Antibacterial Activity of 5-Dialkylaminomethylhydantoins and Related Compounds

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To find new antibacterial leads in the class of hydantoin derivatives, we carried out synthetic investigation and biological evaluation of the title hydantoin derivatives and related compounds. Among the hydantoin derivatives described in this article, compound 3o, in which a 2,6-dichlorophenyl ring was introduced at the *N*-3 position of the hydantoin nucleus, showed the highest levels of antibacterial activity against both *Escherichia coli* NBRC14237 (NIHJ) and *Staphylococcus aureus* ATCC6538P (gram-negative and gram-positive bacteria, respectively) strains.

Key words hydantoin; 2-thiohydantoin; methylenehydantoin; β-aminoalanine; cyclization; antibacterial activity

In connection with our synthetic studies in the search for new bioactive lead compounds, some molecular modifications of β-aminoalanines to the class of oxazolidinones (linezolid mimetic molecules) have been reported.[1,2] Some of the synthesized compounds were evaluated for antibacterial activity with gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) strains, and we found that most of the 4-dialkylaminomethyloxazolidinone-related derivatives (**A**)[1] showed no significant antibacterial activities against either gram-positive or gram-negative strains. Therefore, we carried out further molecular modification of linezolid to the hydantoin analogue (**B**). Molecular modification to the represented structure (**B**) can be considered to be a bioisosteric replacement[3] of the oxazolidinone ring in linezolid by a hydantoin nucleus (Fig. 1).

Through our pre-screening, we found that some hydantoin derivatives in this class showed significant antibacterial activities against both gram-negative (*E. coli*) and gram-positive (*S. aureus*) strains. In the early stage of invasion of bacteria such as *E. coli*, the surface glycans of bacteria recognize host cell lectin.[4] For such molecular recognition of glycans, the major recognition patterns between the host and target guest molecules are through intermolecular hydrogen bonding interactions. This interaction process is a logical path and is thought to direct a controlled biological response.[5] We have been interested in target compounds that interfere with such a recognition process in order to find new leads. In terms of donor-acceptor for hydrogen bondings, compound **B** has both donor and acceptor functionalities in the molecules, in contrast to molecule **A** having no donor for hydrogen bonding. Bioactive linezolid (R_1=H, and R_2=$COCH_3$) as a lead in this study has both donor and acceptor groups for hydrogen bonding in supramolecular interactions.[6—8] From this point of view, further molecular modifications of this class of compounds (**B**) seemed to be interesting in the search for new antibacterial leads. We therefore carried additional synthetic investigation and biological evaluation of these 5-dialkylaminomethylhydantoin derivatives (**B**).

In this article, additional synthetic applications of β-aminoalanines[9—11] to some new hydantoins and biological evaluation of the hydantoin-related derivatives for antibacterial activity with gram-negative (*E. coli*) and gram-positive (*S. aureus*) strains are described.

Synthesis of 5-Dialkylaminomethyl-3-aryl-hydantoins (3) and Related Compounds In our synthetic studies on β-aminoalanines (**1**),[9] we have already reported target molecules of 5-dialkylaminomethyl-3-aryl-hydantoins (**3**) which are easily prepared by cyclization of urea derivatives (**2**) readily obtained by addition of β-aminoalanines to arylisocyanates (or arylisothiocyanates).[10,11] The hydantoin derivatives (**3**) described in this paper were prepared in a manner similar to that reported previously. Synthesis of the compounds (**2**, **3a**, **3b**, **3d**—**3j**, **3n**, **3p**—**3t**, **4**, **5**) has already been reported.[10,11] Preparation of new derivatives (**3c**, **3k**—**3m**, **3o**) and their physical and spectroscopic data are described in the Experimental. The overall reaction stages (**1**→**2**→**3**) for the target 5-dialkylaminomethyl-3-aryl-hydantoins (**3**) are shown in Chart 1. The structures of compounds (**3**) and the results of antibacterial assays are summarized in Tables 1 and 2.

