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A MULTI-CENTERED ELECTROPHILE FORMED FROM A UNIQUE BIOACTIVE CYCLIC HYDROXAMIC ACID, 4-HYDROXY-7-METHOXY-2H-1,4-BENZOXAZIN-3(4H)-ONE

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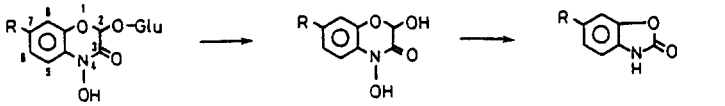
ABSTRACT: 4-Hydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (HMBOA) is a compound of considerable interest because of its pharmacological, agrochemical, and antimicrobial properties. A plausible bioactive metabolite of HMBOA is 4-acetoxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (AMBOA). Electrophilic reactions of AMBOA with phenols, anilines, thiols, heteroaromatics, amino acid derivatives and nucleic acids were investigated in relation to the chemical mechanisms of the biological effects elicited by the compound. The results suggest that HMBOA acts as an alkylating agent of proteins and nucleic acids *in vivo* after metabolic O-acylation.

INTRODUCTION:

During the past three decades, considerable research has been conducted to define the chemical bases of insect resistance, disease resistance, and herbicide tolerance elicited by plants. Early research implicated cyclic arylhydroxamic acids (4-hydroxy-2H-1,4-benzoxazin-3(4H)-ones, benzoxazinoids) contained in several gramineous species including corn, wheat, and rye as chemical resistance factors.[1,2] In the undisturbed plant, benzoxazinoids exist as stable β-glucosides that, upon cell disruption, are enzymatically converted to the corresponding aglycones.[3] In aqueous solutions, and in some organic solvents, the aglycones are converted to 2-(3H)-benzoxazolinones.[4-6] Both the aglycones and the benzoxazolinones exhibit biological activity, whereas the glucosides are essentially inactive.[7,8]

The predominant benzoxazinoid in corn and wheat is 2-(2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one)- β-D-glucopyranoside (1).[9] Compound 1, the corresponding aglycon 3 (DIMBOA), and the benzoxazolinone derivative (MBOA, 5) are the best characterized members of this group of com-

Fig. 1. Structures of Naturally Occurring Benzoxazinoids



1 R = CH_3O
2 R = H

3 (DIMBOA) R = CH_3O
4 R = H

5 (MBOA) R = CH_3O
6 R = H

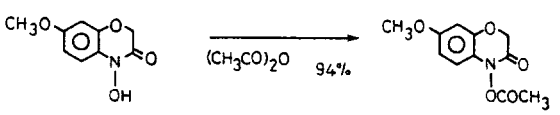
pounds. The respective demethoxy analogues 2, 4, and 6 are also known. Several other benzoxazinoids have been isolated[3] and the analytical methods using high performance liquid chromatography have been developed for these cyclic arylhydroxamic acids and benzoxazolinones.[10,11]

The roles of benzoxazinoids in insect resistance,[12-14] in herbicide tolerance,[12,15-17] and in allelopathy[12,18,19] have been established. Benzoxazinoids also exibit antimicrobial, antifungal, anti-inflammatory, and mutagenic activities.[20-24] In addition, stimulation of reproduction in Microtus montanus by MBOA (5) has been demonstrated.[25] Because of this wide variety of biological activities elicited by natural benzoxazinoids, a number of synthetic benzoxazinoids have been developed for pharmacological and agrochemical use.[26-28]

Though benzoxazinoids have attracted attention because of their interesting biological activities, the chemical mechanisms of the actions elicited by these cyclic arylhydroxamic acids are not well understood. Only the reactions of benzoxazinoids with thiol[29] and simazine (a herbicide),[16,17] and complex formation with divalent metal cations[30] have been described as possible chemical bases for the biological activities.

A suggestive result in connection with elucidation of the chemical mechanism of action elicited by benzoxazinoids was the finding that a 2-dehydroxy derivative of DIMBOA, 4-hydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one (HMBOA, 7) also possesses antimicrobial and mutagenic activities

Fig. 2. Structures of HMBOA and AMBOA



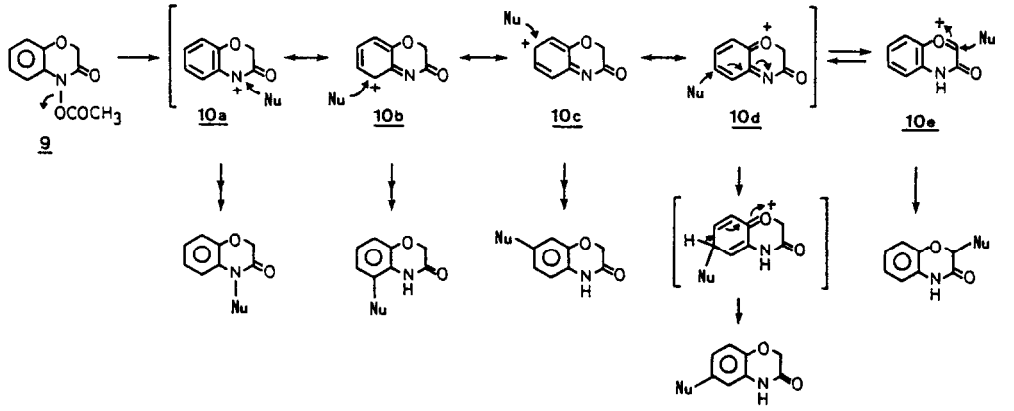
7 HMBOA

8 AMBOA

comparable to those of DIMBOA (3).[24] This result indicates that HMBOA (7) is a good synthetic model compound of DIMBOA (3), and the 2-hydroxy group of benzoxazinoids is not necessary for the biological activities. Another suggestive result came from our previous investigation on the acetoxy

group-rearrangement reactions of 4-acetoxy-2H-1,4-benzoxazin-3(4H)-one (9) (Fig. 3).[31] The results suggest that compound 9 is easily converted to cations 10a-d and 10e by heterolytic cleavage of the N-O bond of the cyclic arylhydroxamic acid and tautomerization, and the cations (10a-d and 10e) are attacked by acetate anion at positions 2, 4, 5, 6 and 7.[31] Under

Fig. 3. Nucleophilic Attack on the Cationic Species Formed from Synthetic Benzoxazinoid[31]



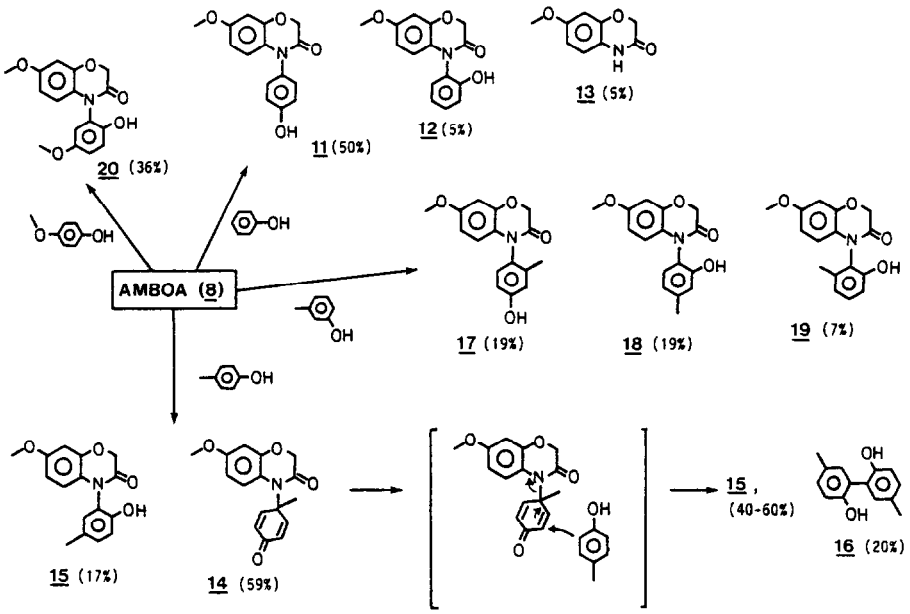
these circumstances, we hypothesized that the chemical basis of the biological activities elicited by HMBOA (7) would be the electrophilic reactivity of the compound, at least in part; i.e., the 4-hydroxy group of HMBOA (7) would act as a good leaving group after metabolic acylation, and the resulting cation would act as an electrophile, reacting with biomacromolecules such as proteins and nucleic acids. In fact, HMBOA (7) showed mutagenic activity toward Salmonella typhimurium TA100 and TA98 only in the presence of a mammalian metabolic enzyme mix (S-9) which contains large amounts of acylating enzymes,[24] suggesting that metabolic activation of the compound is necessary for eliciting the mutagenic activity. A plausible acylated metabolite of HMBOA (7) would be 4-acetoxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (AMBOA, 8, Fig. 2), because O-acetylation is one of the most common metabolic pathways of xenobiotics. In this paper, we describe the electrophilic reactions of AMBOA (8) with phenols, anilines, thiols, heteroaromatics, amino acid derivatives, and nucleic acids in relation to the chemical mechanisms of the biological effects elicited by HMBOA (7).

