Direct \(N^d\)-Selective Alkylation of Hydantoins Using Potassium Bases

Yumi Shintani, Koichi Kato, Masashi Kawami, Mikihsa Takano, and Takuya Kumamoto

\(a\) Department of Synthetic Organic Chemistry, Graduate School of Biomedical and Health Sciences, Hiroshima University; \(b\) Integrative Brain Imaging Center, National Center of Neurology and Psychiatry; \(c\) Department of Pharmaceutics and Therapeutics, Graduate School of Biomedical and Health Sciences, Hiroshima University.

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Hydantoins, including the antiepileptic drug phenytoin, contain an amide nitrogen and an imide nitrogen, both of which can be alkylated. However, due to the higher acidity of its proton, \(N^3\) can be more easily alkylated than \(N^1\) under basic conditions. In this study, we explored methods for direct \(N^d\)-selective methylation of phenytoin and found that conditions using potassium bases [potassium tert-butoxide (BuOK) and potassium hexamethyldisilazide (KHMDS)] in tetrahydrofuran (THF) gave \(N^3\)-monomethylated phenytoin in good yield. The applicable scope of this reaction system was found to include various hydantoins and alkyl halides. To explore the function of methylated hydantoins, the effects of a series of methylated phenytoins on P-glycoprotein were examined, but none of methylated products showed inhibitory activity toward rhodamine 123 efflux by P-glycoprotein.

Key words methylation; regioselective; hydantoin; P-glycoprotein

Introduction

Hydantoins (1) are a major scaffold with various applications\(^{2,3}\) (Fig. 1). The structure is found in pharmaceuticals and pesticides, and modifications such as alkylation, arylation, and halogenation have been made at the \(N^1\), \(N^3\), and 5 positions to obtain desired activities. Phenytoin (1a) is a 5,5-diphenyl analog of \(N^1\) and is used as an anticonvulsant. As frequently seen in antiepileptic drugs, 1a was reported as a weak substrate of P-gp, and is used as an anticonvulsant.\(^1\a\) In silico studies of the X-ray structure of P-gp and docking studies,\(^5,6\) according to the applicable scope of this reaction system was found to include various hydantoins and alkyl halides. Therefore, alkylation of one or both of the \(N^1\) and \(N^3\) positions of these compounds is expected to affect their pharmacokinetic behavior.

In conventional alkylation using alkyl halides under basic condition, \(N^d\) can be more easily alkylated than \(N^1\) because of the more acidic proton on \(N^3\). For example, treatment of 1a with \(\text{CH}_3\text{I}\) in the presence of a weak base such as \(\text{K}_2\text{CO}_3\) gives \(N^3\)-monomethylated product 2, and a mixture of 2 and dimethylated 3 is obtained when stronger bases such as NaH are used.\(^7,8\) For the selective preparation of \(N^1\)-alkylated hydantoins such as 4, including \(N^1\)-monomethylated phenytoin (4a), two main methods have been utilized: 1) hydantoin-ring formation using \(N\)-substituted substrates\(^9\-13\); 2) \(N^1\)-alkylation of \(N^3\)-protected hydantoins by aminals or tert-alky groups followed by deprotection of \(N^3\).\(^14\-18\) To date, examples of \(N^1\)-selective alkylation of unprotected hydantoins are limited.

Crosignani et al.\(^19\) reported the synthesis of \(N^3\)-methylated hydantoins as an intermediate of various \(N^3\)-alkyl-N\(^3\)-methyl-hydantoin derivatives. Positron emission tomography (PET) experiments using \(^{11}\)C-labeled 1a showed increased brain uptake in rat after inhibition of P-gp by tariquidar.\(^20\) PET tracers that are weak P-gp substrates are useful tools for assessing P-gp function based on overexpression. A hydrogen bond between Tyr307 and the \(N^3\)-hydrogen of phenytoin (1a) was estimated by in silico screening.\(^21\) Thus, \(N^3\)-selective alkylation of hydantoins would enable their modification while leaving \(N^1\) unsubstituted, thus allowing for effective hydrogen bonding to P-gp. Furthermore, the development of new methodology for rapid methylation reactions would be useful for preparing compounds labeled with short-lived isotopes such as \(^{11}\)C. In this note, we report our studies on \(N^d\)-selective alkylation of hydantoins 1, primarily phenytoin (1a), using various bases and alkyl halides in order to realize a simple method for the selective and rapid preparation of \(N^3\)-alkylated phenytoin (Fig. 1), and investigate the inhibitory activity of methylated phenytoins toward P-gp using rhodamine 123.