Assays for Antibacterial Activity and Discussion We used gram-negative bacteria (*E. coli*) and gram-positive bac-

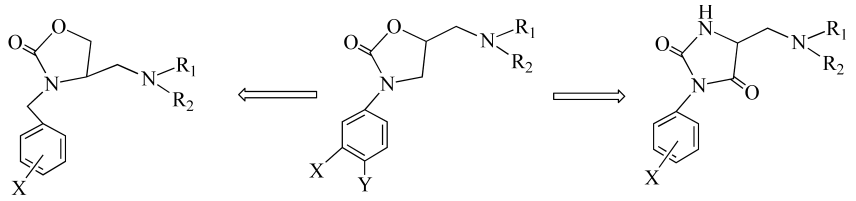

Fig. 1. Structures of **A**, **B** and Linezolid

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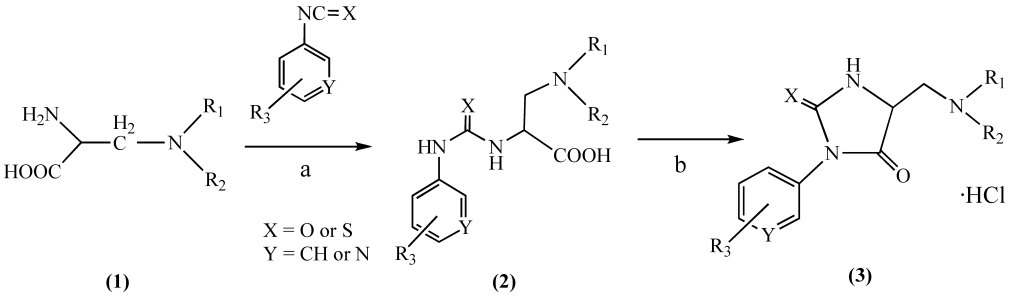
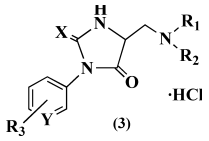
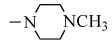


Reaction conditions: a) 3 N-NaOH aq, rt—50 °C, 1—3 h. b) Concentarated HCl for several days, rt.

Chart 1

Table 1. Antibacterial Activities of Compounds **3a**—**3t**



Compd.	$-N\begin{smallmatrix}R_1\\R_2\end{smallmatrix}$	X	Y	R_3	Antibacterial activity[a] MIC (μmol/ml) *E. coli*	*S. aureus*
3a		O	CH	H	0.411	0.411
3b		O	CH	H	0.195	0.391
3c		O	CH	H	0.177	0.355
3d		O	CH	H	0.207	0.414
3e		O	CH	H	0.217	0.433
3f		O	CH	H	0.229	0.458
3g		O	CH	H	0.119	0.237
3h		O	CH	4-Cl	0.048	0.097
3i		O	CH	4-Cl	0.105	0.210
3j		O	CH	4-CH_3	0.207	0.414
3k		O	CH	4-OCH_3	0.197	0.393
3l		O	CH	4-F	0.204	0.408
3m		O	CH	4-Br	0.171	0.171
3n		O	CH	2,4-Cl	0.088	0.088
3o		O	CH	2,6-Cl	0.044	0.088
3p		O	CH	2,4-Cl	0.085	0.085
3q		S	CH	H	>0.373	>0.373
3r		S	CH	H	>0.393	>0.393
3s		S	CH	H	>0.414	>0.414
3t[b]		S	N	H	>0.367	>0.367

a) We used *Staphylococcus aureus* ATCC6538P and *E. coli* NBRC14237 (NIHJ) as target organisms. *b*) Compound **3t** dihydrochloride was used for antibacterial activity assay.

Table 2. Antibacterial Activities of Compounds **2**, **4** and **5**

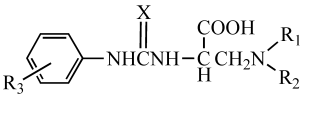
Compd.	Antibacterial activity[a] MIC (μmol/ml) *E. coli*	*S. aureus*
2a	0.098	0.394
2b	0.051	0.205
4a	0.170	0.340
4b	>0.627	>0.627
5	0.340	0.340

a) We used *Staphylococcus aureus* ATCC6538P and *E. coli* NBRC14237 (NIHJ) as target organisms.

