# BENZO-DERIVATIVES OF MUTAGENIC-CARCINOGENIC 2-AMINODIPYRIDO[1,2-a:3',2'-d]IMIDAZOLE

Ching-Shing Lee,[a] Yuichi Hashimoto,[b] Koichi Shudo,*[b] and Minako Nagao[c]

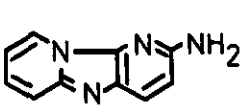
a) Chia Nan Junior College of Pharmacy
72-1 Pao-an Tsun, Jen-te Hsiang, Tainan Hsein, Taiwan, R.O.C.

b) Faculty of Pharmaceutical Sciences, University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

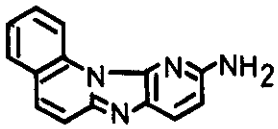
c) National Cancer Center Research Institute
5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Japan

Abstract ——— Benzo-derivatives of mutagenic-carcinogenic 2-aminodipyrido[1,2-a:3',2'-d]imidazole (Glu-P-2) were synthesized in short steps. The interaction of the compounds with DNA and their mutagenicity were investigated.

2-Aminodipyrido[1,2-a:3',2'-d]imidazoles (Glu-P's) are potent mutagen/-carcinogens isolated from a pyrolysate of L-glutamic acid.[1] Glu-P's bind to DNA *in vivo* at the position 8 of guanine residues in DNA after metabolic activation.[2] The chemical modification of DNA with Glu-P's is believed to be responsible for their mutagenic-carcinogenic activity. In the path of the chemical modification of DNA, the intercalation of Glu-P's into DNA base pairs preceding the covalent reaction is one of important steps.[2,3] Recently, we reported the synthesis,[4] physical interaction (intercalation) with DNA,[3] and mutagenicity of methyl-substituted Glu-P's.[4] The molecular shapes of Glu-P-derivatives effect on their intercalative ability and the conformation of the intercalated complex,[5] which seem to be important factors determining their mutagenic activity. In this communication, we describe synthesis and interaction with DNA of benzo-derivatives of Glu-P-2, compounds 1, 2 and 3, which are structurally related to intercalative antitumor agents



Glu-P-2



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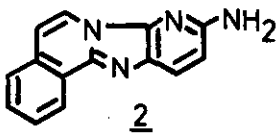

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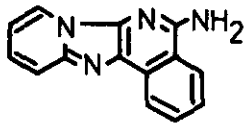


3

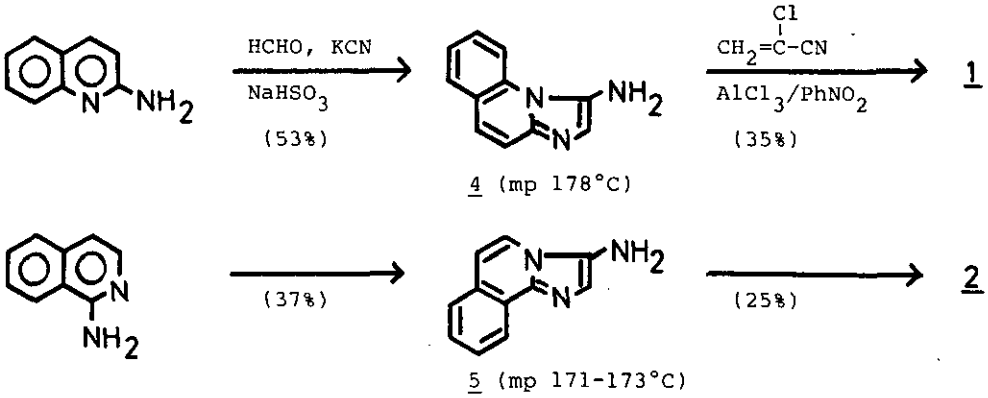
with four-fused-rings such as ellipticine.

The key-step for the short-step synthesis of these benzo-Glu-P's (1-3) is the construction of the 2-aminopyridyl moiety. Two approaches were investigated, that is, (i) Houben-Hoesch reaction approach and (ii) Strecker reaction approach.

(i) Houben-Hoesch reaction approach ——— Synthesis of 10-aminopyrido[3',2';4,5]imidazo[1,2-a]quinoline (1) and 9-aminopyrido[3',2';4,5]imidazo[1,2-a]-isoquinoline (2)

2-Aminopyridyl moiety of the compounds 1 and 2 could be constructed from aminoimidazoquinoline (4) and aminoimidazoisoquinoline (5), respectively, by the reaction with 2-chloroacrylonitrile under Houben-Hoesch conditions (Scheme I). Compounds 4 and 5 were obtained by Strecker reaction from 2-aminoquinoline and 1-aminoisoquinoline, respectively, as shown in Scheme I. Compounds 4 and 5 were heated (100°C) with 2-chloroacrylonitrile (2eq.) in nitrobenzene in the presence of 4 equivalent of $AlCl_3$ for 10 h. The yields of 1 and 2 were 35% and 25%, respectively. Though the yields are not high, the method is generally useful for a large-scale and short-step synthesis of many Glu-P-derivatives.[1a,4]