Reactions with Phenols (Fig. 4):

HMBOA (7) was prepared by the method decribed by Coutts and Pound.[32]

Schotten-Baumann acetylation of HMBOA gave AMBOA (8) in 94% yield (Fig. 2). The reaction of AMBOA with phenol proceeded rapidly in an organic solvent such as methylene chloride or benzene below 25 ℃ to give the p-substituted phenol (11)[33] in the yield of 50%, together with the o-substituted phenol (12, 5%) and 2H-1,4-benzoxazin-3(4H)-one (13) as by-products.

Fig. 4. Reactions of AMBOA with Phenols



In every reaction of AMBOA with nucleophiles described in this paper, compound 13 was produced in 5-10% yield (not shown in figures except when the compound is significant in the reaction). The formation of 13 may be interpreted in terms of participation of a triplet nitrenium ion,[34] electron transfer, or some other mechanisms.[35]

HMBOA did not react with phenol under the same conditions. The necessity of O-acetylation for an efficient reaction of HMBOA with nucleophiles (facilitation of the heterolytic cleavage of the N-O bond of HMBOA) is consistent with the requirement of a metabolic enzyme mix for showing the manifestation of mutagenic activity by HMBOA.[24] The 7-demethoxy analog of AMBOA, 4-acetoxy-2H-1,4-benzoxazin-3(4H)-one (9), reacted with phenol to give a p-substituted phenol in the yield of only 5%,[33] suggesting facilitation of heterolytic cleavage of the N-O bond by the electron-donating ability of the 7-methoxy group. Interestingly, introduction of a 2-hydroxy group into 9 (i.e., the 4-acetoxy derivative of compound 4)

enhanced the electrophilic reactivity, probably because of the stereoelectronic effect of the 2-hydroxy group.[36] Substitution of the oxygen atom at position 1 of 9 with a sulfur atom (4-acetoxy-2H-1,4-benzothiazin-3(4H)-one) also enhanced the electrophilic reactivity of the compound[37] (data not shown).

The reaction of AMBOA with p-cresol also proceeded rapidly to give an ipso-attacked cyclohexadienone derivative (14) in 59% yield. The adduct at the o-position to the hydroxy group, 15, was also obtained (17%). Compound 15 might be formed via compound 14 by rearrangement, at least in part. In fact, treatment of 14 in benzene gave 15 in the yield of 40 to 60%, together with 13 (30-50%) and bis-cresol (16, 20%). Compound 15 may be formed by thermal rearrangement. Compounds 13 and 16 would be produced by a nucleophilic attack on the cyclohexadienone (14) by p-cresol. The results suggest that the benzoxazinone moiety of 14 acts as a nucleofugal leaving group.

The major adducts produced in the reactions of AMBOA with phenol and p-cresol were compounds in which attack had occurred at the p-position of the phenolic hydroxy group (11 and 14). However, the reaction with m-cresol gave three isomeric products; 17 (19%), 18 (19%) and 19 (7%).[33] The reaction with p-methoxyphenol gave 20 (36%) as the only adduct which could be isolated.

Reactions with Anilines (Fig. 5):

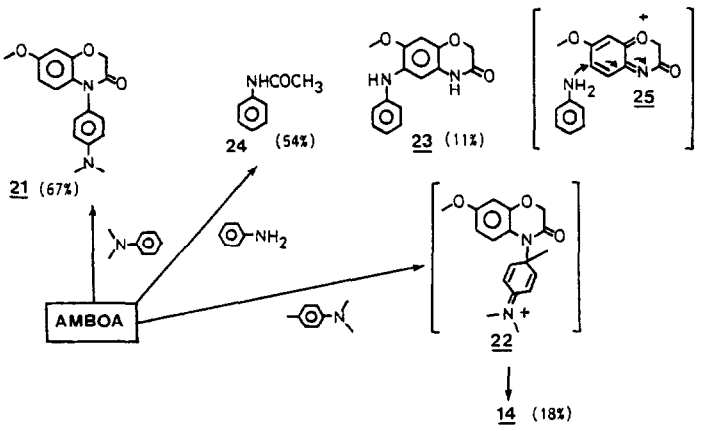
The reaction of AMBOA with dimethylaniline proceeded in a quite similar fashion to the reaction with phenol, giving the p-substituted dimethylaniline (21) as a major product (67%). Dimethyltoluidine also reacted similarly to give a cyclohexadienone derivative (14, 18%) which was probably formed by hydrolysis of an iminium derivative (22). However, the reaction with aniline occurred between the carbon atom at position 6 of AMBOA and the nitrogen atom of aniline to give compound 23 in the yield of 11%. In the reaction with aniline, the major product was the N-acetylaniline (24, 54%) presumably through nucleophilic attack by the nitrogen atom of aniline at the carbonyl carbon of the 4-acetoxy group of AMBOA.

The reaction at the carbon atom at position 6 of AMBOA probably occurs via formation of the cation 25 (corresponding to the cation drawn as 10d in Fig. 3) by heterolytic cleavage of the N-O bond. The structures of the 6-substituted benzoxazinone derivatives and the reaction mechanism of the nucleophilic substitution at position 6 of benzoxazinone were previously investigated and reported.[31, 33]

The structures of the compounds presented in this paper were determined unambiguously by examination of ^{1}H-NMR, ^{13}C-NMR, mass, UV and IR spectra, elemental analysis, alternative synthesis, and/or derivatization

to a known compound.

Fig. 5. Reactions of AMBOA with Anilines

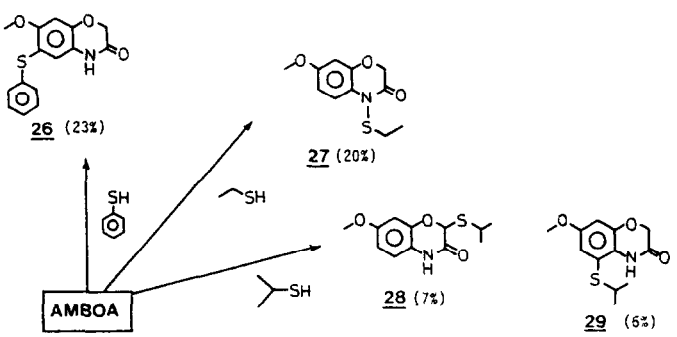


Reactions with Thiols (Fig. 6):

Thiophenol reacted with AMBOA in a similar fashion to aniline to give compound 26 in the yield of 23%; the sulfur atom of thiophenol reacted at position 6 of AMBOA. Aniline and thiophenol reacted with AMBOA at position 6 via their nucleophilic heteroatoms, but phenol reacted with AMBOA at the nitrogen atom of position 4 via its nucleophilic para-positioned carbon atom.

In addition, as shown in Fig. 6, ethylmercaptan reacted with AMBOA at the nitrogen of position 4 via its sulfur atom to give 27 in the yield of 20%. In a more complex process, isopropylmercaptan reacted with AMBOA at positions 2 and 5 via its nucleophilic sulfur atom to give 28 (7%) and 29 (6%), respectively. Formation of these products can be interpreted in

Fig. 6. Reactions of AMBOA with Thiols



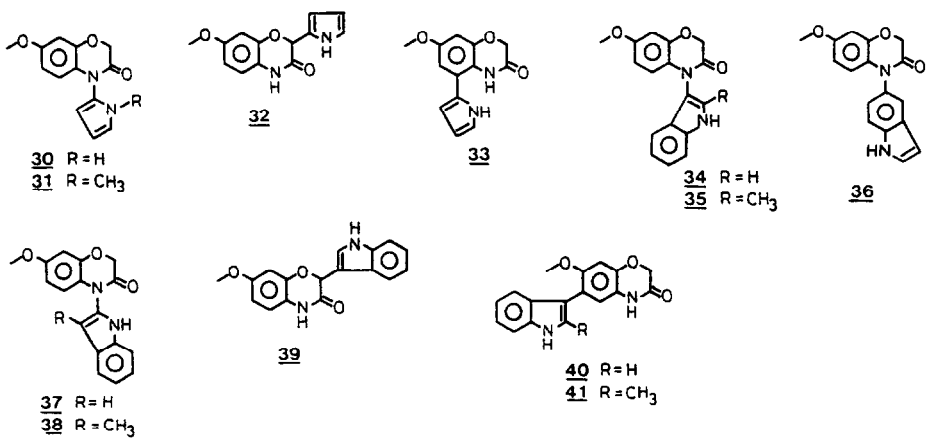
terms of the participation of the cations formed from AMBOA by heterolytic cleavage of the N-O bond (these cations correspond to the cations shown in Fig. 3), though the regio-selectivity of these reactions cannot be interpreted at the present stage. Calculation of the perturbation energy of the mutually interacting reaction sites might give us some information.

The yields of the adducts of the reactions of AMBOA with thiols to give compounds 26-29, were rather low, presumably because of the nature of the nucleophilic sulfur atom, which would attack the carbonyl carbons at position 3 and in the 4-acetoxy group of AMBOA, yielding volatile or benzoxazinone-ring-opened products.

Reactions with Pyrroles and Indoles (Fig. 7):

The electrophilic reactivity of AMBOA discussed above seemed to be strong enough to allow it to react with nucleophilic heterocycles. In fact, AMBOA reacted rapidly with the carbon atom at the α-position of pyrrole in benzene at room temperature to give the 4-substituted benzoxazinone (30) in a high yield (66%) accompanied with the 5-substituted benzoxazinone (33, 3%). When the reaction was performed in DMF, a small amount of the 2-substituted benzoxazinone (32, 4%) was isolated as well as 30 (50%) and 33 (1%). DMF is considered to have a cation-stabilizing effect, allowing the tautomerization of the cation corresponding to 10a-d to the cation whose charge is located at position 2 (the cation corresponding to 10e) (Fig. 3), followed by attack at position 2 by the α-carbon of pyrrole. In the reaction of AMBOA with N-methylpyrrole, the only isolated adduct was the 4-substituted benzoxazinone (31, 73%).