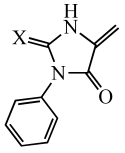
teria (*S. aureus*) as target organisms for the assay of antibacterial activities of the synthesized hydantoin derivatives (**3**). The bioassay for antibacterial activity was carried out by authentic methods according to the Japanese Society of Chemotherapy.[12,13] Synthetic target compounds (**3**) were dissolved in dimethyl sulfoxide (DMSO) for bioassay. The minimum inhibitory concentrations (MICs) of the compounds are summarized in Tables 1 and 2. Most of these compounds showed remarkable antibacterial activity against both *E. coli* and *S. aureus*.

Among the 3-aryl hydantoin derivatives described in this article, compound (**3o**) in which a 2,6-dichlorophenyl ring was introduced at the *N*-3 position of the hydantoin nucleus showed the highest levels of antibacterial activity (0.044—0.088 μmol/ml). Compound (**3h**) that has a 4-dichlorophenyl ring on the hydantoin ring showed levels of antibacterial activity (0.048—0.097 μmol/ml) similar to those of the 2,6-dichloro derivative (**3o**) and higher than those of other 3-aryl hydantoin derivatives having a 4-substituted phenyl group such as F (**3l**), Br (**3m**), CH_3 (**3j**), and OCH_3 (**3k**). It is well known that these 3-aryl hydantoin derivatives form a mixture of enantiomeric or diastereomeric rotational isomers resulting from restricted internal rotation of the aryl and a heterocyclic hydantoin system.[14] Our results may indicate the complexity of the contribution of an electronic state of the phenyl ring and a C–N bond rotational conformer about the C–N pivot bond connecting the two ring systems for antibacterial activities. The biological results for both 2,4-dichlorophenyl derivatives (**3n**) and (**3p**) apparently indicate not only the importance of an electronic effect of a substituent on the 3-aryl group in the hydantoin ring but also conformational isomerism regarding a C–N bond rotation in 3-aryl hydantoins.[15]

2-Thiohydantoin analogues (**3q**—**3t**) showed no signifi-



	X	R_1	R_2	R_3
2a	S	—$(CH_2)_2$–S–$(CH_2)_2$–		H
2b	O	—$(CH_2)_4$–		4-C1



	X
4a	S
4b	O

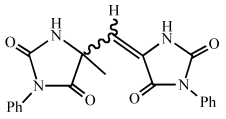


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Chart 2

cant antibacterial activities against either *E. coli* or *S. aureus* strains (>0.367 μmol/ml).

5-Methylenhydantoin (**4a**) and 2-thio-5-methylenehydantoin (**4b**) showed lower levels of antibacterial activity than that of 2,6-dichlorophenyl hydantoin (**3o**). Ring-opened urea intermediate (**2b**), which gives a cyclized hydantoin (**3h**), also showed the same level of antibacterial activity against *E. coli* (gram-negative) (0.051 μmol/ml), but its antibacterial activity against *S. aureus* (gram-positive) (0.205 μmol/ml) was lower than that of the cyclized hydantoin (**3h**). Ring-opened urea derivative (**2a**), which gives a cyclized hydantoin (**3q**), also showed antibacterial activity against *E. coli* (gram-negative) (0.098 μmol/ml), but its antibacterial activity against *S. aureus* (gram-positive) was also weak (0.394 μmol/ml). The cyclized hydantoin (**3q**) obtained from compound **2a** showed no significant antibacterial activity against either *E. coli* or *S. aureus* strains (>0.373 μmol/ml). Compound **5** obtained from dimerization of 5-methylenehydantoin **4a** did not show remarkable antibacterial activity against either the *E. coli* or *S. aureus* strain (Chart 2).

On the basis of these results, further molecular modifications, including isolation of the C–N bond rotational conformer about the C–N pivot bond, concerning the two ring systems in order to find more active antibacterial compounds are under investigation. Optical resolution of the compounds with high levels of activity is also underway.

Experimental

Melting points are uncorrected. IR spectra were measured by a Shimadzu FT/IR-8100 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were obtained by a JEOL JNM A-500 at 35 °C. The chemical shifts were expressed in δ ppm downfield from an internal tetramethylsilane (TMS) signal. The signal assignments were confirmed by ^{1}H–^{1}H two-dimensional (2D) correlation spectroscopy (COSY), ^{1}H–^{13}C heteronuclear multiple quantum coherence (HMQC), and ^{1}H–^{13}C heteronuclear multiple-bond connectivity (HMBC) spectra. High FAB-MS spectra were obtained by a JEOL JMS-HX110 mass spectrometer. The abbreviations Ppz, Pyr and Hyd were used for the piperazine ring, pyrrolidine ring and hydantoin ring, respectively.