Results and Discussion

We began our investigation by using the reaction system reported by Crosignani et al., where \(N\)-unsubstituted hydantoin was selectively methylated at \(N^1\) using lithium bis-(trimethylsilyl)amide (LiHMDS) as base in tetrahydrofuran.

![Fig. 1. Modification of Hydantoins (1) with Alkyl Halides](image-url)
The ratio was estimated from integration in the 1H-NMR spectra of the crude products. a) Yield based on 1a. b) Reactions were carried out using LHMDS (2.2 equiv) and CH3I (1.2 equiv) for 6 h. c) Solid BuONa was used. 1 2 3 NaHMDS 52 : 48 : 0 —

| Run | Base       | Ratio of 1a : 4a : 3a (%) | Isolated 4a (%) |
|-----|------------|---------------------------|-----------------|
| 1   | LiHMDS     | 12 : 83 : 5               | 66              |
| 2   | LiHMDS     | 60 : 40 : 0               | —               |
| 3   | NaHMDS     | 52 : 48 : 0               | —               |
| 4   | KHMS       | 34 : 66 : 0               | 69              |
| 5   | BuOLi      | 84 : 16 : 0               | —               |
| 6   | 'BuOK      | 45 : 55 : 0               | —               |
| 7   | 'BuONa     | 23 : 76 : 0               | 73              |
| 8   | 'BuOK      | 5 : 93 : 2                | 79              |

(a) Compound 1a, base solution (2.0 equiv), and CH3I (0.94 equiv) were used unless otherwise noted. b) Ratio was estimated from integration in the 1H-NMR spectra of the crude products. c) Yield based on 1a. d) Reactions were carried out using LHMDS (2.2 equiv) and CH3I (1.2 equiv) for 6 h. e) Solid BuONa was used. f) 1.2 equiv of alkyl halides (Table 3). Reaction of 5-methyl-5-phenyldihydropyridin 1b gave N2-methylated 4b as the sole product in lower yield (27%) with recovering 1b (56%) (run 1). It could be because N3–H of 1a, which could be activated by two phenyl groups at C5, would be more acidic than that of 1b with one phenyl group. The reactions of phenytoin 1a using other alkyl halides such as benzyl bromide, allyl bromide, and bromoacetate were rather slow. The reaction with benzyl bromide gave the desired N1-benzylated 4c was obtained as the major product (53%) with a small amount of dibenzylation 5 (4%) in 6 h (run 2). Lower reactivity but N1-selectivity were observed when allyl bromide was used, and N2-allylated 4d was obtained in 32% yield with recovering 1a (21%) (run 3). Generation of other by-products were not observed in the crude products in both cases. On the other hand, the desired N3-alkylated product 4e was not obtained when using ethyl bromoacetate, and instead N3-alkylated 6 (55%) and dialkylated 7 (5%) were obtained (run 4). The reactivity of methyl, benzyl, and allyl halides were reported as methyl ≈ allyl < benzyl. The lower reactivity of benzyl and allyl halides in this case would be because of steric effect between alkyl groups in alkyl halides and two phenyl groups in 1a. The origin of the N3-selectivity of this reaction is not clear, but the use of THF as solvent appeared to be a factor in the selectivity. As deduced from the acidity of hydantoin, N3-H will be more easily deprotonated by 'BuOK than N3–H to generate potassium salt. X-ray crystallographic analysis of phenytoin sodium (the corresponding sodium salt of 8)25)
showed the formation of a $N^5$-Na bond, and intramolecular coordination of the oxygen in C(4)=O to Na was also observed. A similar coordinated structure might be formed in the reaction of 1a and $\text{BuOK}$ in THF, which is a less polar solvent than DMF, and the reaction at $N^3$ would then be hampered by the formation of the coordinated structure. The second equivalent of base would deprotonate $N^6$-$\text{H}$, and the more reactive $N^6$-$\text{H}$ in 9 could react with CH$_3\text{I}$ to give $N^6$-methylated 4a as the major product (Fig. 2). Addition of 18-crown-6 would assist the dissociation of the $N^6$-$\text{K}$ bond, leading to the generation of a more naked anion on $N^7$ to give $N^7$-methylated 2 and 1,3-dimethylated 3, as in when using DMF, which is a more polar solvent than THF (Table 2, runs 4 and 5). The reaction using bromoacetate, which has an ester function as the site of coordination to the potassium ion, also gave similar selectivity (Table 3, run 4).

