Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 625–630

BIOORGANIC &
MEDICINAL CHEMISTRY
LETTERS

REACTIONS OF 1-METHYLBENZIMIDAZOLE DERIVATIVES WITH *m*-CHLOROPEROXYBENZOIC ACID

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Abstract: Oxidation of seven 1-methylbenzimidazole (MBI) derivatives (with p*K*a's ranging from 1.6 to 6.0) was carried out with *m*-chloroperoxybenzoic acid and structures of the products formed were identified. (Condensed benzene moiety-hydroxylated)-2-(*m*-chlorobenzyloxy)-MBIs and 2-oxo-MBIs were obtained from MBIs with p*K*a's of more than 5.6 and about 3.3, respectively.

Carcinogens can react with cellular DNA to form carcinogen-DNA adducts. These DNA modifications are known to act as triggers of cancer induction. The imidazole moiety of 2'-deoxyguanosine (dG) is one of the major sites modified by carcinogens. For example, methylating carcinogens methylate mainly at the N-7 position of dG, arylaminating carcinogens arylaminate at the C-8 position of dG, and active oxygen-generating carcinogens give 7,8-dihydro-8-oxo-dG (often termed 8-hydroxy-dG). The reaction mechanisms of arylamination and oxidation of dG, however, are not clearly understood as yet. One of the reasons for this is that the yield of the modified product is as low as 0.1-1%, even in *in vitro* experiments. As a means of elucidating chemical carcinogenesis in the presence of arylaminating carcinogens, we have been studying the electrophilic amination of nucleic acid base components using hydroxylamine-*O*-sulfonic acid and *O*-(2,4-dinitrophenyl)hydroxylamine[1-12] as simple models of aminating agents. We have also used 1-methylbenzimidazole (MBI) derivatives as models of the imidazole moiety of purine bases and studied their amination. As a part of our intensive efforts to elucidate the chemical characteristics of this moiety, we examined here the oxidation of a series of MBI derivatives using *m*-chloroperoxybenzoic acid (MCPBA). Some of the products obtained with MCPBA were not those that were expected. The chemical characteristics of the imidazole moiety are discussed.

Reactions of 1-methylbenzimidazoles with *m*-chloroperoxybenzoic acid

Each MBI derivative (**1-7**) (1 mmol) was dissolved in 20 mL of $CHCl_3$, and MCPBA (Aldrich, 57-86% purity and containing *m*-chlorobenzoic acid and water, 690 mg, 2.3-3.4 mmol) was added (Table 1). The mixture was left standing at room temperature for 12 h and was then washed with aqueous 10% Na_2CO_3 solution. After the solvent was removed by evaporation, the products were separated by preparative thin layer chromatography (alumina, eluted with 2-3% MeOH in $CHCl_3$). Compounds **1-6** with p*K*a values of 3.28 to 6.0 gave small amounts of products **8-13** (separation yields of 5 ~ 10%) and about 70 to 80 % of the unreacted starting material was recovered. Structures of products **8-13** were identified from spectroscopic data.[13] Unequivocal structure determination was carried out with compounds **8** and **11**, and structures of compounds

