, 313	272

The data of Table 1 indicate that there is no major difference in direct-acting mutagenicity between the N-methyl and O-methyl analogs **4** and **5**. Both compounds show significant direct-acting mutagenic effects on *S. typhimurium* TA98, although both compounds are slightly less active in this respect than 7-OH-IQ itself. It thus appears that the mutagenicity of these compounds is not related to their ability to act as Michael acceptors.

A second possible explanation of the mutagenicity of 7-OH-IQ is that it could chelate metal cations and thus facilitate metal-catalyzed oxygen-mediated DNA strand scission, analogous to that observed with a metal chelator such as 1,10-phenanthroline.<sup>11</sup> However, a test of this hypothesis using  $\phi$ X174 DNA and the method described by Hecht<sup>12</sup> failed to reveal any DNA-cleaving activity for 7-OH-IQ. The precise mechanism of action of 7-OH-IQ as a direct-acting mutagen thus remains to be determined.

#### EXPERIMENTAL

General experimental procedures were as previously described.<sup>2b</sup> Uv spectra were measured in ethanol.

#### 6-(N-Acetyl-N-methylamino)-1-methyl-5-nitroquinolin-2-one (7).

6-(N-Acetyl-N-methylamino)-5-nitroquinolin-2-one (**6**)<sup>2b</sup> (250 mg, 0.957 mmol) in MeOH (20 ml) was treated with  $K_2CO_3$  (200 mg). After stirring for 15 min  $CH_3I$  (1.5 ml) was added and the suspension stirred overnight. The solvent was then removed and the residue fractionated between

H<sub>2</sub>O and CHCl<sub>3</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated, and the crude product was purified by flash chromatography (CHCl<sub>3</sub>, then CHCl<sub>3</sub>:MeOH, 98.2), to yield **7** (250 mg, 95%), mp 223-226°C (EtOH): <sup>1</sup>H Nmr (CDCl<sub>3</sub>) 1.86 (3H,s), 3.19 (3H,s), 3.76 (3H, s), 6.89 (1H, d, J=9.9 Hz), 7.51-7.58 (3H, m); eims,  $m/z$  (relative intensity) 275 (M<sup>+</sup>, 4), 232 (55), 229 (100), 187 (45), 172 (15), 159 (16), 130 (20), 84 (35); uv  $\lambda_{\max}$  240 nm (6,100), 238 (44,400); ir (CHCl<sub>3</sub>) 1680, 1545, 1440, 1220 cm<sup>-1</sup>. Anal. Calcd for C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>: C, 56.72; H, 4.72, N, 15.27. Found: C, 56.69; H, 4.70; N, 15.19.

1-Methyl-6-Methylamino-5-nitroquinolin-2-one (8).

Compound **7** (200 mg, 0.727 mmol) was dissolved in 5% HCl (20 ml) and heated to 70°C for 20 h in an N<sub>2</sub> atmosphere. The solution was then basified with 10% NH<sub>4</sub>OH and extracted with CHCl<sub>3</sub>, and the CHCl<sub>3</sub> fractions were combined, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated. Flash chromatography (CHCl<sub>3</sub> then CHCl<sub>3</sub>:MeOH, 98.2) yielded **8** (165 mg, 97%) which was recrystallized from EtOH, mp 237-240°C: <sup>1</sup>H Nmr (CDCl<sub>3</sub>) 3.04 (3H, d, J=5 Hz), 3.73 (3H, s), 6.82 (1H, d, J=10 Hz), 7.07 (1H, bs), 7.13 (1H, d, J=9.6 Hz), 7.55 (1H, d, J=9.6 Hz), 8.26 (1H, d, J=10 Hz); eims  $m/z$ , (relative intensity) 233 (M<sup>+</sup>, 100), 216 (5), 203 (10), 187 (40), 172 (25), 158 (30), 143 (30), 130 (45), 117 (25), 103 (32), 89 (30); uv  $\lambda_{\max}$  460 nm (2,400), 244 (36,000); ir (CHCl<sub>3</sub>) 3450, 2950, 1660, 1615, 1510, 1470, 1350 cm<sup>-1</sup>. Anal. Calcd for C<sub>11</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>: C, 56.65; H, 4.72; N, 18.02. Found: C, 56.65; H, 4.66; N, 17.96.

2-Amino-3,6-dihydro-3,6-dimethyl-7H-imidazo[4,5-f]quinolin-7-one (N-Me-7-OH-10, 4).