We examined the inhibitory activity of 1a and methylated products 2, 3, and 4a toward P-gp. We used LLC-PK1 and LLC-GA5-COL150 cells as low and high P-gp-expressing cells, respectively.$^{26,27}$ Rhodamine 123 (7) and verapamil were used as a substrate and inhibitor of P-gp, respectively, and the fluorescent activity of 7 was measured in the presence of phenytoins. High uptake of 7 was observed in all the LLC-PK1 cells. In contrast, low uptake of 7 was observed in the LLC-GA5-COL150 cells in the presence of a series of methylated phenytoins. High uptake of 7 was observed even in the LLC-GA5-COL150 cells in the presence of verapamil. These results suggest these phenytoins (1a) and the derivatives have no inhibitory activity toward rhodamine 123 efflux by P-gp.

In conclusion, we optimized the reaction conditions for 1-alkylated product after longer reaction times. High uptake of LLC-GA5-COL150 cells in the presence of a series of methylated phenytoins. High uptake of 7 was observed even in the LLC-GA5-COL150 cells in the presence of verapamil. These results suggest these phenytoins (1a) and the derivatives have no inhibitory activity toward rhodamine 123 efflux by P-gp.

Experimental

General Procedure: Methylation of Phenytoin (1a) Using BuOK (Table 1, run 8) To a solution of phenytoin (1a, 100mg, 0.40mmol) in THF (2.0mL), BuOK (Aldrich, 1M solution in THF, 0.80mL, 0.80mmol) was added at r.t. After 3min, CH$_3$I (30 µL, 0.48mmol) in THF (0.1mL) was added at r.t. and the mixture was stirred at r.t. for 5min. One molar HCl (2mL) was added and the whole was extracted with AcOEt ($2 \times 10$, $1 \times 5$mL). The combined organic layer was washed with brine (1 $\times 5$mL) and dried over Na$_2$SO$_4$. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography ($n$-hexane–AcOEt 2:1) to give 4a as colorless solids (83mg, 79%).

1-Methyl-5,5-diphenylimidazolidine-2,4-dione (4a) Colorless solids. mp 216.5–218.0°C (lit$^{33}$ 223–224°C). IR (KBr) ν (cm$^{-1}$) 3024, 1768, 1703. $^1$H-NMR (500MHz, CDCl$_3$) δ (ppm) 2.77 (3H, s, CH$_3$), 7.26–7.31 (4H, m, Ar–H), 7.38–7.42 (6H, m, Ar–H), 9.31 (1H, s, NH). $^{13}$C-NMR (125MHz, CDCl$_3$) δ (ppm) 26.4, 75.8, 128.2, 128.8, 128.9, 136.1, 155.9, 174.1. High resolution electrospray ionization (HRESI)MS m/z 267.1130 (Caled for C$_{13}$H$_{15}$N$_2$O$_2$: 267.1134).

2-Methyl-5,5-diphenylimidazolidine-2,4-dione (2) Pale yellow solids. mp 218.5–219.0°C (lit$^{34}$ 223–224°C). IR (KBr) ν (cm$^{-1}$) 3287, 1773, 1700. $^1$H-NMR (500MHz, CDCl$_3$) δ (ppm) 3.10 (3H, s, CH$_3$), 6.11 (1H, s, NH), 7.32–7.38 (10H, m, Ar–H). $^{13}$C-NMR (125MHz, CDCl$_3$) δ (ppm) 25.0, 70.3, 128.5, 128.7, 128.8, 134.6, 156.3, 173.7.