Fig. 7. Reaction Adducts of AMBOA with Pyrroles and Indoles



The reaction of AMBOA with indole was more complex, giving 5 regioisomers, 34 (41%), 36 (3%), 37 (1%), 39 (2%) and 40(1%). The β -carbon (position 3) of indole (the most nucleophilic center of indole) can attack positions 2 and 6 of AMBOA as well as position 4 (nitrogen atom). Less nucleophilic centers of indole (the α -carbon and the carbon atom at position 5) can attack only the nitrogen atom (position 4) of AMBOA. The results suggest that, in the case of indoles and pyrrole, only strongly nucleophilic centers seem to be able to attack the carbon atoms of AMBOA (positions 2, 5 and 6), though we cannnot yet discuss the regio-selectivity in detail. In fact, an indole analog whose β -position is blocked by a methyl group (i.e., 3-methylindole) reacted with AMBOA to give 38 (10%) as the only adduct.

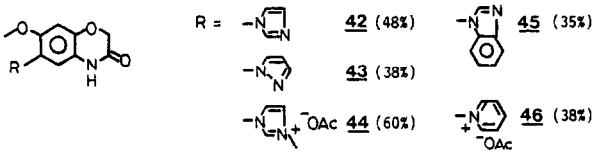
2-Methylindole reacted with AMBOA in benzene to give the 4-substituted benzoxazinone (35, 45%) and the 6-substituted benzoxazinone (41, 9%). The same reaction in methylene chloride gave only the 6-substituted benzoxazinone (41) in 36% yield.

Reactions with Diazoles and Pyridine (Fig. 8):

The nucleophilic center of diazoles and pyridine is the nitrogen atom, and they reacted with AMBOA to give only the 6-substituted benzoxazinone as shown in Fig. 8 (compounds 42-46).

In general, nitrogen nucleophiles reacted with the carbon atom at position 6 of AMBOA. This might be interpreted in terms of instability of the N-N bond (formed if the nitrogen nucleophile attacks at the nitrogen atom of position 4 of AMBOA) or aminoacetal (formed if the nitrogen nucleophile attacks at position 2 of AMBOA).

Fig. 8. Reaction Adducts of AMBOA with Diazoles and Pyridine



A General Rule for Regio-selectivity in the Reactions of AMBOA with Nucleophiles:

As mentioned above, nitrogen atoms (when they act as nucleophilic centers) react with AMBOA at position 6 (Fig. 8). Aniline acts as a nitrogen nucleophile to give 6-substituted benzoxazinone, 23, and N,N-dimethylaniline and N,N-dimethyltoluidine act as carbon nucleophiles to give 4-substituted benzoxazinones, 21 and 14 (via 22), respectively (Fig. 5). Di-

azoles and pyridine act as nitrogen nucleophiles to give 6-substituted benzoxazinones, 42-46.

Carbon nucleophiles generally attack predominantly the nitrogen atom at position 4 of AMBOA. Phenols react with AMBOA to give only 4-substituted benzoxazinones (Fig. 4). However, moderately strong carbon nucleophiles such as the α-carbon of pyrrole and the β-carbon of indoles can also attack the carbon atoms at other positions of AMBOA (positions 2, 5 and 6, Fig. 7). These results suggest that the intrinsically most reactive site of AMBOA is the nitrogen atom at position 4. When the reaction between position 4 of AMBOA and a nucleophile is kinetically unfavorable, the nucleophile attacks the carbon atoms at positions 2, 5 or 6 of AMBOA via its most nucleophilic site. Nitrogen nucleophiles do not bind to position 4 (for thermodynamic reasons), but bind to the carbon atom at position 6, yielding a stable product.

Though the yields of adducts formed in the reactions of AMBOA with sulfur nucleophiles were relatively low, the sulfur nucleophiles can be regarded as intermediate in nature between nitrogen nucleophiles and carbon nucleophiles; thiophenol reacted with AMBOA to give 6-substituted benzoxazinone (26), ethylmercaptan reacted to give 4-substituted benzoxazinone (27, N-S bond formation), and isopropylmercaptan reacted to give 2- and 5-substituted benzoxazinones (28 and 29, respectively).

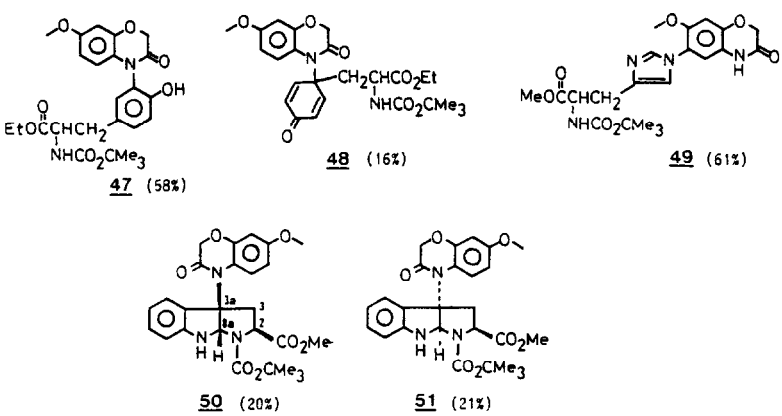
In relation to the multi-centered electrophilic reactivity of AMBOA, we should take account of the electrophilic reactivity of a benzoquinone monoimine, because one of the canonical form of the cation formed by the heterolytic cleavage of the N-O bond of AMBOA can be regarded as a derivative of an o-benzoquinone monoimine as shown in the structure 25 (corresponding to the cation 10d). Shudo et al. reported the electrophilic reactions of p-benzoquinone monoimine with some nucleophiles such as phenol and dimethylaniline.[38] Various sites of p-benzoquinone monoimine including positively charged carbon, nitrogen and oxygen atoms, are attacked by nucleophiles depending on the reaction conditions and the nature of the nucleophiles used.[38] Ortho-benzoquinone monoimine also reacts with various nucleophiles at various positions depending on the reaction conditions and the nature of the nucleophiles (Kagechika et al., unpublished results). In these investigations, an unusual nucleophilic attack on carbonyl oxygen of benzoquinone monoimines (i.e., the reaction of a positively charged oxygen atom) was emphasized. Though the oxygen atom at position 1 of AMBOA is not attacked by nucleophiles because the oxygen atom is already blocked (alkylated to form the benzoxazinone ring), the contribution of the cation with the canonical form of 25 bearing a positively charged oxygen atom is considered to be major in some cases (in

the reactions with aniline, thiophenol, 2-methylindole and nitrogen-heterocycles, 6-substituted benzoxazinones, which are considered to be formed via the cation 25, are the major products). In the case of electrophilic reactions of AMBOA, the positively charged nitrogen atom at position 4 also makes a major contribution in some cases. In view of the considerations described above, we believe that AMBOA with its unique cyclic hydroxamic acid system is the source of an interesting cation with positively charged heteroatoms (oxygen and nitrogen atoms).

Reactions with Amino Acid Derivatives (Fig. 9):

AMBOA possesses electrophilic reactivity which is strong enough to allow reaction with phenols, indoles and imidazoles under quite mild conditions to give the corresponding adducts. Because phenols, indoles and imidazoles can be regarded as nucleophilic fragments of peptides or proteins (i.e., tyrosine, tryptophan, and histidine moieties in peptides or proteins, respectively), it is reasonable to expect that AMBOA (a plausible metabolically activated form of HMBOA) will react with nucleophilic amino acid residues in peptides or proteins *in vivo*, possibly leading to the modification or inactivation of specific enzymes or other important proteins. In fact, AMBOA rapidly reacted with amino acid derivatives such as Boc-L-Tyr-OEt, Boc-L-His-OMe and Boc-L-Trp-OMe to give compounds 47-51 in considerable yields (Fig. 9). The reactions of AMBOA with these amino acid derivatives did not deviate in nature from the reactions between AMBOA and phenols, indoles or imidazoles.

Fig. 9. Reaction Adducts of AMBOA with Amino Acid Derivatives



AMBOA reacted with Boc-L-Tyr-OEt in a quite similar fashion to the reaction with p-cresol, to give 47 (58%, corresponding to 15) and the ipso-attacked adduct 48 (16%, corresponding to 14).[39] The reaction with Boc-L-His-OMe gave the 6-substituted benzoxazinone, 49,[39] in 61% yield; this product corresponds to the adduct obtained in the reaction of AMBOA with imidazole(42). The reaction of AMBOA with Boc-L-Trp-OMe proceeded in a similar fashion to the reaction with indole or 2-methylindole, but not the reaction with 3-methylindole; the adducts isolated were diastereomeric isomers reacting at the β -carbon (position 3) of the indole moiety (50, 20% and 51, 21%).[39] These hexahydropyrroloindoles (50 and 51) were produced by the attack of the β -carbon of the indole moiety at the nitrogen atom (position 4) of AMBOA, followed by cis annulation, which is more sterically favorable than trans annulation. The stereochemistry of 50 and 51 was deduced from their ^{1}H-NMR spectra.[39,40] The results suggest that the adduct obtained in the reaction of AMBOA with 3-methylindole, i.e., 38, might be formed via the position-3-substituted indolenine intermediate, which migrated to give the position-2-substituted indole (38).