Assays for Antibacterial Activity We used *S. aureus* ATCC6538P and *E. coli* NBRC14237 (NIHJ) (gram-positive and gram-negative bacteria, respectively) as target organisms. Synthesized compounds (**2**—**5**) were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1.280 μg/ml. The minimum inhibitory concentration (MIC) of a standard strain was measured by the authentic microdilution method to monitor the bacterial growth turbidity in Muller–Hinton broth according to the Japanese Society of Chemotherapy.[12,13] The determined values of MIC for target compounds by this authentic MIC method are summarized in Tables 1 and 2.

Preparation of 5-{(4-Methylpiperazin-1-yl)methyl}-3-phenylimidazolidine-2,4-dione Hydrochloride (3c) The intermediate urea **2c** was prepared from the reaction of 2-amino-2-(4-methylpiperazin-1-yl)propanoic acid trihydrochloride[9] (5 g, 0.0169 mol) and phenyl isocyanate (4 g, 0.0336 mol) according to the procedure described previously.[10,11] Crude product **2c** was adjusted exactly to pH 5.4 by addition of 1 M HCl, and an electrolytic desalting system (Micro Acilyzer®) was used to remove NaCl from the resulting solution. The obtained product was recrystallized from EtOH. The yield was 55.8%. The mixture of above compound **2c** (0.5 g, 0.00163 mol) in c-HCl (10 ml) was kept for 1 d at room temperature. Evaporation of the solvent gave the desired compound **3c** in 91.5%. The physical spectroscopic data of compounds **2c** and **3c** are shown below.

3-(4-Methylpiperazin-1-yl)-2-(3-phenylureido)propanoic Acid (2c) mp 143 °C (dec.). IR (KBr) cm^{-1}: 3339, 1599. FAB-MS (positive) *m/z*: 307 (M+H)$^+$. ^{1}H-NMR (D_2O) δ: 2.82 (3H, s, Ppz-NC$\underline{H}_3$), 2.84—2.95 (2H, m, C$\underline{H}_2$-Ppz), 3.23 (8H, br, Ppz-H), 4.30—4.33 (1H, m, C$\underline{H}$COOH), 7.15—7.19 (1H, m, Ar H-4), 7.33—7.41 (4H, m, Ar H-2, H-3, H-5, H-6), ^{13}C-NMR (D_2O) δ: 26.0 (Ppz-NC$\underline{H}_3$), 36.2, 36.2 (Ppz-C), 36.3 (C$\underline{H}$COOH), 104.2 (Ar C-2, C-6), 107.1 (Ar C-4), 112.4 (Ar C-3, C-5), 121.4 (Ar C-1), 140.6 (NH$\underline{C}$ONH), 161.1 (C$\underline{O}$OH). *Anal.* Calcd for $C_{15}H_{22}N_4O_3\cdot 2H_2O$: C, 52.62; H, 7.65; N, 16.36. Found: C, 52.62; H, 7.46; N, 16.28.

5-{(4-Methylpiperazin-1-yl)methyl}-3-phenylimidazolidine-2,4-dione Dihydrochloride (3c) mp 192 °C (dec.). IR (KBr) cm^{-1}: 1785, 1715. FAB-MS (positive) *m/z*: 289 (M+H)$^+$. ^{1}H-NMR (DMSO-d_6) δ: 2.78 (3H, s, Ppz-NC$\underline{H}_3$), 3.22—3.70 (11H, m, Ppz-H, C$\underline{H}_2$-Ppz, H$^+$), 4.62 (1H, br, Hyd H-5), 7.35—7.41 (3H, m, Ar H-3, H-4, H-5), 7.47—7.50 (2H, m, Ar H-2, H-6), 8.57 [1H, s, Hyd N(1)-H], 11.41 (1H, br, NH$^+$). ^{13}C-NMR (DMSO-d_6) δ: 41.7 (Ppz-NC$\underline{H}_3$), 48.9, 49.1, 49.4, 50.8 (Ppz-C), 54.5 (Hyd C-5), 56.9 (Ppz-$\underline{C}H_2$), 126.6 (Ar C-3, C-5), 127.8 (Ar C-4), 128.6 (Ar C-2, C-6), 132.0 (Ar C-1), 155.5 (Hyd C-2), 171.2 (Hyd C-4). HR-FAB-MS *m/z*: 289.1666 (Calcd for $C_{15}H_{21}N_4O_2$: 289.1665).

