Bioorganic & Medicinal Chemistry 24 (2016) 4144–4151

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins as potent NADPH oxidase (NOX) inhibitors

Yun Soo Bae [a,†], Sun Choi [b,†], Jung Jae Park [c], Jung Hee Joo [a], Minghua Cui [b], Hyunsung Cho [c], Won Jae Lee [d], Sang Hyup Lee [c,*]

[a] Department of Life Science, College of Natural Sciences, Ewha Womans University, Seoul 03760, Republic of Korea
[b] College of Pharmacy and Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 03760, Republic of Korea
[c] College of Pharmacy and Innovative Drug Center, Duksung Women's University, Seoul 01369, Republic of Korea
[d] Department of Life Science, Seoul National University, Seoul 08826, Republic of Korea

ARTICLE INFO

Article history:
Received 2 June 2016
Revised 28 June 2016
Accepted 29 June 2016
Available online 30 June 2016

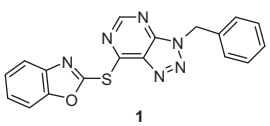
Keywords:
Thiohydantoin
Benzylidene
Aluminum chloride
NADPH oxidase (NOX)

ABSTRACT

We report the synthesis of novel 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins **3**, and their biological evaluation using NADPH oxidase (NOX) 1 and 4. Based on structural and pharmacophore analyses of known inhibitors such as hydroxypyrazole **2**, we envisioned interesting 2-thiohydantoin compounds, 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins **3** that would be expected to well match the structural features in **2**. Efficient synthesis of eighteen target compounds **3** were achieved through the synthetic pathway of **4** → **11** → **3**, established after consideration of several plausible synthetic pathways. The inhibitory activities of compounds **3** against NOX 1 and 4 were measured, with some of the target compounds showing similar or higher activities compared with reference **2**; in particular, compounds **3bz**, **3cz**, and **3ez** were found to be promising inhibitors of both NOX 1 and 4 with modest isozyme selectivities, which highlights the significance of the 2-thiohydantoin substructure for inhibition of NOX 1 and 4. This marks the first time these compounds have been applied to the inhibition of NOX enzymes.

1. Introduction

The 5-benzylidene-2-thiohydantoin compounds have been found to display a range of biological activities, such as antimycobacterial,[1] antiviral,[2] anticonvulsant,[3] and antitumor properties.[4] Among various thiohydantoin compounds we were interested in 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins as potential inhibitors of NADPH oxidase (NOX) 1 and 4. NOX enzymes are known to generate reactive oxygen species (ROS) that play a significant role in many kinds of pathogenesis such as inflammation and vascular and fibrotic disorders.[5] NOX 1 seems to play a significant role in CNS diseases.[3] NOX 4 appears to be the most abundant isoform in endothelial and vascular smooth muscle cells,[6,7] and a strong correlation between expression of NOX 4 and total NOX activities in human coronary arteries was observed.[8] Various natural and synthetic compounds display NOX inhibitory activities,[9] and the general results were reviewed in detail by Borbely et. al.[10] A triazolo[4,5-*d*]pyrimidine derivative **1** (VAS2870)[9,11] was found to inhibit oxLDL-induced superoxide release from human endothelial cells.[12] More significantly, a hydroxypyrazole derivative **2**[13–16] was recently disclosed to have potent activities against NOX enzymes.[15]



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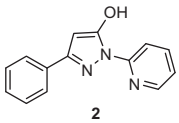

2

Although the results thus far seem reasonably good and a substantial amount of information has been accumulated, there are still no potent and selective inhibitors of NOX 1 and 4. Therefore, with the aim to develop promising inhibitors against NOX 1 and 4, we performed preliminary studies through analysis of the structural features of known inhibitors such as **1** and **2**, library searches, and molecular modeling, which eventually guided us to focus on the thiohydantoin substructure. Here, we present studies on the synthesis of 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins

* Corresponding author. Tel.: +82 2 901 8393.
E-mail address: sanghyup@duksung.ac.kr (S.H. Lee).
† These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.bmc.2016.06.056
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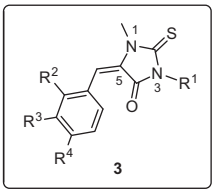
3 and their inhibitory activities against NOX 1 and 4, which is the first application of these target compounds to the inhibition of NOX enzymes.



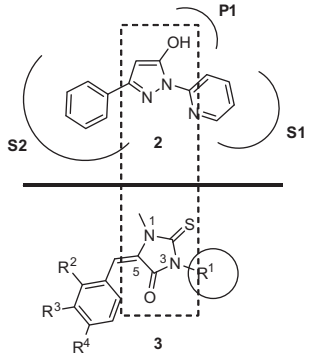


Figure 1. Pharmacophores of **2** and comparison with **3**.