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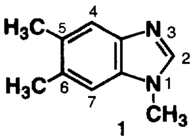
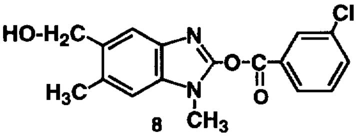
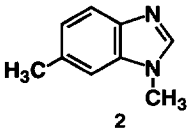
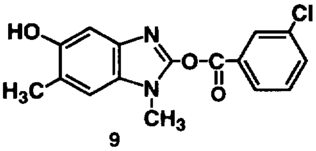
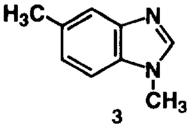
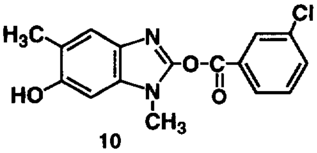
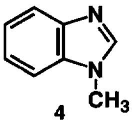
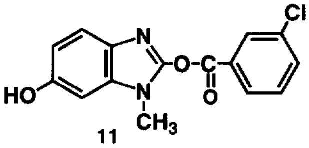
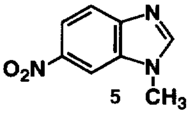
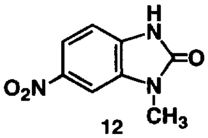
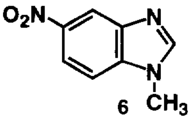
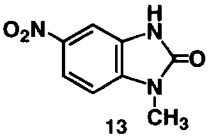
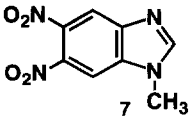
PII: S0960-894X(98)00082-1

Table 1

Starting material	p*K*a	Product[a]
 1	6.0	 8
 2	5.70	 9
 3	5.65	 10
 4	5.6	 11
 5	3.35	 12
 6	3.28	 13
 7	1.6	No reaction

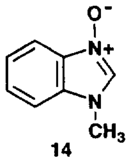
[a] Separation yields were 5-10%

9 and **10** were determined by referring to the data obtained with compounds **8** and **11**. Determination of the position of the OH group of compounds **8-11** was carried out by ^{13}C- and/or ^{1}H-NMR spectroscopy. NOE and ^{1}H-^{13}C HMBC techniques were used for identification of compounds **8** and **11**. Thus, the assignment of the 7-H proton of compounds **8** and **11** was carried out by NOE after 1-methyl protons were irradiated. Using HMBC, the carbon at the 7a-C could be assigned from three-bond couplings to a position between the protons of the 1-methyl group and the ring carbon atoms. The 4-H proton was assigned by HMBC to a position between the ring-protons and the carbon at the 7a-C. The position of the C-methyl group of compound **8** was determined by NOE between 7-H and 6-CH_3 protons. The position of the OH group of compound **11** was determined by HMBC by the assignment of protons at the 4- and 5-positions based on the 7a- and 3a-carbons, respectively. Acid hydrolysis of compound **11** gave 6-hydroxy-1-methyl-2-oxobenzimidazole whose structure was identified by NMR and mass spectroscopy. The position of the OH group of compounds **9** and **10** was determined from the ^{1}H-NMR spectra in which two singlets were observed without *meta* coupling. Structures of compounds **12**[14] and **13**[14] were identified by comparing spectral data with those of the authentic specimens. Structure determination of the products revealed two types. One consisted of (condensed benzene moiety-hydroxylated)-2-(*m*-chlorobenzoyloxy)-MBI derivatives **8-11** which were obtained from MBIs **1-4** with p*K*a's of more than 5.6, and the other consisted of 2,3-dihydro-2-oxo-MBI derivatives **12** and **13** which were obtained from MBIs **5** and **6** with p*K*a's of 3.28 and 3.35, respectively. No reaction took place with 5,6-dinitro-1-methylbenzimidazole **7** which has a low p*K*a value of 1.6. In reactions of MBIs **1-4** with MCPBA, the main products were (condensed benzene moiety-hydroxylated)-2-(*m*-chlorobenzoyloxy)-MBIs **8-11**, and no other products modified at only the 2 position or at the benzene moiety of MBI were obtained. This suggests that both hydroxylation and *m*-chlorobenzoyloxidation proceeded simultaneously. We have never observed a reaction with MCPBA in which *m*-chlorobenzoyloxidation was found to proceed. With respect to the formation of products **12** and **13**, oxidation by MCPBA at the carbon adjacent to the nitrogen was reported in quinazoline derivatives.[15] The quantitiy of MCPBA employed in our study was that usually used for oxidation of substrate. When a quantitiy 1.5 times that of the regular amount of MCPBA (3.5-5.1 mmol) was employed, precipitation of the reagent occurred and no increase in product formation was observed.