3-(4-Bromophenyl)-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione Hydrochloride (3m) 4-Bromophenyl isocyanate (1.8 g, 0.0091 mol) was added dropwise to a solution of β-pyrrolidinoalanine dihydrochloride[9] (1.5 g, 0.0065 mol) in 3 M-NaOH (10 ml) with vigorous stirring at 50 °C and stirring was continued for 30 min. The reaction mixture was separated by filtration in a crystalline material and mother liquid layer. The obtained crystalline material was dissolved in c-HCl and insoluble material was filtered off. The filtrate was warmed at 70 °C for 30 min and then kept at rt to give crystalline precipitates. The isolated precipitates were washed with water to give 4-bromophenylhydantoin (1.03 g, 42.4%). The mother liquid obtained directly from the reaction mixture was concentrated *in vacuo*, and c-HCl was added to the resulting residue. This mixture was warmed at 70 °C for 30 min and then cooled to give a crystalline precipitate. The collected precipitate was washed with water also to give 4-bromophenylhydantoin (0.89 g, 36.6%). Total yield was 79.0%. An analytical sample was obtained by recrystallization from 1 M-HCl, mp 193—196 °C (dec.). IR (KBr) cm^{-1}: 1774, 1713. FAB-MS (positive) *m/z*: 338 (M+H)$^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.84—2.07 (4H, m, Pyr H-3, H-4), 2.49—2.50 (2H, m, Pyr H_A-2, H_A-5), 3.51—3.63 (4H, m, Pyr H_B-2, H_B-5, C$\underline{H}_2$-Pyr), 4.77—4.94 (1H, m, Hyd H-5), 7.35—7.40 (2H, m, Ar H-3, H-5), 7.68—7.72 (2H, m, Ar H-2, H-6), 8.73 [1H, s, Hyd N(1)-H], 10.74 (1H, br s, NH$^+$). ^{13}C-NMR (DMSO-d_6) δ: 22.4, 22.6 (Pyr C-3, C-4), 53.7 (Hyd C-5), 54.1, 54.9 (Pyr C-2, C-5), 54.2 (Pyr-$\underline{C}H_2$), 120.7 (Ar C-4), 128.5 (Ar C-3, C-5), 131.1 (Ar C-1), 131.7 (Ar C-2, C-6), 155.0 (Hyd C-2), 169.9 (Hyd C-4). *Anal.* Calcd for $C_{14}H_{16}N_3O_2Br\cdot$HCl: C, 44.88; H, 4.57; N, 11.22. Found: C, 44.60; H, 4.55; N, 11.18.

Other hydantoin derivatives (**3k**, **3l**, **3o**) were also obtained in a manner similar to that described above.

3-(4-Methoxyphenyl)-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione Hydrochloride (3k) Total yield was 69.7%. mp 181—182 °C (MeOH). IR (KBr) cm^{-1}: 1786, 1718. FAB-MS (positive) *m/z*: 290 (M+H)$^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.92—2.04 (4H, m, Pyr H-3, H-4), 3.08 (2H, m, Pyr H_A-2, H_A-5), 3.62—3.75 (4H, m, Pyr H_B-2, H_B-5, C$\underline{H}_2$-Pyr), 3.79

(3H, s, OCH_3), 4.80—4.82 (1H, m, Hyd H-5), 7.03 (2H, d, J=8.8 Hz, Ar H-3, H-5), 7.27 (2H, d, J=8.8 Hz, Ar H-2, H-6), 8.68 [1H, s, Hyd N(1)-H], 11.25 (1H, br s, NH^+). ^{13}C-NMR (DMSO-d_6) δ: 22.4, 22.7 (Pyr C-3, C-4), 53.2, 54.0 (Pyr C-2, C-5), 53.7 (Hyd C-5), 55.2 (Pyr-$\underline{C}H_2$), 114.0 (Ar C-3, C-5), 124.4 (Ar C-1), 128.0 (Ar C-2, C-6), 155.4 (Hyd C-2), 158.7 (Ar C-4), 170.1 (Hyd C-4). *Anal*. Calcd for $C_{15}H_{19}N_3O_3 \cdot HCl$: C, 55.30; H, 6.19; N, 12.90. Found: C, 55.12; H, 6.15; N, 12.89.