2. Results and discussion

2.1. Synthesis of 2-thiohydantoins 3

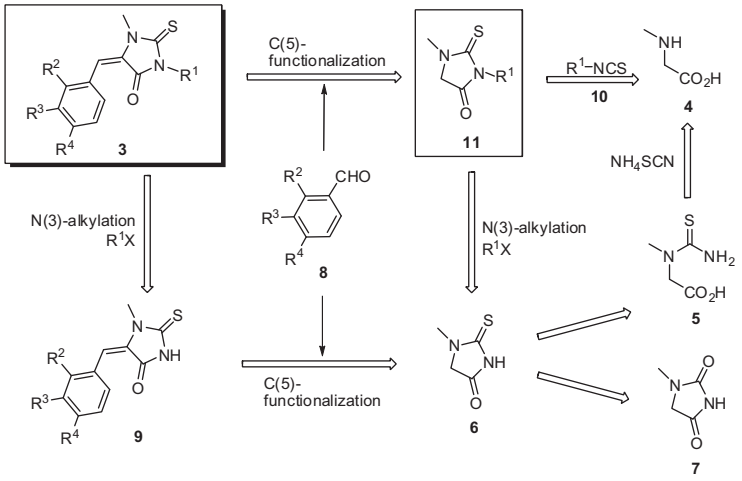
We began a program to develop potent inhibitors of NOX 1 and 4 starting from known analogues such as hydroxypyrazole **2**. Based on the structural analysis of **2** and related derivatives (data not shown), we obtained information on the structural features and important pharmacophores. For example, a consecutive arrangement of rigid ring structures, a hydrophilic region including hydrogen bond acceptors (P1), hydrophobic side chains (S1) near N(3), and extra hydrophobic pockets (S2) near C(5) may be important for activity (Fig. 1). Through these studies, we envisioned a structurally similar moiety, thiohydantoin, and consequently, suggested novel 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins **3** as NOX inhibitors. When we compared thiohydantoins **3** with the reference **2**, we recognized that the thiohydantoin core, ring substituent R^1 at N(3), and benzylidene group at C(5) in **3** would correlate well with the hydroxypyrazole, pyridinyl, and phenyl groups in **2**, respectively. In particular, we tried to align the thiohydantoin ring to best overlap with the hydroxypyrazole ring according to the pharmacophore analysis. Thus, we introduced ring substituents such as cycloalkyl or aryl group at N(3) and incorporated the benzylidene group at C(5) for a better match with the reference compound, to finally afford the target thiohydantoins **3**. Although the benzylidene group appeared to be slightly different from the phenyl group in **2**, we believed that a large group could be accommodated in this region (S2) according to the previous studies.[15]

In order to establish an efficient synthetic method of target compounds **3**, we evaluated several retrosynthetic pathways, as shown in Scheme 1. First, a starting material sarcosine (**4**) could be transformed to thiourea **5**,[17] which could be further converted to 2-thiohydantoin **6**.[17] Alternatively, compound **6** could be prepared from hydantoin **7**. Compound **6** needs to undergo both C(5)-functionalization and N(3)-alkylation to yield target compounds **3**. Thus, C(5)-functionalization using the corresponding benzaldehydes **8** would give 5-benzylidene-2-thiohydantoins **9**, and N(3)-alkylation using alkyl halide (R^1X) would lead to the generation of compounds **3**. Alternatively, N(3)-alkylation of **6** would give the key intermediate, 3-substituted thiohydantoins **11**, and further C(5)-functionalization would provide **3**. Additionally, the key intermediates **11** could also be synthesized from coupling of **4** and alkyl isothiocyanates **10**.

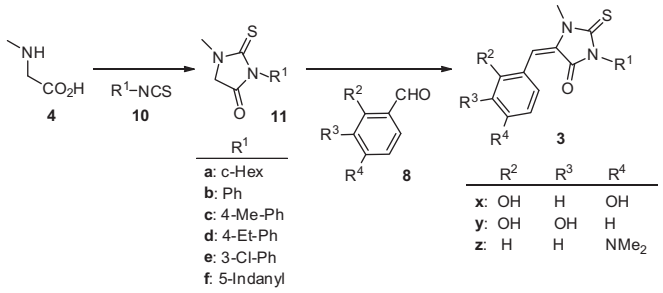
In order to investigate the feasibility of these synthetic pathways, we briefly checked their practical applicability by carrying out several model reactions. By treating **4** and ammonium thiocyanate (NH_4SCN) as previously described,[17] we obtained the thiohydantoin **6** in relatively low yield (30–40%). We then applied another method in which hydantoin **7** was treated with phosphorous pentasulfide (P_2S_5)[18] to give compound **6** in moderate yield (~60%). Next, we attempted N(3)-alkylation to convert **6** to **11** using an analogous procedure;[19] however, the N(3)-alkylation of **6** with alkyl halide (e.g., cyclohexyl bromide) did not provide good results, as several side products were generated along with the desired product. We found that the reaction proceeded with the formation of several polar spots on TLC; the side product(s) might be generated by S-alkylation, which derives some support from previous work.[20,21] If this is the case, then N(3)-alkylation could be a problematic step in the whole pathway. In addition, we believed that, in order to avoid interference of N(3)H in C(5)-functionalization, it would be more advantageous to perform the N(3)-alkylation prior to C(5)-functionalization rather than the reverse order of transformations.