1,3-Dimethyl-5,5-diphenylimidazolidine-2,4-dione (4b) Colorless solids, mp 185–186.5°C (lit$^{20}$ 186–188°C). IR (KBr) ν (cm$^{-1}$) 3024, 1768, 1703. 1H-NMR (500MHz, CDCl$_3$) δ (ppm) 1.85 (3H, s, CH$_3$), 2.81 (3H, s, CH$_3$), 7.30–7.55 (5H, m, Ar–H). $^{13}$C-NMR (125MHz, CDCl$_3$) δ (ppm) 25.3, 26.7, 74.6, 128.2, 128.6, 128.8, 134.6, 156.3, 173.7.

1,5-Dimethyl-5,5-diphenylimidazolidine-2,4-dione (4c) Yellow solids. mp 317.7, 1773, 1707. $^1$H-NMR (500MHz, CDCl$_3$) δ (ppm) 4.54 (2H, s, CH$_2$), 6.77 (2H, d, J = 7.3Hz, Ar–H), 6.99–7.06 (3H, m, Ar–H), 7.25–7.33 (10H, m, Ar–H), 9.21 (1H, s, NH). $^{13}$C-NMR (125MHz, CDCl$_3$) δ (ppm) 45.2, 76.9, 126.9, 127.8, 127.9, 128.6, 128.7, 128.9, 136.3, 136.4, 156.3, 173.7. HRESIMS m/z 343.1443 (Caled for C$_{28}$H$_{29}$N$_2$O$_2$: 343.1447).

1-Benzyl-5,5-phenylimidazoline-2,4-dione (5) A colorless oil. IR (neat) ν (cm$^{-1}$) 1765, 1719. $^1$H-NMR (500MHz, CDCl$_3$) δ (ppm) 4.54 (2H, s, CH$_2$), 6.77 (2H, d, J = 6.9Hz, Ar–H), 6.95–7.06 (3H, m, Ar–H), 7.17–7.19 (4H, m, Ar–H), 7.25–7.39 (11H, m, Ar–H). $^{13}$C-NMR (125MHz, CDCl$_3$) δ (ppm) 43.0, 45.4, 75.5, 126.9, 127.8, 127.9, 128.0, 128.2, 128.4, 128.6, 128.8, 136.1, 136.5, 136.7, 156.3, 173.5. HRESIMS m/z 433.1911 (Caled for C$_{27}$H$_{27}$N$_2$O$_2$: 433.1916).

1-Allyl-5,5-diphenylimidazoline-2,4-dione (4d) Colorless solids. mp 187.5–188.0°C. IR (KBr) ν (cm$^{-1}$) 3209, 1773, 1719. $^1$H-NMR (500MHz, CDCl$_3$) δ (ppm) 3.93–3.94 (2H, m, –CH$_2$–), 4.77–4.83 (2H, m, =CH$_2$), 5.10–5.18 (1H, m, =CH),
7.30–7.41 (10H, m, Ar–H), 7.83 (1H, s, N–H). 13C-NMR (125 MHz, CDCl3) δ (ppm) 44.1, 76.2, 117.6, 128.4, 128.7, 129.0, 131.6, 136.7, 155.8, 174.2. HRESIMS m/z 293.1285 (Calcd for C18H17N2O2: 293.1290).

**Ethyl 2-(2,5-Dioxo-4,4-diphenylimidazolidin-1-yl)acetate (7)** (Yellow solids. mp 184.5–185.5 °C (lit20 185–186 °C). IR (KBr) 3180, 1773, 1752, 1718. 1H-NMR (500 MHz, CDCl3) δ (ppm) 1.25 (3H, t, J = 7.1 Hz, CH3), 4.22 (2H, q, J = 7.1 Hz, CH2), 6.63 (1H, s, NH), 7.34–7.43 (10H, m, Ar–H). 13C-NMR (125 MHz, CDCl3) δ (ppm) 14.2, 40.0, 62.2, 70.9, 127.3, 128.9, 190.0, 138.9, 155.7, 167.0, 173.3. HRESIMS m/z 339.1340 (Calcd for C19H19N2O4: 339.1345).

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials (NMR charts, P-gp assay).

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