3-(4-Fluorophenyl)-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione Hydrochloride (3l) Total yield was 77.8%. mp 165—166 °C (MeOH). IR (KBr) cm^{-1}: 1776, 1714. FAB-MS (positive) m/z: 278 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.93—2.04 (4H, m, Pyr H-3, H-4), 3.09 (2H, br, Pyr H_A-2, H_A-5), 3.63—3.70 (4H, m, Pyr H_B-2, H_B-5, $C\underline{H}_2$-Pyr), 4.82 (1H, t, J=5.5 Hz, Hyd H-5), 7.33—7.35 (2H, m, Ar H-3, H-5), 7.42—7.45 (2H, m, Ar H-2, H-6), 8.75 [1H, s, Hyd N(1)-H], 11.20 (1H, br s, NH^+). ^{13}C-NMR (DMSO-d_6) δ: 22.5, 22.7 (Pyr C-3, C-4), 53.7 (Hyd C-5), 53.3, 54.1 (Pyr C-2, C-5), 55.1 (Pyr-$\underline{C}H_2$), 115.5 (d, J=23 Hz, Ar C-3, C-5), 128.0 (Ar C-1), 128.8 (d, J=9.0 Hz, Ar C-2, C-6), 155.1 (Hyd C-2), 161.1 (d, J=245 Hz, Ar C-4), 169.9 (Hyd C-4). *Anal.* Calcd for $C_{14}H_{16}N_3O_2F \cdot HCl$: C, 53.59; H, 5.46; N, 13.39. Found: C, 53.51; H, 5.50; N, 13.40.

3-(2,6-Dichlorophenyl)-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione Hydrochloride (3o) Total yield was 73.7%. mp >225 °C (dec.). IR (KBr) cm^{-1}: 1802, 1735. FAB-MS (positive) m/z: 328 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.92—2.06 (4H, m, Pyr H-3, H-4), 3.12 (2H, br s, Pyr H_A-2, H_A-5), 3.61—3.73 (4H, m, Pyr H_B-2, H_B-5, $C\underline{H}_2$-Pyr), 5.18 (1H, d, J=9.0 Hz, Hyd H-5), 7.60 (1H, t, J=8.2 Hz, Ar H-4), 7.70—7.72 (2H, m, Ar H-3, H-5), 9.14 [1H, s, Hyd N(1)-H], 11.20 (1H, br s, NH^+). ^{13}C-NMR (DMSO-d_6) δ: 22.4, 22.6 (Pyr C-3, C-4), 53.0, 54.1 (Pyr C-2, C-5), 54.3 (Hyd C-5), 55.3 (Pyr-$\underline{C}H_2$), 127.0 (Ar C-1), 128.3, 128.9 (Ar C-3, C-5), 132.4 (Ar C-4), 134.1, 134.3 (Ar C-2, C-6), 152.9 (Hyd C-2), 168.8 (Hyd C-4). *Anal.* Calcd for $C_{14}H_{15}N_3O_2Cl_2 \cdot HCl$: C, 46.11; H, 4.42; N, 11.52. Found: C, 45.86; H, 4.32; N, 11.39.

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15) In the ^{13}C-NMR spectroscopic analysis of the compounds (**3n**, **3o**, **3p**), compound **3o** showed a singlet resonance pattern ascribable to all 2,6-dichlorophenyl ring and hydantoin nucleus carbons; however, 2,4-dichlorophenyl compounds (**3n**, **3p**) showed a doublet resonance pattern for all carbons as a mixture of two rotational conformers as noted in a previous paper (see ref. 11 for spectroscopic data of **3n**, **3p**).