Considering the efficiency of each pathway and the preliminary results of the model reactions, we adopted the pathway of **4** → **11** → **3**. Therefore, for the synthesis of key intermediates **11**, we employed the previous procedures[22,23] with minor modifications. As shown in Scheme 2, sarcosine (**4**) was treated with alkyl isothiocyanates **10** to provide 3-substituted 1-methyl-2-thiohydantoins **11** in modest yields. In these reactions, cyclohexyl (**a**), phenyl (**b**), 4-methylphenyl (**c**), 4-ethylphenyl (**d**), 3-chlorophenyl (**e**), and indan-5-yl group (**f**) were used as a set of R^1 groups at N(3). For the synthesis of indan-5-yl isothiocyanate (**10f**), *N*-indan-5-yl-*O*-phenyl thiocarbamate, prepared from 5-aminoindane and phenyl chlorothionoformate, was treated with trichlorosilane and triethylamine to afford **10f** in 50% yield (for two steps).[24] The reactions to synthesize 2-thiohydantoins **11** generally provided good results (more than 90% yield), except in the cases of the cyclohexyl (**11a**, 66% yield) and 3-chlorophenyl groups (**11e**, 74% yield), probably due to their electronic and steric effects on the reactivity of nitrogen in isothiocyanate. As a result, the six key intermediates, **11a**,[25] **11b**,[22,26] **11c**,[27] **11d**,[28] **11e**, and **11f**[22] were efficiently synthesized.

We then investigated several methods to perform the condensations of key intermediates **11** with aldehydes **8** to provide the products **3**. Here, three aromatic aldehydes, 2,4-dihydroxybenzaldehyde (**8x**), 2,3-dihydroxybenzaldehyde (**8y**), and 4-(*N*,*N*-dimethylamino)benzaldehyde (**8z**) were employed. At first, the method using melt sodium acetate in acetic acid[19] was applied to our substrates, which gave unsuccessful results probably due to the different structures in the thiohydantoins and aldehydes. Next, microwave-assisted synthesis[29] was found to give moderate results with a limited range of substrates. Furthermore, the condensation reactions were conducted by simply using a secondary amine such as piperidine or pyrrolidine; however, we were only partially successful in obtaining the product with some unhindered aldehydes in moderate to low yield (less than 60% yield). Acid catalysts have also been used for similar reactions with different substrates (not thiohydantoins), and furthermore, Greg et al. achieved improved results by employing Lewis acid catalysts[23,30] compared to the same reactions without acid catalysts.



Scheme 1. Retrosynthetic analysis of 2-thiohydantoins **3**.



Scheme 2. Synthesis of 2-thiohydantoins **3**.

Therefore, we investigated reaction conditions using our substrates and acid catalysts in the presence of piperidine at 25–80 °C. First, reactions of **11a** and **8x** using acid catalysts such as $SnCl_4$, $TiCl_4$, $AlCl_3$, and $MgBr_2$ to afford **3ax** were examined. When a catalytic amount (0.1–0.5 equiv) of $SnCl_4$ or $TiCl_4$ was employed, the starting materials **11a** and **8x** gradually disappeared; however, although several side products were generated, product **3ax** was not formed. When $MgBr_2$ was used, the results of the reaction were inconclusive; a small amount of product formed but subsequently disappeared. When $AlCl_3$ was applied, the product **3ax** was formed in good to modest yields (~60%) with an appropriate reaction time and temperature. Considering the varying results with substrate and reaction conditions, we believed that polar hydroxy groups might affect the function of the acid catalyst, presumably by lowering its activity. Thus, the reaction was tested using **8z**, that does not contain a hydroxy group, to afford **3az** in an improved yields (~85%) compared to **3ax** (~60%).

Having chosen to use $AlCl_3$, we synthesized the target compounds **3** with minor modifications to the reaction conditions and work-up procedures. Thus, $AlCl_3$ (0.1 equiv) in the presence of piperidine (1.5 equiv) at 65–80 °C was used for the condensation of **11** and **8** to synthesize the target compounds **3**. In general, the reaction mixtures were directly applied to a column chromatography after completion of the reaction. However, in some cases (e.g., **3ey** and **3ez**) the reaction mixtures were subjected to work-up procedures to give the crude products that were subsequently applied to column chromatography to afford **3**. Notably, compounds **3** contain an exocyclic C=C bond and could therefore exist as *Z*/*E*-isomers; however, information on the isomeric mixture of 5-benzylidene-2-thiohydantoins has not been revealed except in a few cases.[29,31] In our experiments, we observed the formation of a single isomer in some cases and a mixture of isomers in the other cases based on ^1H and ^{13}C NMR analyses. However, the identification of isomer structures and their separation would require further intensive work, which was not within the scope of our present studies. Consequently, we have achieved the efficient synthesis of eighteen 2-thiohydantoin compounds **3**. Among these, compound **3fz** was previously reported[23] and compound **3bz** was simply introduced without any characterization.[3] Compounds **3ax**, **3az**, **3by**, **3fx**, and **3fy** were known as members of chemical libraries,[28,32] but no information on their preparation, isomer ratio, and spectral data were disclosed. To the best of our knowledge, the other eleven compounds have not been previously identified with appropriate characterization.