Reactions with Nucleic Acids (Fig. 10):

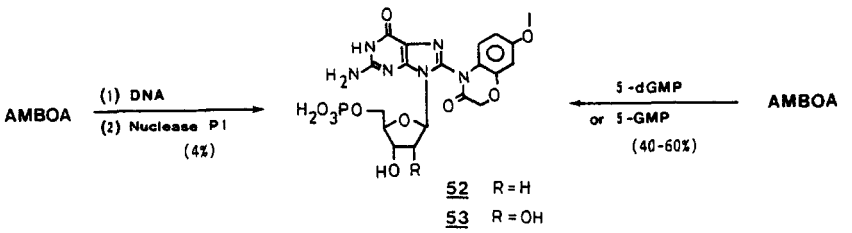
AMBOA is a plausible metabolically activated form of a mutagenic compound, HMBOA (7), with an O-acylated arylhydroxamic acid structure. Recent advances in the area of muta-carcinogenic chemistry have established the importance of O-acylated arylhydroxamic acids as metabolically activated forms of muta-carcinogens which react with DNA.[41,42] Chemical modification of DNA is considered to play an important role in the muta-carcinogenicity elicited by such compounds. Therefore, we anticipated that AMBOA would react with DNA.

In fact, AMBOA covalently bound to calf thymus DNA efficiently in a mixture of water and DMF (5:1 v/v) at room temperature. Enzymatic hydrolysis of the modified DNA using Nuclease P1 yielded a modified nucleotide, 52; AMBOA bound at the nitrogen atom (position 4) to the carbon atom at position 8 of aguanine residue. The structure of 52 was unambiguouly determined by examination of the ^{1}H-NMR, ^{13}C-NMR, UV and IR spectra.[43] The amount of 52 was as much as 4% of the total guanine residues in calf thymus DNA under the experimental conditions used. Position 8 of guanine residues in DNA is known as a major reaction site attacked by various electrophilic muta-carcinogens (or their metabolically activated forms).[41]

The same modified nucleotide, 52, was also obtained by the reaction of AMBOA with 5'-deoxyguanylic acid (5'-dGMP) in the yield of 58%. Hydrolysis of 52 thus obtained with trifluoroacetic acid gave a guanine-benzoxazinone adduct, 4-(guanin-8-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (54, 80%) which was recrystallized from methanol and gave appropriate analytical

values. Compound 54 was also obtained by reaction of AMBOA with guanine,

Fig. 10. Reactions of AMBOA with Nucleic Acids



52 R=H
53 R=OH

though the yield was low (6%) because of the very low solubility of guanine. AMBOA also reacted with 5'-guanylic acid (5'-GMP) to give the corresponding modified nucleotide, 53, in the yield of 47%. Hydrolysis of 53 with trifluoroacetic acid gave 54.

AMBOA did not react with other nucleotides such as adenylic acid, thymidylic acid, or cytidylic acid; i.e., the modification of DNA with AMBOA is guanine-specific. The mode of binding is similar to the binding to DNA of carcinogenic 2-acetylaminofluorene[41] and of some muta-carcinogenic heteroaromatic amines isolated from food pyrolysates.[42]

CONCLUSION:

HMBOA (7) possesses a wide variety of biological activities. AMBOA, the 4-O-acetylated derivative of HMBOA, is a plausible metabolically activated form of HMBOA. The electrophilic reactivity of AMBOA was investigated. AMBOA reacted with phenols, anilines, thiols, pyrroles, indoles, diazoles and pyridine at various sites (at positions 2, 4, 5, 6, and 7 of AMBOA). Through the investigation of the structures and the yields of the reaction adducts, a general rule for the regio-selectivity of the electrophilic reaction of AMBOA was deduced. This rule should contribute to the understanding of the chemistry of the unique cyclic arylhydroxamic acid systems.

In relation to the chemical mechanisms of the biological actions elicited by HMBOA, the reactions of AMBOA with amino acid derivatives and nucleic acids were investigated. AMBOA reacted with nucleophilic aromatic amino acid derivatives, and the structures of the adducts were determined. AMBOA also reacted with DNA. The site of modification of DNA by AMBOA was determined to be position 8 of guanine residues in DNA. These reactions suggest that chemical modification of bio-macromolecules such as proteins and nucleic acids by AMBOA (or HMBOA after metabolic activation) *in vivo* plays an important role in eliciting the biological activities, at least in part.

EXPERIMENTAL SECTION:

4-Hydroxy-2H-1,4-benzoxazin-3(4H)-one (HMBOA, 7): The title compound was prepared by the reductive cyclization of the ethyl ester of 5-methoxy-2-nitrophenoxyacetic acid as described previously in the yield of 82%.[31,32] mp 122℃, IR (KBr): 2850, 2770, 1650, 1502 cm^{-1}. Anal. Calcd for $C_9H_9NO_4$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.25; H, 4.62; N, 7.17.

4-Acetoxy-2H-1,4-benzoxazin-3(4H)-one (AMBOA, 8): HMBOA (7, 500 mg, 2.56 mmole) was dissolved in 5 ml of ice-cold aqueous K_2CO_3 (1.40 g, 10 mmole), and 10 ml of an ice-cold ether solution of $(CH_3CO)_2O$ (0.71 ml, 7.5 mmole) was added. The mixture was vigorously shaken under ice-cooling for 15 min. The ether layer was then separated and dried over Na_2SO_4, and concentrated under reduced pressure (0 ℃), and the addition of n-hexane resulted in the precipitation of colorless prisms of AMBOA (570 mg, yield 94%). AMBOA is unstable at room temperature, and for studies the reactions of AMBOA, the compound was freshly prepared. dp ca. 30℃, ^{1}H-NMR ($CDCl_3$, -20℃): 2.44 (3H, s), 3.80 (3H, s), 4.79-4.81 (2H), 6.54 (1H, dd, J=4, 9 Hz), 6.60 (1H, d, J=4 Hz), 6.79 (1H, d, J=9 Hz).

General Procedure (reactions with phenols, anilines, thiols, pyrroles, indoles, diazoles and pyridine): Freshly prepared AMBOA (1.5 g, 6.33 mmole) was dissolved in 40 ml of an organic solvent (benzene, CH_2Cl_2, or DMF). To this solution, 8 equivalents of nucleophile (50.6 mmole) was added at below 25℃, and the mixture was stirred for 30 min at the same temperature. The solution was then evaporated under reduced pressure at below 25℃, and the residue was separated by silica gel column chromatography (usually CH_2Cl_2-AcOEt). Fractions were evaporated and the residue was recrystalized from an appropriate organic solvent.

The structures of the all products presented in this paper were unambiguously determined by examination of the NMR, UV, IR and elemental analysis data (and in a few cases by mass spectroscopy), comparison of these spectroscopic data with those of related compounds or alternatively synthesized (model) compounds, and/or derivatization to other compounds including known compounds. The essential data for the structural determination are given below.

4-(4-Hydroxyphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (11): Colorless needles (AcOEt), mp 228-229 ℃, IR (KBr): 3300, 1663cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.77 (3H, s), 4.78 (2H, s), 6.30 (1H, d, J=8.7 Hz), 6.51 (1H, dd, J=2.6, 8.7 Hz), 6.68 (1H, d, J=2.6 Hz), 6.91 (2H, d, J=8.8 Hz), 7.09 (2H, d,

J=8.8 Hz), 9.75 (1H, s). The presence of proton signals (positions 2 (2H), 5, 6 and 8 of the benzoxazinone moiety) suggests that the nitrogen atom at position 4 of benzoxazinone is the binding site. Regarding phenol moiety, two doublets with a coupling constant of 8.8 Hz (2H each) are consistent with the para-substituted phenol structure. Anal.Calcd for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.83; N, 5.16. Found: C, 66.19; H, 4.92; N, 5.22. Acetylation with acetic anhydride occurred at the phenolic hydroxy group to give an O-acetylated derivative which gave appropriate elemental analytical data.

4-(2-Hydroxyphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (12): Colorless needles (AcOEt/n-hexane), mp 206℃ , IR (KBr): 3280, 1672cm^{-1}. UV (EtOH)/ λ_{max}(ε): 247 nm (7.4×10^3), 270 nm (6.5×10^3). ^{1}H-NMR (DMSO-d_6): 3.68 (3H, s), 4.74 (2H, s), 6.16 (1H, d, J=8.9 Hz), 6.46 (1H, dd, J=2.7, 8.9 Hz), 6.65 (1H, d, J=2.7 Hz), 6.80-7.40 (4H, m), 9.67 (1H, s). The proton signals due to the benzoxazinone ring are similar to those of compound 11. Four proton signals at higher magnetic field (δ 7.40) and the presence of the hydroxyl function are consistent with the proposed structure. Anal. Calcd for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.83; N, 5.16. Found: C,66.56; H, 4.82; N, 5.22.