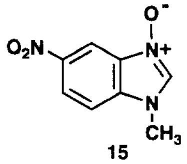
The reactions of MCPBA with adenosine and guanosine were not successful because of the insolubility of these nucleosides in $CHCl_3$. When magnesium monoperoxyphthalic acid (MMPP), with a reactivity known to be similar to that of MCPBA,[16] was used instead and allowed to react with adenosine or guanosine in neutral buffer, the reaction gave adenosine 1-oxide and no product, respectively. On the other hand, the reaction of guanosine with Fenton's reagent gave 7,8-dihydro-8-oxoguanine in a trace yield.

Preliminary experiments examining reaction mechanisms

Since the main reactivities of MCPBA are known to be *N*-oxidation of the tertiary ring nitrogen and epoxidation at the ene residues of the substrate,[16] we considered the possibility that the 3-oxide of MBI derivatives was initially formed as an intermediate. We then prepared 1-methylbenzimidazole 3-oxide (**14**)[17] and 1-methyl-5-nitrobenzimidazole 3-oxide (**15**)[18] and examined the reactions of each with MCPBA under the same conditions as employed with compounds **1-7**. Unlike reactions with compounds **1-6**, the reactions with **14** and **15** proceeded very rapidly and were completed within 2 h, giving products **11** and **13**, respectively, although the yields were 20 to 30%. When *m*-chlorobenzoic acid was used instead of MCPBA, there was no reaction. These results suggest that MCPBA was responsible for the initial N3-oxidation of MBIs and that other MCPBA molecules reacted with the oxide derivative to form the products. In order to examine whether *N*-oxides **14** and **15** were formed in the MCPBA-reaction with **4** and **6**, respectively, product analysis was carried out using HPLC. However, we failed to find peaks that corresponded to *N*-oxides **14** and **15**. The *N*-oxides formed may react rapidly with MCPBA to form products **11** and **13**. However, the low yields of **11** and **13** from **14** and **15**, respectively, suggests the involvement of other mechanisms. For formation of **12** and **13** from **5** and **6**, there is a possibility of an alternative mechanism by which electrophilic attack of the *m*-chloroperoxybenzoic acid anion at the 2-position of the protonated **5** and **6** and subsequent release of the *m*-chlorobenzoyloxy group gave **12** and **13** (Scheme 1). Further investigation on the reaction mechanisms is in progress.

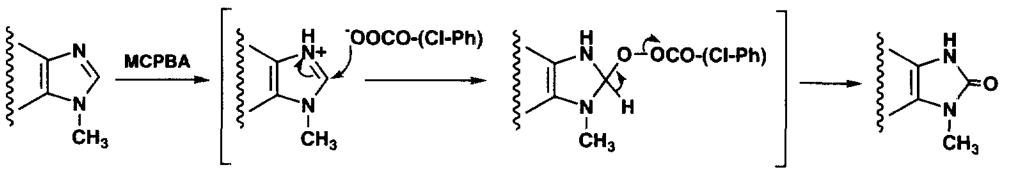


14



15

Scheme 1

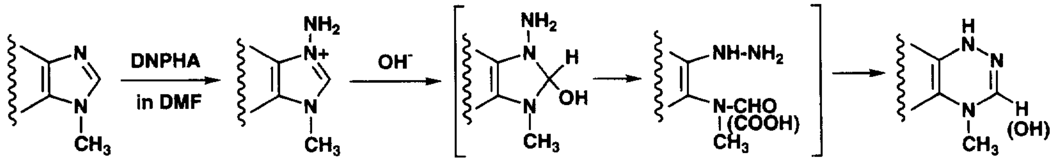


5, 6 **12, 13**

Comparisons of the reactivities of *m*-chloroperoxybenzoic acid and *O*-(2,4-dinitrophenyl)-hydroxylamine toward 1-methylbenzimidazole derivatives and of the reactivities of *N*-aminated 1-methylbenzimidazoles and 7-amino-9-ethylguanines in response to alkaline treatment.