2.2. Evaluation of NOX 1 and 4 inhibitory activities and SAR studies

We evaluated the NOX 1 and 4 inhibitory activities of the synthesized compounds **3** along with hydroxypyrazole derivative **2** as a reference. This marks the first time these compounds have been applied to the inhibition of NOX enzymes. To obtain specific inhibition of NOX 1 and 4 enzymes, we established transgenic fly lines overexpressing human NOX 1 or human NOX 4 in a *Drosophila* DUOX knockdown condition according to our previous procedure.[33] Transgenic flies were homogenated with ice-cold PBS containing protease inhibitors, and the membranes enriched human NOX 1 or 4 were harvested. The membranes with human NOX 1 or 4 served as ROS production monitoring by lucigenin chemiluminescence in the absence or presence of chemical compounds.[34] The results of inhibitory activities for all of the thiohydantoins against NOX 1 and 4 were shown in Table 1. We first evaluated **3ax**, **3ay**, and **3az** (**3a** series) containing a cyclohexyl group at N(3) as an aliphatic chain (R^1) and three benzylidene groups at C(5), respectively. For NOX 1, compound **3ay** that contains a 2,3-dihydroxybenzylidene group exhibited higher activity than **3ax** and **3az**, but, lower activity than reference **2**. Inhibitory activities of the compounds in the **3a** series also displayed similar trends for NOX 4. Next, we evaluated **3bx**, **3by**, **3bz** (**3b** series), **3cx**, **3cy**, **3cz** (**3c** series), and **3dx**, **3dy**, **3dz** (**3d** series) containing phenyl, 4-methylphenyl, and 4-ethylphenyl groups at N(3) as nonpolar aromatic groups (R^1), and the same three benzylidene groups at C(5), respectively. In the **3b** series, while compounds **3bx** and **3by** displayed moderate to low activities for NOX 1 and 4, compound **3bz** with a 4-(*N*,*N*-dimethylamino)benzylidene group showed excellent activities that were similar or higher compared

Table 1
Inhibitory activities of compounds **3** for NOX 1 and 4

Compound		Inhibitory activity (K_i, μM)	
		NOX 1	NOX 4
3a series	**3ax**	26.0 ± 1.2	37.6 ± 0.76
	3ay	7.39 ± 3.6	6.35 ± 2.4
	3az	12.3 ± 19.3	35.6 ± 14
3b series	**3bx**	33.8 ± 4.7	27.7 ± 12
	3by	49.7 ± 4.4	24.0 ± 6.3
	3bz	1.95 ± 0.07	0.88 ± 0.01
3c series	**3cx**	27.1 ± 7.6	34.6 ± 4.4
	3cy	50.3 ± 33	27.0 ± 2.8
	3cz	1.02 ± 0.07	2.33 ± 0.05
3d series	**3dx**	8.40 ± 0.27	2.39 ± 0.02
	3dy	27.9 ± 6.1	26.4 ± 5.7
	3dz	4.39 ± 0.06	2.63 ± 0.13
3e series	**3ex**	34.5 ± 3.3	33.8 ± 8.1
	3ey	7.65 ± 1.4	9.31 ± 0.10
	3ez	0.35 ± 0.04	0.84 ± 0.06
3f series	**3fx**	6.58 ± 0.33	6.83 ± 8.6
	3fy	6.63 ± 6.0	2.36 ± 1.9
	3fz	8.04 ± 2.5	13.1 ± 6.3
2[a]		1.45 ± 0.15[a]	3.12 ± 0.52[a]

[a] Ref. 15.

to reference **2** [~3.5 fold (NOX 4)]. Trends in the **3c** series were similar to the **3b** series, but compound **3cz** exhibited higher activities in both NOX 1 and 4 than reference **2** [~1.4 fold (NOX 1) and ~1.3 fold (NOX 4)]. A similar trend was observed in the **3d** series. Compound **3dz** showed similar or higher activities compared to reference **2** and notably, **3dx** containing a 2,4-dihydroxybenzylidene group also displayed higher activity than **2** against NOX 4. We then evaluated **3ex**, **3ey**, and **3ez** (**3e** series) containing 3-Cl-phenyl group at N(3) as a slightly different group to see the effect of bulky *meta*-substituents. Surprisingly, among the compounds tested, compound **3ez** displayed the highest activities that were higher than reference **2** against both NOX 1 and 4 [~4.1 fold (NOX 1) and ~3.7 fold (NOX 4)]. Finally, we evaluated **3fx**, **3fy**, and **3fz** (**3f** series) containing indan-5-yl group at N(3) to assess the effect of an additional fused ring. In general, these compounds showed good to modest activities, and compound **3fy** displayed higher activities than **2** for NOX 4. As for isozyme selectivities between NOX 1 and 4, compounds **3cz** and **3ez** showed modest selectivities for NOX 1 (2.3 and 2.4 fold, respectively), and compounds **3bz**, **3dx** and **3fy** showed modest selectivities for NOX 4 (2.2, 3.5 and 2.8 fold, respectively).