7-Methoxy-2H-1,4-benzoxazin-3(4H)-one (13): Colorless prisms (EtOH), mp 165-166 ℃ , IR (KBr): 3190, 1678cm^{-1}. UV (EtOH)/λ_{max}(ε): 260 nm (8.55×10^3), 287 nm (sh). ^{1}H-NMR (DMSO-d_6): 3.69 (3H, s), 4.51 (2H, s), 6.78 (1H, d, J=9.0 Hz), 6.49 (1H, dd, J=2.8, 9.0 Hz), 6.53 (1H, d, J=2.8 Hz), 8.81 (1H, s); identical with an authentic sample. Anal. Calcd for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.14; H, 5.02; N, 7.76.

7-Methoxy-4-(1-methyl-4-oxo-2,5-cyclohexadien-1-yl)-2H-1,4-benzoxazin-3(4H)-one (14): Colorless prisms (CH_2Cl_2/n-hexane), mp 97-99℃ , IR (KBr): 1627, 1670 cm^{-1}. UV (EtOH)/λ_{max}(ε): 232 nm (1.4×10^4), 290 nm (sh). ^{1}H-NMR ($CDCl_3$): 2.04 (3H, s), 3.74 (3H, s), 4.45 (2H, s), 6.81 (1H, d, J=9.0 Hz), 6.43 (1H, dd, J=2.5, 9.0 Hz), 6.61 (1H, d, J=2.5 Hz), 6.31 (2H, d, J=10.2 Hz), 7.22 (2H, d, J=10.2 Hz). ^{13}C-NMR ($CDCl_3$): 27.53 (q), 55.30 (q), 59.58 (s), 70.48 (t), 103.21 (d), 107.40 (d), 119.46 (d), 123.35 (s), 127.44 (2C, d), 149.03 (s), 151.46 (2C, d), 156.47 (s), 170.19 (s), 183.57 (s). The presence of two carbonyl carbons (^{13}C-NMR) and two doublets (2H each) with the coupling constant of 10.2 Hz (^{1}H-NMR) suggests the cyclohexadienyl structure. Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.22; H, 5.28; N, 5.20.

4-(2-Hydroxy-5-methylphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (15): Colorless needles (AcOEt), mp 230 ℃ , IR (KBr): 3320, 1673cm^{-1}. UV (EtOH)/ λ_{max}(ε): 275 (8.4×10^3). ^{1}H-NMR (DMSO-d_6): 2.24 (3H, s), 3.70 (3H, s), 4.74 (2H, s), 6.18 (1H, d, J=8.9 Hz), 6.47 (1H, dd, J=2.8, 8.9 Hz), 6.63 (1H, d, J=2.8 Hz), 6.88 (1H, d, J=8.1 Hz), 6.93 (1H, d, J=2.2 Hz), 7.10

(1H, dd, J=2.2, 8.1 Hz), 9.44 (1H,s). The proton signals at the aromatic region indicate the presence of two 1,2,4-trisubstituted phenyl ring systems, suggesting the structure. Comparison of these ^{1}H-NMR data with those of compound 12 also supports the structure. ^{13}C-NMR (DMSO-d_6): 19.80 (q), 55.21 (q), 67.46 (t), 102.29 (d), 107.20 (d), 116.15 (d), 116.59 (d), 121.84 (s), 123.30 (s), 128.16 (s), 129.92(d), 130.01 (d), 144.80 (s), 151.32 (s), 155.31 (s), 162.46 (s). Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.28; H, 5.37; N, 4.86.

4-(4-Hydroxy-2-methylphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (17): Colorless needles (AcOEt), mp 163 ℃, IR (KBr): 3320, 1665cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.33 (3H, s), 3.52 (3H, s), 4.75 (2H, s), 6.23 (1H, br.s), 6.46 (2H, d, J=2.1 Hz), 6.63 (1H, t, J=2.1 Hz), 6.75 (1H, dd, J=2.3, 9.0 Hz), 6.87 (1H, d, J=2.3 Hz), 7.02 (1H, d, J=9.0 Hz). Comparison of these NMR data with those of compound 11 supports the structure. Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.02; H, 5.28; N, 4.75.

4-(2-Hydroxy-4-methylphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (18): Colorless needles (AcOEt), mp 166 ℃, IR (KBr): 3340, 1668cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.00 (3H, s), 3.75 (3H, s), 4.76 (2H, s), 6.26 (1H, d, J=8.5 Hz), 6.43 (1H, dd, J=2.2, 8.5 Hz), 6.63 (1H, d, J=2.2 Hz), 6.61 (1H, dd, J=2.0, 9.0 Hz), 6.65 (1H, d, J=2.0 Hz), 6.92 (1H, d, J=9.0 Hz). Comparison of these NMR data with those of compounds 12 and 15 supports the structure. Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.35; H, 5.43; N, 4.70.

4-(2-Hydroxy-6-methylphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (19): Colorless needles (AcOEt), mp 236 ℃, IR (KBr): 3350, 1675cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.12 (3H, s), 3.79 (3H, s), 4.79 (2H, s), 6.25 (1H, d, J=8.8 Hz), 6.45 (1H, dd, J=2.0, 8.8 Hz), 6.59 (1H, d,J=2.0 Hz), 6.82-7.18 (3H, m). Comparison of these NMR data with those of compounds 12, 15 and 18 supports the structure. Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.01; H, 5.25; N, 4.67.

4-(2-Hydroxy-5-methoxyphenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (20): Colorless plates (AcOEt/n-hexane), mp 157-158 ℃, IR(KBr): 3350, 1673 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.70 (6H, sx2), 4.75 (2H, s), 6.21 (1H, d, J=8.8 Hz), 6.21 (1H, dd, J=2.7, 8.8 Hz), 6.65 (1H, d, J=2.7 Hz), 6.70-7.00 (3H, m), 9.21 (1H, s). Three higher magnetic field protons and the presence of a phenolic proton suggest the structure, as in the cases above. Anal. Calcd for $C_{16}H_{15}NO_5$: C, 63.78; H, 5.01; N, 4.65. Found: C, 63.65; H, 5.32; N, 4.48.

4-(4-Dimethylaminophenyl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (21): Colorless plates (AcOEt/n-hexane), mp 158-159 ℃, IR (KBr): 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.00 (6H, s), 3.75 (3H, s), 4.71 (2H, s), 6.38 (2H, m), 6.57

(1H, m), 6.75 (2H, d, J=9.0 Hz), 7.10 (1H, d, J=9.0 Hz). The presence of proton signals of the benzoxazinone ring and the para-substituted N,N-dimethylaniline moiety gave the structure. Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N,9.39. Found: C, 68.40; H, 6.00; N, 9.33.

7-Methoxy-6-phenylamino-2H-1,4-benzoxazin-3(4H)-one (23): Colorless plates (AcOEt/n-hexane), mp 170-171℃, IR (KBr): 3420, 3192, 1680 cm^{-1}. ^1H-NMR ($CDCl_3$): 3.86 (3H, s), 4.00 (1H, s), 4.56 (2H, s), 6.63 (1H, s), 6.80 (1H, s), 6.85-7.45 (5H, m), 8.23 (1H, s). Two singlet proton signals of the benzoxazinone moiety suggest the 6-substituted benzoxazinone ring structure. The aniline moiety possesses five protons. Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.60; H,5.22; N, 10.36. Found: C, 66.40; H, 5.13; N, 10.25.

7-Methoxy-6-phenylthio-2H-1,4-benzoxazin-3(4H)-one (26): Colorless needles (AcOEt/n-hexane), mp 166-167℃, IR (KBr): 3170, 1680cm^{-1}. ^1H-NMR (DMSO-d_6): 3.74 (3H, s), 4.52 (2H, s), 6.78 (1H,s), 6.88 (1H, s), 6.00-7.50 (5H, m), 10.48 (1H, s). Two singlet proton signals of the benzoxazinone moiety suggest the 6-substituted benzoxazinone ring structure. The thiophenol moiety possesses five protons. Anal. Calcd for $C_{15}H_{13}N_3O_3S$: C, 62.70; H, 4.56; N,4.88. Found: C, 62.64; H, 4.56; N, 4.64.

4-Ethylthio-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (27): Oil. (M^+)239. IR (KBr): 1705 cm^{-1}. ^1H-NMR ($CDCl_3$): 1.24 (3H, t, J=8.0 Hz), 1.92 (2H, q, J= 8.0 Hz), 3.76 (3H, s), 4.65 (2H, s), 6.52 (1H, d, J=3.0 Hz), 6.58 (1H, dd, J=3.0, 9.0 Hz), 7.55 (1H, d, J=9.0 Hz). Three aromatic protons of the benzoxazinone ring were assigned in addition to the protons of the thioethyl group.

2-Isopropylthio-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (28): Colorless needles (AcOEt/n-hexane), mp 161-162℃, IR (KBr): 3180, 1680cm^{-1}. ^1H-NMR ($CDCl_3$): 1.38 (6H, d, J=7.0 Hz), 3.32 (1H, hept., J=7.0 Hz), 3.78 (3H, s), 5.91 (1H, s), 6.57 (1H, dd, J=2.5, 9.0 Hz), 6.59 (1H, d, J=2.5 Hz), 6.76 (1H, d, J=9.0 Hz), 8.83 (1H, s). Regarding the benzoxazinone moiety, the presence of the NH signal, 3 aromatic proton signals (positions 5, 6 and 8), and the signal of the position-2 proton integrating as one proton (instead of two protons of the starting compound) suggest the structure. Anal. Calcd for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.97; N, 5.53. Found: C, 56.63; H, 5.87; N, 5.48.