Compound **8** (60 mg, 0.257 mmol) was dissolved in EtOH (15 ml) and Raney Ni (1/2 spatula) was added. The reaction mixture was hydrogenated for 20 min at room temperature with stirring. The catalyst was filtered off, and the filtrate was evaporated to 8 ml and treated with BrCN (45 mg, 0.42 mmol), with stirring for 14 h at room temperature. The crude hydrobromide product was filtered off, basified with 10% NH<sub>4</sub>OH, and again filtered off. The residue was washed with water and finally with EtOH. Recrystallization from EtOH afforded **4** (39 mg, 67%), mp > 330°C: <sup>1</sup>H Nmr (DMSO) 3.53 (3H, s), 3.60 (3H, s), 6.50 (1H, d, J=9.5 Hz), 6.70 (2H, s), 6.98 (1H, d, J=8.7 Hz), 7.41 (1H, d, J=8.7 Hz), 8.15 (1H, d, J=9.5 Hz); eims  $m/z$  (relative intensity) 228 (M<sup>+</sup>, 100), 213 (10), 199 (20), 185 (12), 129 (5), 114 (5), 83 (8); uv  $\lambda_{\max}$  365 nm (4,100), 327 (11,000), 320 (12,800), 270 (16,500), 222 (45,800); ir (KBr) 3400, 3150, 1638, 1600, 1540, 1440, 1330, 1235, 1200 cm<sup>-1</sup>. Calcd for C<sub>12</sub>H<sub>12</sub>N<sub>4</sub>O: M<sup>+</sup> 228.1011. Found: M 228.1010.

6-Bromo-2-methoxy-5-nitroquinoline (10) and 6-bromo-1-methyl-5-nitro-2(1H)-quinolinone (11).

A large excess of CH<sub>3</sub>I was added to 6-bromo-5-nitro-2(1H)-quinolinone (**9**)<sup>2b</sup> (0.9 g, 3.383 mmol) and K<sub>2</sub>CO<sub>3</sub> (0.92 g) in 100 ml of DMF, and the mixture was stirred for 18 h at room temperature.

Solvent was then removed in vacuo and the residue was dissolved in  $\text{CHCl}_3$  and washed with water. The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ), evaporated, and purified by flash chromatography ( $\text{CH}_2\text{Cl}_2$ ). Early fractions afforded **10** (122 mg, 13%), mp 123-125°C (EtOH):  $^1\text{H Nmr}$  ( $\text{CDCl}_3$ ) 4.07 (3H, s), 7.04 (1H, d,  $J=9.2$  Hz), 7.76-7.86 (3H, m); eims  $m/z$  (relative intensity) 284, 282 ( $\text{M}^+$ , 98, 100), 255, 253 (25, 30), 238, 236 (25, 30), 208, 206 (18, 18), 192 (30), 177 (25), 157 (55), 127 (50), 114 (100); uv,  $\lambda_{\text{max}}$  332 nm (2,100), 328 (2,100), 222 (64,500); ir ( $\text{CHCl}_3$ ) 1600, 1530, 1480, 1360, 1300  $\text{cm}^{-1}$ . Later fractions of the column yielded **11** (0.77 g, 82%) which was crystallized from EtOH, mp 175-178°C:  $^1\text{H Nmr}$  ( $\text{CDCl}_3$ ) 3.72 (3H, s), 6.84 (1H, d,  $J=9.7$  Hz), 7.36 (1H, d,  $J=8.9$  Hz), 7.45 (1H, d,  $J=8.9$  Hz), 7.77 (1H, d,  $J=9.7$  Hz); eims  $m/z$  (relative intensity) 284, 282 ( $\text{M}^+$ , 38, 40), 238, 236 (16, 18), 226, 224 (10, 10), 210, 208 (12, 14), 198 (10), 167 (10), 157 (20), 145 (18), 114 (40), 102 (35), 87 (48), uv  $\lambda_{\text{max}}$  343 nm (5,700), 240, (45,500); ir ( $\text{CHCl}_3$ ) 1660, 1580, 1535, 1360, 1230  $\text{cm}^{-1}$ .

6-Methylamino-5-nitro-2-chloroquinone (13).