From the inhibition results, it was evident that the three types of substituents at C(5) strikingly affected the activities. While compounds containing a dihydroxybenzylidene group (**x** or **y**) at C(5) gave low to modest activities, compounds containing a 4-(*N*,*N*-dimethylamino)benzylidene group (**z**) provided the highest activities in most of the series. Considering these results, a nonpolar hydrogen bond acceptor (e.g., *N*,*N*-dimethylamino group) at C(5) was highly preferable rather than a polar hydrogen bond donor (e.g., dihydroxy group). However, the six types of substituents at N(3) did not seem to significantly affect the activities, and the 3-chlorophenyl (**3e** series) and indan-5-yl (**3f** series) groups gave slightly better results than the other series. Consequently, it was found that compounds **3bz**, **3cz** and **3ez** displayed the highest level of activities against both NOX 1 and 4, similar or higher compared to reference **2**, along with modest isozyme selectivity. Thus, we have successfully developed improved, promising inhibitors of NOX 1 and 4 using a combination of a 4-(*N*,*N*-dimethylamino)benzylidene group at C (5) and a phenyl, 4-methylphenyl, or 3-chlorophenyl group at N (3), respectively.

3. Conclusion

In this study, we synthesized novel 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins **3** and investigated their inhibitory activities against NOX 1 and 4. Compounds **3** were designed based on an analysis of the structures of existing inhibitors such as reference **2** and information on their pharmacophores, and thus, were expected to match the structural features found in **2**. We achieved the efficient synthesis of target compounds **3** through the synthetic pathway of **4** → **11** → **3**, to provide eighteen 2-thiohydantoin compounds **3**. We then measured the inhibitory activities of compounds **3** against NOX 1 and 4, and found that compounds **3bz**, **3cz** and **3ez** showed the highest levels of activity with modest isozyme selectivities that were similar or higher compared to reference **2**, which suggests they are promising compounds as inhibitors of NOX 1 and/or 4. This marks the first time these compounds have been applied to the inhibition of NOX enzymes. Thus, the targeted 2-thiohydantoin moiety was demonstrated to be a meaningful substructure for the inhibition of NOX 1 and 4.

4. Experimental section

4.1. General information

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Bruker DRX 300 spectrometer. Mass spectra were obtained by the CI, EI or FAB ionization method. FT-IR spectra were run on a Perkin-Elmer Spectrum GX spectrometer. Melting points were determined in open capillary tubes using a Buchi B-545 melting point apparatus and were uncorrected. Thin layer chromatography was performed with general purpose silica gel plates (20 × 20 cm; Aldrich No. Z12272-6). Deionized water was obtained with a Milli-Q (18 MΩs) water system (Millipore). The solvents and reactants were of the highest commercial grade available and were used without further purification unless noted.

4.2. General procedure[22,23] for preparation of 3-substituted 1-methyl-2-thiohydantoins 11

To a stirred solution of sarcosine (**4**, 180 mg, 2.0 mmol) in anhydrous ethanol (6 mL) was added the appropriate isothiocyanate (**10**, 2.0 mmol). The reaction mixture was refluxed for 5 h and then was allowed to cool to room temperature. The solvent was removed in vacuo, and the resulting residue was purified by column chromatography (EtOAc/hexanes) to afford the title compound.

4.2.1. 3-Cyclohexyl-1-methyl-2-thiohydantoin (11a)

Use of cyclohexyl isothiocyanate (**10a**) and the general procedure (85 °C, 6 h) afforded the title compound as a yellow solid: yield, 281 mg (66%); Mp 160–162 °C; R_f 0.36 (1:2 EtOAc/hexanes); ^{1}H NMR (300 MHz; DMSO-d_6) δ: 0.93–1.35 (m, 3H, *c*-Hx), 1.50–1.65 (m, 3H, *c*-Hx), 1.68–1.84 (m, 2H, *c*-Hx), 1.98–2.20 (m, 2H, *c*-Hx), 3.18 (s, 3H, N(1)CH_3), 4.17 (s, 2H, C(5)CH_2); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 24.8 (*c*-Hx), 25.5 (*c*-Hx), 28.2 (*c*-Hx), 33.9 (N(1)CH_3), 53.5 (C(5)), 55.0 (N(3)CH), 171.3 (C=O), 182.1 (C=S). MS *m*/*z* 212 $[M]^+$. HRMS (EI+) calcd for $C_{10}H_{16}N_2OS$ $[M]^+$: 212.0983; found 212.0982.

4.2.2. 3-Phenyl-1-methyl-2-thiohydantoin (11b)[22]

Use of phenyl isothiocyanate (**10b**) and the general procedure (85 °C, 2 h) afforded the title compound as a yellow solid: yield, 375 mg (91%); Mp 163–165 °C; R_f 0.25 (1:2 EtOAc/hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ: 3.34 (s, 3H, N(1)CH_3), 4.16 (s, 2H, C(5)

H_2), 7.18–7.26 (m, 2H, Ph), 7.34–7.48 (m, 3H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 34.7 (N(1)CH_3), 54.7 (C(5)), 128.7, 129.5, 129.6, 133.7 (Ph), 170.0 (C=O), 183.6 (C=S). MS *m*/*z* 206 $[M]^+$. HRMS (EI+) calcd for $C_{10}H_{10}N_2OS$ $[M]^+$: 206.0513; found 206.0515.