5-Isopropylthio-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (29): Colorless prisms (AcOEt/n-hexane), mp 70-72 ℃, IR (KBr): 3180, 1680cm^{-1}. ^1H-NMR ($CDCl_3$): 1.26 (6H, d, J=7.0 Hz), 3.22 (1H, hept, J= 7.0 Hz), 3.76 (3H, s), 4.58 (2H, s), 6.55 (1H, d, J=2.8 Hz), 6.72 (1H, d, J=2.8 Hz), 8.30 (1H, s). Since the coupling constant of two aromatic protons suggests meta coupling, the substitution position seems to be position 5. Anal. Calcd

for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.97; N, 5.53. Found: C, 56.72; H, 5.81; N, 5.47.

7-Methoxy-4-(pyrrol-2-yl)-2H-1,4-benzoxazin-3(4H)-one (30): Colorless flakes (EtOH), mp 154-155 ℃, IR (KBr): 3275, 1678 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.70 (3H, s), 4.75 (2H, s), 6.31 (1H, d, J=8.8 Hz), 6.52 (1H, dd, J=2.9, 8.8 Hz), 6.65 (1H, d, J=2.9 Hz), 5.99 (1H, dd, J=1.8, 3.9 Hz), 6.12 (1H, dd, J=2.8, 3.9 Hz), 6.78 (1H, dd, J=1.8, 2.8 Hz). ^{13}C-NMR (DMSO-d_6): 55.35 (q), 67.95 (t), 102.39 (d), 105.26 (d), 107.64 (2C, d), 116.73 (d), 116.93 (d), 121.26 (s), 124.18 (s), 144.95 (s), 155.94 (s), 163.96 (s). The lack of an α-proton (pyrrole) signal suggests the structure. Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.65; H, 4.92; N, 11.36.

7-Methoxy-4-(1-methylpyrrol-2-yl)-2H-1,4-benzoxazin-3(4H)-one (31): Colorless prisms (CH_2Cl_2/n-hexane): mp 93-94℃, IR (KBr): 1690 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.32 (3H, s), 3.72 (3H, s), 4.82 (2H, s), 6.22 (1H, d, J=9.0 Hz), 6.57 (1H, dd, J=2.7, 9.0 Hz), 6.67 (1H, d, J=2.7 Hz), 6.02 (1H, dd, J=1.9, 4.1 Hz), 6.11 (1H, dd, J=3.0, 4.1 Hz), 6.86 (1H, dd, J=1.9, 3.0 Hz). Anal. Calcd for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.84. Found: C, 65.07; H, 5.46; N, 10.62.

7-Methoxy-2-(pyrrol-2-yl)-2H-1,4-benzoxazin-3(4H)-one (32): Colorless prisms (AcOEt/n-hexane), dp 190 ℃, IR (KBr): 3260, 3190, 1678cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.65 (3H, s), 5.57 (1H, s), 6.46 (1H, d, J=2.8 Hz), 6.50 (1H, dd, J=2.8, 8.6 Hz), 6.82 (1H, d, J=8.6 Hz), 5.83 (1H, dd, J= 1.7, 3.6 Hz), 5.90 (1H, dd, J=2.4, 3.6 Hz), 6.74 (1H, dd, J=1.7, 2.4 Hz), 10.67 (1H, s), 11.07 (1H, s). The presence of one proton at position 2 of the benzoxazinone moiety and the lack of one α-proton of the pyrrole indicate the structure. Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 63.93; H, 4.95; N, 11.47. Found: C, 64.16; H, 4.95; N, 11.30.

7-Methoxy-5-(pyrrol-2-yl)-2H-1,4-benzoxazin-3(4H)-one (33): Colorless flakes (AcOEt/n-hexane), mp 196-197 ℃, IR (KBr): 3320, 3240, 1660 cm^{-1}. ^{1}H-NMR (DMSO-d_6/CF_3COOD): 3.78 (3H, s), 4.55 (2H, s), 6.55 (1H, d, J=2.8 Hz), 6.70 (1H, d, J=2.8 Hz), 6.23 (1H, dd, J=2.3, 3.3 Hz), 6.40 (1H, dd, J=1.5, 3.3 Hz), 6.94 (1H, dd, J=1.5, 2.3 Hz). Two meta coupling proton signals of the benzoxazinone moiety suggest the 5-substituted benzoxazinone structure. Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.79; H, 4.94; N, 11.41.

4-(1H-Indol-3-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (34): Colorless needles (CH_3CN), mp 234-235 ℃, IR (KBr): 3280, 1673cm^{-1}. ^{1}H-NMR ($CDCl_3$): 3.70 (3H, s), 4.80 (2H, s), 6.33 (1H, dd, J=2.6, 8.7 Hz), 6.50 (1H, d, J=8.7 Hz), 6.62 (1H, d, J=2.6 Hz), 6.90-7.40 (5H, m), 8.67 (1H, s). The lack of the characteristic 3-H (indole) proton signal and the presence of 2-H

(indole) proton signal suggest the structure. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.26; H, 4.84; N, 9.63.

7-Methoxy-4-(2-methyl-1H-indol-3-yl)-2H-1,4-benzoxazin-3(4H)-one (35): Colorless needles (AcOEt/n-hexane), mp 191-192℃, IR (KBr): 3290, 1668 cm^{-1}. ^1H-NMR ($CDCl_3$): 2.18 (3H, s), 3.70 (3H, s), 4.86 (2H, s), 6.27 (1H, d, J=9.0 Hz), 6.43 (1H, dd, J=2.5, 9.0 Hz), 6.68 (1H,d, J=2.5 Hz), 6.78-7.15 (3H, m), 7.33 (1H, m), 11.30 (1H, s). The presence of a 3-H (indole) proton signal and the lack of a 2-H (indole) proton signal suggest the structure. Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.08. Found: C, 69.91; H, 5.20; N, 8.91.

4-(1H-Indol-5-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (36): Colorless needles (EtOH), mp 227-228℃, IR (KBr): 3320, 1678 cm^{-1}. ^1H-NMR (DMSO-d_6): 3.70 (3H, s), 4.80 (2H, s), 6.22 (1H, d, J=9.1 Hz), 6.44 (1H, dd, J=2.8, 9.1 Hz), 6.67 (1H, d, J=2.8 Hz), 6.52 (1H, d, J=3.4 Hz), 6.82 (1H, dd, J=2.0, 8.2 Hz), 7.32 (1H, m), 7.46 (1H, d, J=3.4 Hz), 7.63 (1H, d, J=8.2 Hz). The aromatic proton signals indicate the presence of two 1,2,4-tri-substituted phenyl ring systems, suggesting the structure. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.21; H, 4.83; N, 9.42.

4-(1H-Indol-2-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (37): Light violet plates (CH_2Cl_2/n-hexane), mp 200-201℃, IR (KBr): 3290, 1668 cm^{-1}. ^1H-NMR ($CDCl_3$): 3.76 (3H, s), 4.71 (2H, s), 6.40 (1H, dd, J=2.8, 8.9 Hz), 6.62 (1H, d, J=2.8 Hz), 6.82 (1H, d, J=8.9 Hz), 6.47 (1H, d, J=2.0 Hz), 7.00-7.36 (3H, m), 7.58 (1H, m), 8.67 (1H, s). Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.09; H, 4.76; N, 9.44.

7-Methoxy-4-(3-methyl-1H-indol-2-yl)-2H-1,4-benzoxazin-3(4H)-one (38): Colorless prisms (AcOEt), mp 213-214℃, IR (KBr): 3310, 1674cm^{-1}. ^1H-NMR (DMSO-d_6): 2.03 (3H, s), 3.69 (3H, s), 4.81 (1H, d, J=15 Hz), 4.87 (1H, d, J=15 Hz), 6.25 (1H, d, J=8.9 Hz), 6.49(1H, dd, J=2.7, 8.9 Hz), 6.69 (1H, d, J=2.7 Hz), 6.90-7.53 (3H, m), 7.53 (1H, m), 11.18 (1H, s). Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.08. Found: C, 70.16; H, 5.19; N, 9.15.

2-(1H-Indol-3-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (39): Colorless needles (CH_3CN), mp 241 ℃, IR (KBr): 3400, 3160, 1667cm^{-1}. ^1H-NMR (DMSO-d_6): 3.63 (3H, s), 5.91 (1H, s), 6.44 (1H, d, J=2.8 Hz), 6.50 (1H, dd, J=2.8, 8.8 Hz), 6.85 (1H, d, J=8.8 Hz), 6.94-7.13 (2H, m), 7.17 (1H, d, J=2.7 Hz), 7.37 (1H, dd, J=2.2, 6.0 Hz), 7.61 (1H, dd, J=2.2, 6.0 Hz), 10.66 (1H,s), 11.09 (1H, s). The signal at 5.91 ppm can be assigned to the proton at position 2 of the benzoxazinone moiety. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.24; H, 4.71; N, 9.39.

6-(1H-Indol-3-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (40): Colorless flakes (CH_2Cl_2/n-hexane), mp 222-224℃, IR (KBr): 3400, 3180, 1689 cm^{-1}.