Scheme I

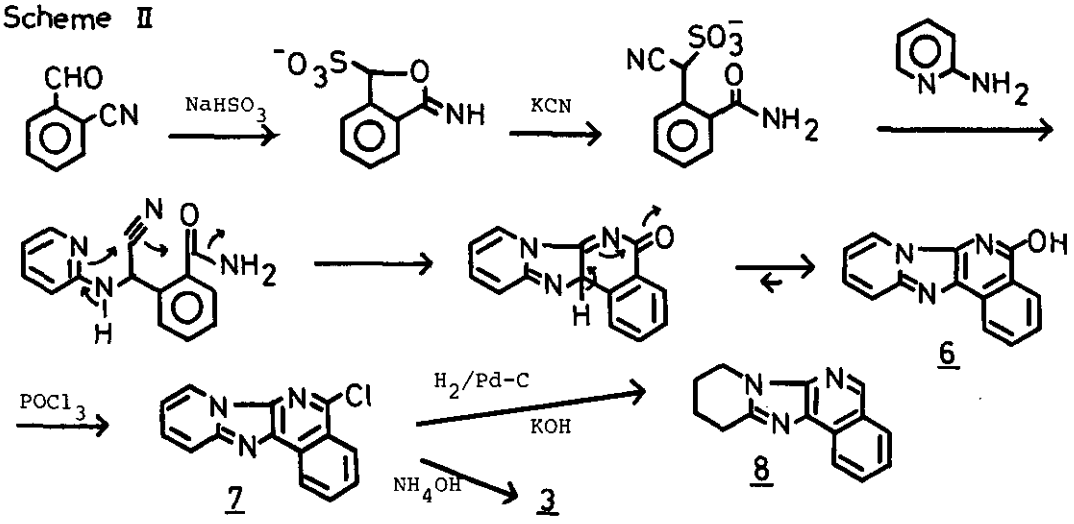


(ii) Strecker reaction approach ——— Synthesis of 5-aminopyrido[1',2';1,2]-imidazo[5,4-c]isoquinoline (3)

The retro-Strecker synthesis of 3 gives 2-aminopyridine and o-cyanobenzaldehyde. Previously, we reported that Strecker reaction of 2-aminopyridine with KCN and acrylaldehyde failed to give Glu-P-2.[1b,4] But in this case, the Strecker reaction of 2-aminopyridine (1eq.) with KCN (2eq.), o-cyanobenzaldehyde (2.5eq.) and $NaHSO_3$ (1eq.) in water gave 2-hydroxy derivative (6) in the yield of 31%: four bonds are formed in one step as shown in Scheme II. The 2-hydroxy group of 6 was converted to a chloro group by a treatment with $POCl_3$ to give 7. The structure of 7 was deduced from its NMR, UV, IR, mass spectroscopy and elemental analysis. The structure was supported by the hydrogenation of 7 to a 6,7,8,9-tetrahydro-derivative (8): this reaction is characteristic to Glu-P's. The 2-chloro group of 7 was converted to an amino group by the usual method, giving 3.

The physical constants, mp, mass, $^1$H-NMR, UV and elemental analysis, of these synthesized benzo-Glu-P's are shown in Table I.

Scheme II




The physical binding of the synthesized benzo-Glu-P's (1-3) was measured by the shift of UV absorption in the presence of double stranded calf thymus DNA. The binding constants with DNA under the conditions used in the experiment are shown in Table II. The mutagenicity of compounds 1-3 were tested by Ames' assay using Salmonella typhimurium TA98 in the presence of rat liver metabolizing enzyme systems (Table II).

Table I. The physical constants of synthesized benzo-Glu-P's (1-3)

| |
|---|
| 1: mp 261-263°C. $M^+$234. NMR($CD_3OD$): δ 6.79(d,J=8Hz,1H), 7.18(d,J=7Hz,1H), 7.6-7.8(m,3H), 7.91(d,J=8Hz,1H), 8.32(d,J=7Hz,1H), 8.54(m,1H). UV(EtOH in nm, log ε): 231(4.49), 239(4.49), 274(4.18), 312(3.29), 324(3.59), 339(3.38), 375(2.57). Anal.Calcd for $C_{14}H_{10}N_4$: C;71.79, H;4.27, N;23.93. Found: C;71.61, H;4.28, N;24.04. |
| 2: mp 217-219°C. $M^+$234. NMR($CD_3OD$): δ 6.78(d,J=8Hz,1H), 7.16(d,J=8Hz,1H), 7.3-7.6(m,3H), 7.91(d,J=8Hz,1H), 8.32(d,J=8Hz,1H), 8.50(m,1H). UV(EtOH): 230 (4.43), 263(4.55), 284(4.35), 297(4.44), 358(4.24). Anal. Found: C;71.75, H;4.20, N;24.04. |
| 3: mp 254-256°C. $M^+$234. NMR($CDCl_3$): δ7.0(m,1H), 7.3-7.9(m,4H), 8.24(d,J=8Hz, 1H), 8.46(d,J=8Hz,1H), 8.60(d-like,J=8Hz,1H). UV(EtOH): 238(4.34), 243 (4.37), 261(4.49), 299(3.59), 313(3.63), 374(4.07), 385(4.12), 403(3.97). Anal. Found: C;71.63, H;4.23, N;23.93. |