4.2.3. 3-(4-Methylphenyl)-1-methyl-2-thiohydantoin (11c)[27]

Use of 4-methylphenyl isothiocyanate (**10c**) and the general procedure (85 °C, 2 h) with recrystallization (EtOAc/hexanes) instead of column chromatography afforded the title compound as a yellow solid: yield, 405 mg (92%); Mp 147–149 °C; R_f 0.29 (1:2 EtOAc/hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ: 2.32 (s, 3H, PhCH_3), 3.31 (s, 3H, N(1)CH_3), 4.13 (S, 2H, C(5)H_2), 7.10 (d, *J* = 6.3 Hz, 2H, Ph), 7.22 (d, *J* = 6.3 Hz, 2H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 21.4 (PhCH_3), 34.4 (N(1)CH_3), 54.5 (C(5)), 128.1, 130.0, 130.8, 139.5 (Ph), 170.0 (C=O), 183.4 (C=S). MS *m*/*z* 220 $[M]^+$. HRMS (EI+) calcd for $C_{11}H_{12}N_2OS$ $[M]^+$: 220.0670; found 220.0668.

4.2.4. 3-(4-Ethylphenyl)-1-methyl-2-thiohydantoin (11d)

Use of 4-ethylphenyl isothiocyanate (**10d**) and the general procedure (85 °C, 1 h) afforded the title compound as a yellow solid: yield, 422 mg (90%); Mp 115–117 °C; R_f 0.16 (1:2 EtOAc/hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ: 1.28 (t, *J* = 7.6 Hz, 3H, PhCH$_2$CH_3), 2.71 (q, *J* = 7.6 Hz, 2H, PhCH_2), 3.42 (s, 3H, N(1)CH_3), 4.23 (s, 2H, C(5)H_2), 7.22 (d, *J* = 8.2 Hz, 2H, Ph), 7.34 (d, *J* = 8.2 Hz, 2H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 15.3 (PhCH_2CH_3), 28.8 (PhCH_2), 34.5 (N(1)CH_3), 54.4 (C(5)), 128.2, 128.9, 131.0, 145.6 (Ph), 170.0 (C=O), 183.6 (C=S). MS *m*/*z* 234 $[M]^+$. HRMS (EI+) calcd for $C_{12}H_{14}N_2OS$ $[M]^+$: 234.0826; found 234.0825.

4.2.5. 3-(3-Chlorophenyl)-1-methyl-2-thiohydantoin (11e)

Use of 3-chlorophenyl isothiocyanate (**10e**) and the general procedure (85 °C, 3 h) with recrystallization (EtOAc/hexanes) instead of column chromatography afforded the title compound as a yellow solid: yield, 356 mg (74%); Mp 192–194 °C; R_f 0.16 (1:2 EtOAc/hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ: 3.41 (s, 3H, N(1)CH_3), 4.24 (s, 2H, C(5)H_2), 7.47–7.21 (m, 4H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 34.5 (N(1)CH_3), 54.4 (C(5)), 126.8, 128.8, 129.6, 130.2, 134.4, 134.7 (Ph), 169.5 (C=O), 182.6 (C=S). MS *m*/*z* 240 $[M]^+$. HRMS (EI+) calcd for $C_{10}H_9ClN_2OS$ $[M]^+$: 240.0124; found 240.0122.

4.2.6. 3-(Indan-5-yl)-1-methyl-2-thiohydantoin (11f)[22]

Use of indan-5-yl isothiocyanate (**10f**) and the general procedure (85 °C, 1 h) afforded the title compound as a yellow solid: yield, 473 mg (96%); Mp 158–160 °C; R_f 0.14 (1:2 EtOAc/hexanes); ^{1}H NMR (300 MHz; $CDCl_3$) δ: 2.07–2.28 (m, 2H, CH_2-indanyl), 2.87–3.01 (m, 4H, CH_2-indanyl), 3.43 (s, 3H, N(1)CH_3), 4.23 (s, 2H, C(5)H_2), 7.04 (dd, *J* = 7.8, 1.2 Hz, 1H, Ph-indanyl), 7.12 (s, 1H, Ph-indanyl), 7.34 (d, *J* = 7.8 Hz, 1H, Ph-indanyl); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 25.8, 33.1, 33.2 (CH_2-indanyl), 34.7 (N(1)CH_3), 54.7 (C(5)), 124.6, 125.3, 126.5, 131.6, 145.9, 146.2 (Ph-indanyl), 170.4 (C=O), 184.0 (C=S). MS *m*/*z* 246 $[M]^+$. HRMS (EI+) calcd for $C_{13}H_{14}N_2OS$ $[M]^+$: 246.0826; found 246.0828.

4.3. General procedure[23] for 3-substituted 5-benzylidene-1-methyl-2-thiohydantoins 3

To a stirred solution of thiohydantoin (**11**, 0.20 mmol), aldehyde (**8**, 0.24 mmol) and piperidine (30 μL, 0.30 mmol) in dioxane (0.6 mL) was added aluminum chloride (2.7 mg, 0.020 mmol). The stirred reaction mixture was heated to 65–80 °C until completion of the reaction. In cases where the reaction was incomplete, more aluminum chloride (0.1–0.2 equiv) was added along with the corresponding amount of piperidine in the same ratio, and the reaction mixture was refluxed for additional time. After cooling to room temperature, the reaction mixture was directly subjected to column chromatography (gradient elution with appropriate EtOAc/hexanes mixtures) to give the title compounds **3**. In the cases of **3ey** and **3ez**, the reaction mixture was worked-up prior to column chromatography. The reaction mixture was evaporated to approximately half of the original volume. H_2O (50 mL) was slowly added, and the resulting mixture was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo to give an oily residue. Purification of the residue by column chromatography (gradient elution with appropriate EtOAc/hexanes mixtures) afforded the title compound.