^{1}H-NMR (DMSO-d_6): 3.73 (3H, s), 4.55 (2H, s), 6.73 (1H, s), 7.13 (1H, s), 6.88-7.20 (2H, m), 7.40 (1H, dd, J= 3.0, 6.8 Hz), 7.47 (1H, d, J=2.9 Hz), 7.69 (1H, dd, J=2.5, 6.0 Hz), 10.47 (1H, s), 11.14 (1H, s). Two singlet signals (δ 6.73 and 7.13) of the benzoxazinone moiety indicate the 6-substituted benzoxazinone structure. The signal of the 3-position proton of the indole moeity is absent. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.08; H, 4.79; N, 9.47.

7-Methoxy-6-(2-methyl-1H-indol-3-yl)-2H-1,4-benzoxazin-3(4H)-one (41): Colorless prisms (CH_3CN), mp 292-293℃ , IR (KBr): 3360, 3190, 1690 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 2.24 (3H, s), 3.66 (3H, s), 4.59 (2H, s), 6.75 (1H, s), 6.85 (1H, s), 6.84-7.34 (4H, m), 10.48 (1H, s), 10.96 (1H, s). Comparison of these NMR data with those of compound 40 supports the structure. Anal. Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.08. Found: C, 70.07; H, 5.21; N, 9.02.

6-(1H-Imidazol-1-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (42): Colorless needles (EtOH), mp 286-287℃ , IR (KBr): 3120, 1686 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.74 (3H, s), 4.60 (2H, s), 6.86 (1H, s), 6.90(1H, s), 7.02 (1H, br.s), 7.30 (1H, br.s), 7.78 (1H, br.s), 10.58 (1H, s). Two singlet signals of the benzoxazinone ring (δ 6.86 and 6.90) indicate the 6-substituted benzoxazinone structure. The protons of positions 2, 4 and 5 of the imidazole moiety can be assigned. ^{13}C-NMR (D_2O/CH_3COOH): 56.23 (q), 66.30 (t), 101.70 (d), 113.04 (d), 117.27 (s), 119.02 (s), 119.36 (d), 122.72(d), 135.41 (d), 145.04 (s), 148.89 (s), 167.71 (s). Anal. Calcd for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13. Found: C, 58.67; H, 4.49; N, 16.93.

7-Methoxy-6-(1H-pyrazol-1-yl)-2H-1,4-benzoxazin-3(4H)-one (43): Colorless needles (EtOH), mp 233-234℃ , IR (KBr): 3170, 3120, 1686cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.72 (3H, s), 4.64 (2H, s), 7.00 (2H, s), 6.40 (1H, dd, J=1.7, 2.5 Hz), 7.61 (1H, d, J=1.7 Hz), 8.02 (1H, d, J=2.5 Hz), 10.61 (1H, s). Anal. Calcd for $C_{12}H_{11}N_3O_3$: C, 58.77; H, 4.52; N, 17.13. Found: C, 58.85; H, 4.58; N, 16.90.

1-(7-Methoxy-2H-1,4-benzoxazin-3(4H)-on-6-yl)-3-methyl-1H-imidazol-3-ium acetate (44): Light brown prisms (CH_3CN), mp 140℃ , IR (KBr): 3460, 3120, 1690 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 1.70 (3H, s), 3.80 (3H, s), 3.97 (3H, s), 4.58 (2H, s), 6.91 (1H, s), 7.49 (1H, s), 7.91 (2H, m), 9.60 (1H, s), 10.80 (1H, s). Anal. Calcd for $C_{15}H_{16}N_3O_5$ H_2O: C, 53.41; H, 5.68; N, 12.46. Found: C, 53.68; H, 5.52; N, 12.43.

6-(1H-Benzimidazol-1-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (45): Colorless prisms (EtOH), mp 259-261 ℃ , IR (KBr): 3140, 1673cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.78 (3H, s), 4.59 (2H, s), 6.86 (1H, s), 7.20 (1H, s), 7.13-7.40 (3H, m), 7.63-7.90 (1H, m), 8.23 (1H, s), 10.48 (1H, s). Anal. Calcd for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 64.80; H, 4.41; N, 14.13.

1-(7-Methoxy-2H-1,4-benzoxazin-3(4H)-on-6-yl)-pyridin-1-ium acetate (46): Analyzed as the chlorate (46 was recrystallized from a mixture of EtOH and concentrated HCl). Yellow needles (i-PrOH), dp 225℃, IR (KBr): 3400, 3110, 1690 cm^{-1}. ^{1}H-NMR (DMSO-d_6): 3.79 (3H, s), 4.71 (2H, s), 7.08 (1H, s), 7.28 (1H, s), 8.27 (2H, dd, J=6.2, 7.5 Hz), 8.75 (1H, t, J=7.5 Hz), 9.14 (2H, d, J=6.2 Hz), 11.11 (1H, s). Anal. Calcd for $C_{14}H_{13}ClN_2O_3$ H_2O: C, 54.12; H, 4.87; N, 9.01. Found: C, 53.78; H, 4.72; N, 8.87.

Reaction with t-butoxycarbonyl-L-tyrosine ethyl ester (Boc-L-Tyr-OEt): AMBOA (120 mg, 0.51 mmole) and Boc-L-Tyr-OEt (1.3 g, 8 eq) were suspended in 20 ml of cold benzene. Then the mixture was stirred for 30 min at room temperature. The mixture was evaporated under reduced pressure, and the residue was separated by silica gel column chromatography (CH_2Cl_2/MeOH) to give compounds 47 (58%) and 48 (16%).

Compound 47: Colorless amorphous solid. IR (KBr): 3360, 1735, 1680cm^{-1}. UV (EtOH)/ λ_{max} (ε): 258 nm (8.20×10^3), 270 nm (8.15×10^3). ^{1}H-NMR (DMSO-d_6): 1.12 (3H, t, J=7.0 Hz), 1.33 (9H, s), 2.84 (2H, m), 3.70 (3H, s), 4.04 (2H, q, J=7.0 Hz), 4.73 (2H, s), 6.19 (1H, d, J=8.9 Hz), 6.42 (1H, dd, J= 2.5, 8.9 Hz), 6.65 (1H, d, J=2.5 Hz), 6.90 (1H, d, J=8.0 Hz), 6.97 (1H, s), 7.15 (1H, dd, J=2.0, 8.0 Hz), 7.24 (1H, d-like), 9.56 (1H, br.s). The protons of the tyrosine moiety can be assigned. The UV spectrum is very similar to those of 12 and 15. Anal. Calcd for $C_{25}H_{30}N_2O_8$: C, 61.72; H, 6.22; N, 5.76. Found: C, 61.48; H, 6.28; N, 5.53.

Compound 48: Colorless syrup. (M^+) 486.1988 (Calcd for $C_{25}H_{30}N_2O_8$: 486.1999). ^{1}H-NMR ($CDCl_3$): 1.26 (3H, t, J=7.0 Hz), 1.39 (9H, s), 2.50-3.40 (2H, m), 3.75 (3H, s), 4.16 (2H, q, J=7.0 Hz), 4.47 (2H, s), 4.80 (1H, m), 5.00 (1H, m), 6.39 (2H, m), 6.41 (1H, dd, J=2.8, 9.2 Hz), 6.58 (1H, d, J= 2.8 Hz), 6.71 (1H, d, J=9.2 Hz), 7.34 (2H,m). The dienone structure can be deduced from the NMR and UV spectra (by comparison with those of compound 14).

Reaction with t-butoxycarbonyl-L-histidine methyl ester (Boc-L-His-OMe): AMBOA (102 mg) and Boc-L-His-OMe (0.92 g, 8 eq) were dissolved in 15 ml of cold DMF. Then the solution was stirred for 30 min at room temperature. The solution was evaporated under reduced pressure, and the residue was separated by silica gel column chromatography (CH_2Cl_2/MeOH) to give compound 49 (117 mg, 61%). Light brown amorphous solid. (M^+) 446.1933 (Calcd for $C_{21}H_{26}N_4O_7$: 446.1833). IR (KBr): 3370, 1737 (sh), 1719 (sh), 1695, 1517 cm^{-1}. UV (95% EtOH)/λ_{max} : pH 3, 265, 298 nm; pH 7, 263, 300 nm; pH 11, 279, 308 nm. ^{1}H-NMR ($CDCl_3$): 1.43 (9H, s), 3.12 (2H, d, J=5 Hz), 3.72 (3H, s), 3.79 (3H, s), 4.54 (1H, m), 4.64 (2H, s), 5.92 (1H, d, J= 8Hz), 6.68 (1H, s), 6.81 (1H, s), 6.92 (1H, br.s), 7.69 (1H, br.s), 9.24 (1H,

br.s). Comparison of these NMR data with those of compound 42 supports the structure. Anal.Calcd for $C_{21}H_{26}N_4O_7 \cdot \frac{1}{4}H_2O$: C, 55.93; H, 5.92; N, 12.42. Found: C, 55.93; H, 5.92; N, 12.19.

Reaction with t-butoxycarbonyl-L-tryprophan methyl ester (Boc-L-Trp-OMe): AMBOA (380 mg, 1.60 mmole) and Boc-L-Tyr-OEt (1.0 g, 2eq) were suspended in 10 ml of ice-cold CH_2Cl_2. Then the mixture was stirred for 30 min at room temperature. The mixture was evaporated under reduced pressure, and the residue was separated by silica gel column chromatography (CH_2Cl_2/AcOEt) to give compounds 50 (20%) and 51 (21%).