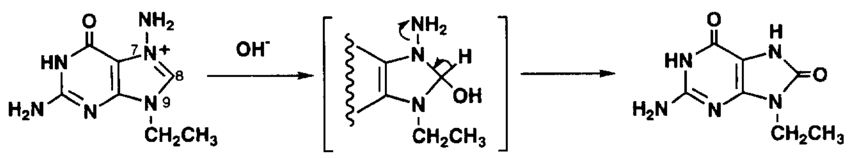
When the MBIs **1**, **4-6**, and **7** were each allowed to react with *O*-(2,4-dinitrophenyl)hydroxylamine (DNPHA), *N*-aminated benzimidazonium salts (**16**) were obtained in all but **7** and the yield was proportional to the p*K*a values of MBIs (Scheme 2).[8] With alkaline treatment, *N*-aminated benzimidazonium salts derived from MBIs **1** and **4** were stable and no product was obtained, while those derived from MBIs **5** and **6** gave benzotriazines (**17**) by imidazole ring-opening and subsequent re-closure.[8] With MCPBA oxidation, MBIs **1** and **4-6** yielded products **8** and **11-13**, respectively, and products **8** and **11** were similar as were products **12** and **13**. These results suggest that the electronegativity of the imidazole ring has a role in its reactivity, and that p*K*a values (approximately 3.35 to 5.6) of MBIs appear crucial in determining the reaction pathway. In terms of 9-ethylguanine derivatives, amination proceeded at the N7-position with p*K*a values of 3.2 and 3.4.[8] Unlike *N*-aminated MBI derivatives, alkaline treatment of 7-amino-9-ethylguaninium salt (**18**) derivatives afforded 7,8-dihydro-9-ethyl-8-oxoguanine derivatives (**19**) via C8-hydroxylation and the concomitant elimination of the 7-amino group (Scheme 3).[8] This indicates that the pyrimidine moiety of the purine nucleus also has a role

Scheme 2



1, 4-6 16 17

Scheme 3



18 19

in the reactivity of the imidazole moiety, in addition to the contribution of the basicity of the imidazole moiety. Further investigation along these lines is in progress.

Acknowledgements: We would like to thank Professor Y. Kawazoe of Nagoya City University for his helpful advice and encouragement. We also thank Professor Y. Uozumi of Nagoya City University for discussion. This work was supported in part by a Grant-in-Aid for Scientific Research (C), No.07672280, from the Ministry of Education, Science, Sports and Culture of Japan.

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13. **8**: Recrystallization from $CHCl_3$-hexane gave a light brown powder. mp 213-215 °C. ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3H, 6-CH_3), 3.40 (s, 3H, N-CH_3), 5.39 (s, 2H, 5-C*H*$_2$OH), 6.84 (s, 1H, 7-H), 7.13 (s, 1H,

4-H), 7.37 (t, 1H, J = 7.8 Hz, 5'-H), 7.52 (d, 1H, J = 7.8 Hz, 4'-H), 7.92 (d, 1H, J = 7.8 Hz, 6'-H), 8.00 (bs, 1H, 2'-H), 8.41 (bs, 1H, 5-CH$_2$O*H*); ^{13}C NMR (CDCl$_3$) δ 19.2 (q, C-*C*H$_3$), 26.8 (q, N-CH$_3$), 65.8 (t, CH$_2$), 109.5 (d, 7-C), 111.3 (d, 4-C), 126.0 (s, 3a-C), 126.9 (s, 6-C), 127.8 (d, 6'-C), 129.7 (d, 2'-C and 5'-C), 130.8 (s, 5-C), 131.4 (s, 7a-C), 131.9 (s, 1'-C), 133.0 (d, 4'-C), 134.6 (s, 3'-C), 155.9 (s, 2-C), 165.2 (s, CO); MS *m/z* 330 (M$^+$). Anal. Calcd for $C_{17}H_{15}ClN_2O_3 \cdot 0.5H_2O$: C, 60.09; H, 4.75; N, 8.24. Found: C, 59.73; H, 4.65; N, 8.13.