4.3.1. 3-Cyclohexyl-5-(2,4-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3ax)

Use of thiohydantoin **11a** and aldehyde **8x** in the general procedure (80 °C, 3 h) afforded the title compound as a red solid: yield, 43 mg (64%); Mp 205–207 °C; R_f 0.13 (1:2 EtOAc/hexanes); IR (KBr) 3434, 2090, 1637, 1384, 1096 cm^{-1}; ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.10–1.35 (m, 3H, *c*-Hx), 1.56–1.70 (m, 3H, *c*-Hx), 1.75–1.87 (m, 2H, *c*-Hx), 2.15–2.29 (m, 2H, *c*-Hx), 3.53 (s, 3H, N(1)CH_3), 4.53–4.66 (m, 1H, N(3)CH), 6.28 (dd, *J* = 8.8, 2.2 Hz, 1H, Ph), 6.37 (d, *J* = 2.2 Hz, 1H, Ph), 6.97 (s, 1H, CH=C), 8.43 (d, *J* = 8.8 Hz, 1H, Ph), 10.10 (br s, 2H, OH); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 24.9 (*c*-Hx), 25.6 (*c*-Hx), 28.2 (*c*-Hx), 31.0 (N(1)CH_3), 54.8 (N(3)CH), 101.7, 107.0, 110.8, 116.9, 125.2, 132.1, 158.8, 161.4 (Ph, CH=C), 161.2 (C=O), 174.2 (C=S). MS *m*/*z* 332 $[M]^+$. HRMS (EI+) calcd for $C_{17}H_{20}N_2O_3S$ $[M]^+$: 332.1194; found 332.1195.

4.3.2. 3-Cyclohexyl-5-(2,3-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3ay)

Use of thiohydantoin **11a** and aldehyde **8y** in the general procedure (80 °C, 9 h) afforded the title compound as a red solid: yield, 46 mg (72%); Mp 95–97 °C; R_f 0.40 (1:2 EtOAc/hexanes); IR (KBr) 3433, 2930, 2854, 1635, 1471, 1402, 1372, 1283, 1188, 1146, 1099, 728, 530 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ: 1.20–1.46 (m, 3H, *c*-Hx), 1.63–1.79 (m, 3H, *c*-Hx), 1.81–1.93 (m, 2H, *c*-Hx), 2.15–2.35 (m, 2H, *c*-Hx), 3.32, 3.66 (2s, 3H, N(1)CH_3), 4.56–4.84 (m, 1H, N(3)CH), 6.64, 6.84 (2s, 1H, CH=C), 6.69–6.83 (m, 1H, Ph), 6.87–7.08 (m, 2H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 25.2 (*c*-Hx), 26.1 (*c*-Hx), 28.8 (*c*-Hx), 31.7, 34.5 (N(1)CH_3), 56.4, 56.7 (N(3)CH), 109.7, 116.2, 117.8, 120.2, 120.3, 122.0, 122.4, 122.6, 124.8, 128.6, 130.8, 143.1, 143.3, 144.2, 148.7 (Ph, CH=C, one peak was not detected and believed to overlap with the observed peaks), 163.9, 165.4 (C=O), 176.1, 180.5 (C=S). MS *m*/*z* 332 $[M]^+$. HRMS (EI+) calcd for $C_{17}H_{20}N_2O_3S$ $[M]^+$: 332.1194; found 332.1195.

4.3.3. 3-Cyclohexyl-5-(4-(*N*,*N*-dimethylamino)benzylidene)-1-methyl-2-thiohydantoin (3az)

Use of thiohydantoin **11a** and aldehyde **8z** in the general procedure (65 °C, 6 h) afforded the title compound as a red solid: yield, 59 mg (86%); Mp 215–217 °C; R_f 0.57 (1:2 EtOAc/hexanes); IR (KBr) 2925, 1701, 1588, 1530, 1443, 1395, 1341, 1233, 1185, 1078, 950, 875, 807, 515 cm^{-1}; ^{1}H NMR (300 MHz; DMSO-d_6) δ: 1.09–1.42 (m, 3H, *c*-Hx), 1.57–1.73 (m, 3H, *c*-Hx), 1.76–1.89 (m, 2H, *c*-Hx), 2.12–2.35 (m, 2H, *c*-Hx), 2.98, 3.01 (2s, 6H, N(CH_3)$_2$), 3.41, 3.56 (2s, 3H, N(1)CH_3), 4.41–4.67 (m, 1H, N(3)CH), 6.68–6.85 (m, 3H, Ph, CH=C), 7.38 (d, *J* = 9.0 Hz, 1H, Ph), 8.16 (d, *J* = 9.0 Hz, 1H, Ph); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 24.5, 24.9 (*c*-Hx), 25.5, 25.6 (*c*-Hx), 28.2, 28.3 (*c*-Hx), 31.1, 35.6 (N(1)CH_3), 54.8, 55.2 (N(3)CH), 111.2, 111.4, 116.8, 118.7, 119.9, 123.8, 124.6, 125.8, 132.0, 133.4, 150.6, 151.3 (Ph, CH=C), 161.1, 163.1 (C=O), 173.5, 178.6 (C=S). MS *m*/*z* 343 $[M]^+$. HRMS (EI+) calcd for $C_{19}H_{25}N_3OS$ $[M]^+$: 343.1718; found 343.1718.