Table II. Binding constants (K) with DNA and mutagenicity (revertants/μg) toward Salmonella typhimurium TA98 in the presence of metabolic enzyme systems (S-9 mix).

| compound | $K(\times 10^4 M^{-1})^{a)}$ | rev./μg[b)] | compound | $K(\times 10^4 M^{-1})^{a)}$ | rev./μg[b)] |
|---|---|---|---|---|---|
| Glu-P-2 | 2.00 | 430 | 2 | 20.00 | 3800 |
| 1 | 0.24 | 281 | 3 | 0.03 | 5 |

a) Binding constants (K) were measured in a phosphate buffer (1mM, pH 7.3) at room temperature. Concentrations of the compounds: 0.98-1.18 x $10^{-5}$M. Calf thymus DNA: 0-0.81 mM, nine concentrations. b) The mutation test with Salmonella typhimurium TA98 was carried out as described previously.[6] The metabolizing enzymes (S-9) were prepared from the liver of rats injected with polychlorinated biphenyls (PCB).

The binding constants (K) are very different each other reflecting the geometrical structures of benzo-Glu-P's. Interestingly, 2 shows higher affinity toward DNA and mutagenicity than Glu-P-2: the other two benzo-Glu-P's (1 and 3) are similar in their molecular size with 2, but exibit lower affinity and mutagenicity than Glu-P-2. The binding constants of benzo-Glu-P's decrease in the order of 2 > 1 > 3, which is the same order of the decreasing mutagenicity: there seems to exist a correlation between their mutagenicity and the binding

constants with DNA in this case, while any relationship between mutagenicity and binding constants was not found in the case of methyl-substituted Glu-P's,[3,4] where the conformation of an intercalated complex formed from DNA and metabolically activated Glu-P's seems to be a more important factor rather than the affinity of Glu-P's toward DNA.[3]

In conclusion, three benzo-derivatives of Glu-P-2 (1-3) were synthesized in short steps. The shape of the synthesized benzo-Glu-P's affects on the binding ability with DNA and their mutagenicity.

REFERENCES

1. a) T.Yamamoto, K.Tsuji, T.Kosuge, T.Okamoto, K.Shudo, K.Takeda, Y.Iitaka, K.Yamaguchi, Y.Seino, T.Yahagi, and T.Sugimura, Proc.Japan Acad., 1978, 54B, 248. b) K.Takeda, K.Shudo, T.Okamoto and T.Kosuge, Chem.Pharm.Bull., 1978, 26, 2924.

2. a) Y.Hashimoto, K.Shudo and T.Okamoto, J.Am.Chem.Soc., 1982, 104, 7637. b) Y.Hashimoto and K.Shudo, Biochem.Biophys.Res.Commun., 1983, 116, 1100. c) Y.Hashimoto, K.Shudo and T.Okamoto, Chem.Pharm.Bull., 1979, 27, 2532. d) idem, Biochem.Biophys.Res.Commun., 1980, 92, 971. e) idem, Acc.Chem.Res., in press.

3. a) M.Imamura, K.Takeda, K.Shudo, T.Okamoto, C.Nagata and M.Kodama, Biochem. Biophys.Res.Commun., 1980, 96, 611. b) M.Imamura, K.Shudo, T.Okamoto and T.Andoh, ibid, 1980, 97, 968. c) C.-S.Lee, Y.Hashimoto, T.Ohta, K.Shudo and T.Okamoto, Chem.Pharm.Bull., 1982, 30, 3046. d) Y.Hashimoto, C.-S.Lee, K.Shudo and T.Okamoto, Tetrahedron Lett., 1983, 24, 1523.

4. K.Takeda, K.Shudo, T.Okamoto, M.Nagao, K.Wakabayashi, and T.Sugimura, Carcinogenesis, 1980, 1, 889.

5. K.Yamaguchi et al., unpublished results.

6. T.Yahagi, M.Nagao, Y.Seino, T.Matsushima, T.Sugimura and M.Okada, Mutation Res., 1977, 48, 121.

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