6-Methylamino-5-nitro-2(1H)-quinolinone (**12**)<sup>2b</sup> (200 mg, 0.913 mmol) was added to  $\text{POCl}_3$  (5 ml) and heated to 100°C for 1 h. The reaction mixture was then poured into ice water and extracted with  $\text{CH}_2\text{Cl}_2$ , and the organic fractions were combined, washed with water, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. Purification by flash chromatography ( $\text{CH}_2\text{Cl}_2$ ) afforded **13** (166 mg, 77%) as a yellow solid, mp 140-142°C ( $\text{CH}_2\text{Cl}_2$ ):  $^1\text{H Nmr}$  ( $\text{CDCl}_3$ ) 3.19 (3H, d,  $J=5$  Hz), 7.35 (1H, d,  $J=9.7$  Hz), 7.47 (1H, d,  $J=8.9$  Hz), 8.00 (1H, d,  $J=9.7$  Hz), 9.00 (1H, bs) 9.12 (1H, d,  $J=8.9$  Hz); eims  $m/z$  (relative intensity) 239, 237 ( $\text{M}^+$ , 45, 100), 222, 220 (8, 18), 204 (10), 191 (35), 176 (20), 164 (30), 155 (18), 127 (40), 97 (42), 83 (40), 71 (50); uv  $\lambda_{\text{max}}$  432 nm (2,900), 320 (2,500), 260 (12,200); ir ( $\text{CHCl}_3$ ) 3420, 2960, 1620, 1570, 1500, 1400, 1340, 1200, 1170  $\text{cm}^{-1}$ . Anal. Calcd for  $\text{C}_{10}\text{H}_8\text{N}_3\text{O}_2\text{Cl}$ : C, 50.42; H, 3.36; N, 17.64. Found: C, 50.50; H, 3.16; N, 17.45.

2-Methoxy-6-methylamino-5-nitroquinoline (14).

Method a. Compound **13** (130 mg, 0.548 mmol) was added to a solution of sodium methoxide prepared by dissolving 2.8 g of Na in 80 ml of anhydrous methyl alcohol, and the reaction mixture was refluxed for 2 h, after which time tlc showed the absence of starting material. The solvent was removed, the residue fractionated between water and  $\text{CHCl}_3$ , and the chloroform fractions combined, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. Crystallization of the residue from EtOH gave **14** as needles (78 mg, 61%) mp 163-165°C:  $^1\text{H Nmr}$  ( $\text{CDCl}_3$ ) 3.14 (3H, d,  $J=5$  Hz), 4.00 (3H, s), 6.99 (1H, d,  $J=9.5$  Hz), 7.23 (1H, d,  $J=9.3$  Hz), 7.90 (1H, d,  $J=9.3$  Hz), 8.72 (1H, bs), 8.99 (1H, d,  $J=9.5$  Hz); eims  $m/z$  (relative intensity) 233 ( $\text{M}^+$ , 100), 216 (15), 203 (10), 187 (25), 172 (15), 158 (22), 144 (30), 129 (25), 116 (12), 102 (15), 89 (10); uv  $\lambda_{\text{max}}$  445 nm (10,300), 325 (8,400), 255 (38,300), 235 (43,000); ir ( $\text{CHCl}_3$ ) 3000, 1635, 1515, 1485, 1400, 1380, 1280  $\text{cm}^{-1}$ . Anal. Calcd for

$C_{11}H_{11}N_3O_3$ : C, 56.65; H, 4.72; N, 18.02. Found: C, 56.54; H, 4.59; N, 17.86.

Method b. 6-Bromo-2-methoxy-5-nitroquinoline (10) (44 mg, 0.154 mmol) was treated with 33% methylamine in EtOH (5 ml) and the solution was refluxed for 24 h. Solvent was then removed in vacuo and the residue was purified by flash chromatography ( $CHCl_3$ ) to afford 14 (30 mg, 83%), identical with that prepared by method a.

2-Amino-7-methoxy-3-methyl-3H-imidazo[4,5-f]quinoline (O-Me-7-OH-IQ, 5).

A stirred heterogeneous mixture of 14 (33 mg, 0.141 mmol) and Raney Ni (1/4 spatula) in 10 ml of EtOH was hydrogenated for 15 min at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to 5 ml and treated with BrCN (30 mg, 0.28 mmol) for 14 h. The hydrobromide was then filtered off, basified with 10%  $NH_4OH$  and again filtered off. The residue was washed with water and with MeOH and crystallized from MeOH to yield 5 (17 mg, 52%), mp 293-296°C:  $^1H$  Nmr (DMSO) 3.58 (3H, s), 3.93 (3H, s), 6.51 (2H, s), 6.90 (1H, d,  $J=8.8$  Hz), 7.31 (1H, d,  $J=8.7$  Hz), 7.55 (1H, d,  $J=8.7$  Hz), 8.41 (1H, d,  $J=8.8$  Hz); eims  $m/z$  (relative intensity) 228 ( $M^+$ , 100), 199 (28), 185 (20), 167 (10), 157 (10), 129 (12), 98 (50), 84 (72); uv  $\lambda_{max}$  345 nm (4,600), 310 (5,700), 300 (6,900), 260 (48,300), 219 (44,800); ir ( $CHCl_3$ ) 2950, 1615, 1580, 1580, 1460, 1430, 1410, 1350  $cm^{-1}$ . Calcd for  $C_{12}H_{12}N_4O$ :  $M^+$  228.1011. Found:  $M$  228.1011.

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