Compound 50: Colorless prisms (ether/n-hexane), mp 185-186℃ . (M^+)495. IR (KBr): 3360, 1750, 1683 cm^{-1}. UV (EtOH): 237, 286, 310 (sh) nm. ^{1}H-NMR ($CDCl_3$): 1.39 (3H, s), 3.31 (2H, m), 3.70 (3H, s), 3.72 (3H, s), 4.13 (1H, dd, J=7.0, 8.2 Hz), 4.28 (1H, d, J=15.5 Hz), 4.57 (1H, d, J=15.5 Hz), 5.82 (1H, s), 6.39 (1H, dd, J=2.8, 8.9 Hz), 6.57 (1H, d, J=2.8 Hz), 6.90 (1H, d, J=8.9 Hz), 6.60-6.82 (2H, m), 7.13 (1H, m), 7.56 (1H, m). ^{13}C-NMR ($CDCl_3$): 28.07 (q), 43.19 (t), 52.04 (q), 55.38 (q), 58.83 (d), 70.55 (t), 74.83 (s), 79.05 (d), 81.34 (s), 103.31 (d), 107.82 (d), 109.46 (d), 119.78 (2C, d), 122.71 (s), 125.81 (d), 128.22 (s), 130.15 (d), 147.67 (s), 148.96 (s), 153.71 (s), 156.40 (s), 170.06 (s), 171.64 (s). There appeared to be no signal attributable to the proton of the indole 2-position. Instead, a singlet at 5.82 ppm was found and assigned to the 8a proton of 1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole, suggesting the structure. The stereochemistry of 50 and 51 was also deduced from the ^{1}H-NMR spectra. The methyl signal of the ester group of 50 was observed at lower field (3.70 ppm) than that of 51 (3.26 ppm), and the signal of hydrogen (position 2 of the hexahydropyrroloindole moiety) of 50 (4.13 ppm) was observed at higher field than that of 51 (4.58 ppm). The molecular model studies suggest that the methyl ester of the structure 51 (the anti isomer between the 2-carbomethoxy group and the 3a-benzoxazinone ring) can be shielded by the benzene ring of the hexahydropyrroloindole. In addition, the spectral features of 50 and 51 correspond to the reported data for the syn and anti isomers of racemic 3a-hydroxy derivatives.[40]

Compound 51: Colorless powder (n-hexane), mp 85-95℃ . (M^+) 495. IR(KBr): 3360, 1745, 1688 cm^{-1}. UV (EtOH): 241, 286, 310 (sh) nm. ^{1}H-NMR ($CDCl_3$): 1.41, 1.51 (3H, sx2), 3.10 (1H, dd, J=1.4, 9.3 Hz), 3.60 (1H, m), 3.26 (3H, s), 3.72 (3H, s), 4.60 (1H, m), 4.28 (1H, d, J=15.0 Hz), 4.63 (1H, d, J=15.0 Hz), 5.58 (1H, s), 6.41 (1H, dd, J=2.8, 8.9 Hz), 6.55 (1H, d, J=2.8 Hz), 6.60-6.80 (2H, m), 6.92-7.22 (2H, m), 7.52 (1H, m). ^{13}C-NMR ($CDCl_3$): 28.19 (q), 43.48 (t), 51.86 (q), 55.38 (q), 59.30 (d), 70.61 (t), 75.48 (s), 78.11 (d), 80.93 (s), 103.19 (d), 107.88 (d), 109.52 (d), 119.37(d), 119.95 (d), 122.77 (s), 126.34 (d), 126.81 (s), 130.39 (d), 148.73 (s),

149.25 (s), 153.59 (s), 156.46 (s), 169.88 (s), 170.94 (s).

Reaction with nucleotides: AMBOA (244 mg, 1.03 mmole) and 2 equivalents of a nucleotide sodium salt (2'-deoxyguanosine 5'-phosphate: 5'-dGMP or guanosine 5'-phosphate: 5'-GMP) were dissolved in 20 ml of a mixture of water and DMF (4:1~ 10:1 v/v) under ice-cooling. The solution was stirred at room temperature for 30 min. Then, 120 ml of water and 150 ml of CH_2Cl_2 were added to the solution. The separated aqueous phase was taken, washed with CH_2Cl_2 to remove DMF, and lyophilized. The residue thus obtained was separated by Sephadex LH-20 column chromatography (H_2O) and by high-performance liquid chromatography (Polygosil $_5C_{18}$, CH_3CN/aqueous NH_4OH). The fractions which contained an AMBOA-nucleotide adduct were lyophilized. The residue was dissolved in 1 ml of MeOH, and addition of 3 ml of EtOH resulted in precipitation of compound 52 (58%) or 53 (47%).

Compound 52: Colorless powder, dp >220℃ . m/e 328. UV (H_2O)/λ_{max}(ε): pH 1.5, 259 nm (2.0×10^4), 278 nm (sh); pH 7.0, 259 nm (2.0×10^4), 278 nm (sh); pH 11.0, 278 nm (1.7×10^4), 310 nm (sh). ^{1}H-NMR (D_2O): 1.82-2.30 (1H, m), 2.93 (1H, ddd, J=6, 7, 14 Hz), 3.70 (3H, s), 3.76-4.12 (3H, m), 4.79 (1H, q, J=14 Hz), 4.81 (1H, q, J=14 Hz), 5.89 (1H, t-like, J=7 Hz), 6.24-6.60 (2H, m), 6.54 (1H, m). The lack of an 8-H (guanine) proton signal and the presence of all the C-H protons of the benzoxazinone moiety (positions 2 (2H), 5, 6 and 8) indicate the structure.

Compound 53: Colorless powder, m/e 328. UV (H_2O)/ λ_{max}(ε): pH 1.5, 260 nm (2.2×10^4), 274 nm (sh); pH 7.0, 260 nm (2.2×10^4), 274 nm (sh); pH 11.0, 278 nm (1.9×10^4), 310 nm (sh). ^{1}H-NMR (D_2O): 3.70 (3H, s), 3.80-4.10 (3H, m), 4.24-4.46 (1H, m), 4.82 (1H, q, J=14 Hz), 4.84 (1H, q, J=14 Hz), 5.13 (1H, t-like, J=6 Hz), 5.55(1H, d-like, J=6 Hz), 6.40-6.70 (2H, m), 6.54 (1H, d, J=2 Hz). ^{13}C-NMR (D_2O): 55.67 (q), 64.63 (t), 67.51 (t), 70.24 (d), 71.12 (71.55)*(d), 83.64 (d), 88.27 (d), 103.33 (d), 108.30 (d), 115.08 (s), 116.68 (117.07)(d), 121.41 (121.56)(s), 135.23 (135.84)(s), 144.85 (s), 151.73 (s), 153.72 (s), 156.84 (156.94)(s), 158.31 (s), 167.37 (s) (*peaks of a rotational isomer of 53).

4-(Guanin-8-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (54): Compounds 52 and 53 could be hydrolyzed in CF_3COOH (20 mg/ml, 15hr at room temperature) to give 4-(guanin-8-yl)-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (54) in the yields of 80%. Colorless needles (MeOH), mp >300℃ , UV (H_2O)/λ_{max}(ε): pH 1.5, 254 nm (2.0×10^4), 285 nm (sh); pH 7.0, 253 nm (2.3×10^4), 280 nm (sh); pH 11.0, 255nm (2.1×10^4), 274 nm (2.0×10^4), 321 nm (sh). ^{1}H-NMR (DMSO-d_6): 3.71 (3H, s), 4.81 (2H, s), 6.42 (2H, br.s), 6.54 (2H, br.s), 6.69 (1H,d, J=2 Hz), 10.63 (1H, br.s), 12.72 (1H, br.s). ^{13}C-NMR (DMSO-d_6): 55.46, 67.45, 103.33, 108.15, 114.36, 116.70, 122.13, 135.42, 144.97, 152.89, 153.66, 155.91, 156.37, 164.05. Anal. Calcd for $C_{14}H_{12}N_6O_4\cdot$ C,

51.22; H, 3.69; N, 25.60. Found: C, 51.35; H, 3.81; N, 25.38.

The same compound (54) could also be prepared by the reaction of AMBOA with guanine. Guanine (500 mg, 3.3 mmole) was suspended in a mixture of DMSO and water (3:2 v/v, 50 ml), and 400mg of AMBOA (1.65 mmole) was added. The suspension was stirred vigorously for 30 min at room temperature. Then the mixture was evaporated under reduced pressure, and the resultant residue was extracted with EtOH (20 ml). The extract was evaporated, and the residue was recrystallized from MeOH to give 34 mg of 54 (6%).

Reaction with calf thymus DNA: Calf thymus DNA (500 mg, 1.56 mmole P) was dissolved in a mixture of water and DMF (5:1 v/v, 240 ml). The solution was cooled (0 ℃) and 370 mg (1.56 mmole) of AMBOA was added. The solution was stirred at room temperature for 30 min, and then 600 ml of EtOH and 10 ml of brine were added. The resulting precipitate of DNA was dissolved in 120 ml of H_2O containing a small amount of CH_3COOH (to adjust the pH to 5.5), then 2 mg of Nuclease P1 (Yamasa Co.) was added, and the mixture was incubated at 55 ℃ for 1 hr. The solution was lyophilized, and the residue thus obtained was separated by Sephadex LH-20 column chromatography (H_2O). The fraction containing modified nucleotide (52) was lyophilized, and the residue was purified by repeated precipitations from a mixture of MeOH and EtOH to give 7.3 mg of 52.

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