9: ^{1}H NMR (CDCl$_3$) δ 2.26 (s, 3H, 6-CH$_3$), 3.40 (s, 3H, N-CH$_3$), 6.85 and 6.88 (each s, each 1H, 4- and 7-H), 7.48 (t, 1H, J = 8.0 Hz, 5'-H), 7.63 (d, 1H, J = 8.0 Hz, 4'-H), 8.11 (d, 1H, J = 8.0 Hz, 6'-H), 8.20 (bs, 1H, 2'-H), 8.58 (s, 1H, 5-OH); MS *m/z* 316 (M$^+$).

10: ^{1}H NMR (CDCl$_3$) δ 2.23 (s, 3H, 5-CH$_3$), 3.39 (s, 3H, N-CH$_3$), 6.79 and 6.95 (each s, each 1H, 4- and 7-H), 7.48 (t, 1H, J = 8.0 Hz, 5'-H), 7.64 (d, 1H, J = 8.0 Hz, 4'-H), 8.11 (d, 1H, J = 8.0 Hz, 6'-H), 8.21 (s, 1H, 2'-H), 8.35 (s, 1H, 5-OH); MS *m/z* 316 (M$^+$).

11: Recrystallization from CHCl$_3$-hexane gave a gray powder. mp 242-246 °C. ^{1}H NMR (CDCl$_3$) δ 3.42 (s, 3H, CH$_3$), 6.89 (d, 1H, J = 2.0 Hz, 7-H), 6.90 (dd, 1H, J = 2.0, 8.5 Hz, 5-H), 7.11 (d, 1H, J = 8.5 Hz, 4-H), 7.47 (t, 1H, J = 8.0 Hz, 5'-H), 7.62 (d, 1H, J = 8.0 Hz, 4'-H), 8.09 (d, 1H, J = 8.0 Hz, 6'-H), 8.19 (bs, 1H, 2'-H), 10.2 (s, 1H, 6-OH); ^{13}C NMR (CDCl$_3$) δ 27.0 (q, CH$_3$), 102.0 (d, 7-C), 109.7 (d, 5-C), 114.6 (d, 4-C), 125.9 (s, 3a-C), 128.3 (d, 6'-C), 129.9 (d, 5'-C), 130.2 (d, 2'-C), 131.3 (s, 7a-C or 1'-C), 131.5 (s, 7a-C or 1'-C), 133.7 (d, 4'-C), 134.8 (s, 3'-C), 145.6 (s, 6-C), 156.2 (s, 2-C), 164.5 (s, CO); MS *m/z* 302 (M$^+$). Anal. Calcd for $C_{15}H_{11}ClN_2O_3$: C, 59.52; H, 3.66; N, 9.25. Found: C, 59.34; H, 3.72; N, 9.35.

Hydrolysis of **11** (10 mg) in a solution consisting of 1 mL MeOH and 1mL c-HCl at 70 °C for 7 h gave 6-hydroxy-1-methyl-2-oxobenzimidazole in a 4 mg (74%) yield. ^{1}H NMR (Me$_2$SO-*d*$_6$) δ 3.30 (s, 3H, CH$_3$), 6.39 (dd, 1H, J = 2.0, 8.3 Hz, 5-H), 6.47 (d, 1H, J = 2.0 Hz, 7-H), 6.73 (d, 1H, J = 8.3 Hz, 4-H), 8.98 and 10.43 (each bs, each 1H, NH and 6-OH); MS *m/z* 164 (M$^+$).

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