4.3.4. 3-Phenyl-5-(2,4-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3bx)

Use of thiohydantoin **11b**, aldehyde **8x**, and additional $AlCl_3$ (0.020 mmol) and piperidine (0.30 mmol) in the general procedure (80 °C, 1 d and additionally, 10 h) afforded the title compound as a red solid: yield, 37 mg (57%); Mp 263–265 °C; R_f 0.25 (1:1 EtOAc/hexanes); IR (KBr) 3162, 2918, 2849, 2348, 2110, 1635, 1454, 1396, 1260, 1023, 873, 749, 538 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 3.61 (s, 3H, N(1)CH_3), 6.23 (dd, J = 8.8, 2.2 Hz, 1H, Ph), 6.37 (d, J = 2.2 Hz, 1H, Ph), 7.05 (s, 1H, CH=C), 7.28–7.63 (m, 5H, Ph), 8.41 (d, J = 8.8 Hz, 1H, Ph), 10.01, 10.27 (2s, 2H, OH); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 30.6 (N(1)CH_3), 101.7, 106.9, 110.9, 117.3, 125.2, 128.5, 128.7, 128.9, 132.0, 133.9, 158.9, 161.4 (Ph, CH=C), 160.9 (C=O), 174.1 (C=S). MS m/z 326 $[M]^+$. HRMS (EI+) calcd for $C_{17}H_{14}N_2O_3S$ $[M]^+$: 326.0725; found 326.0724.

4.3.5. 3-Phenyl-5-(2,3-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3by)

Use of thiohydantoin **11b** and aldehyde **8y** in the general procedure (80 °C, 7 h) afforded the title compound as a red solid: yield, 47 mg (71%); Mp 155–156 °C; R_f 0.18 (1:6:12 MeOH/EtOAc/hexanes); IR (KBr) 3355, 3062, 2938, 1732, 1595, 1471, 1350, 1283, 1233, 1154, 1052, 1004, 735, 529 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 3.35, 3.63 (2s, 3H, N(1)CH_3), 6.88, 7.04 (2s, 1H, CH=C), 6.56–6.87 (m, 2H, Ph), 7.32–7.73 (m, 6H, Ph), 9.07, 9.17 (2s, 1H, OH), 9.53, 9.65 (2s, 1H, OH); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 30.4, 33.8 (N(1)CH_3), 112.0, 116.1, 116.3, 116.7, 117.8, 118.7, 119.5, 119.6, 120.9, 121.0, 127.5, 127.6, 127.7, 128.4, 128.5, 128.6, 128.7, 133.5, 133.6, 144.3, 144.7, 145.1, 145.2 (Ph, CH=C, one peak was not detected and believed to overlap with the observed peaks), 160.6, 162.6 (C=O), 175.4, 179.0 (C=S). MS m/z 326 $[M]^+$. HRMS (EI+) calcd for $C_{17}H_{14}N_2O_3S$ $[M]^+$: 326.0725; found 326.0722.

4.3.6. 3-Phenyl-5-(4-(*N*,*N*-dimethylamino)benzylidene)-1-methyl-2-thiohydantoin (3bz)

Use of thiohydantoin **11b** and aldehyde **8z** in the general procedure (65 °C, 4 h) afforded the title compound as a red solid: yield, 61 mg (92%); Mp 238–240 °C; R_f 0.36 (1:2 EtOAc/hexanes); IR (KBr) 3748, 2368, 1715, 1581, 1530, 1501, 1478, 1393, 1344, 1194, 1147, 1056, 819, 763, 518 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 3.00 (s, 6H, N$(CH_3)_2$), 3.63 (s, 3H, N(1)CH_3), 6.72 (d, J = 9.0 Hz, 2H, Ph), 6.89 (s, 1H, CH=C), 7.36 (d, J = 7.8 Hz, 2H, Ph), 7.42–7.54 (m, 3H, Ph), 8.18 (d, J = 9.0 Hz, 2H, Ph); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 30.8 (N(1)CH_3), 39.6 (N$(CH_3)_2$), 111.0, 119.9, 124.2, 124.4, 128.5, 128.7, 128.9, 133.4, 134.0, 151.4 (Ph, CH=C), 160.9 (C=O), 173.4 (C=S). MS m/z 337 $[M]^+$. HRMS (EI+) calcd for $C_{19}H_{19}N_3OS$ $[M]^+$: 337.1248; found 337.1248.

4.3.7. 3-(4-Methylphenyl)-5-(2,4-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3cx)

Use of thiohydantoin **11c** and aldehyde **8x** in the general procedure (80 °C, 8 h) afforded the title compound as a red solid: yield, 44 mg (65%); Mp 275–279 °C; R_f 0.36 (1:1 EtOAc/hexanes); IR (KBr) 3748, 3444, 2917, 2381, 2324, 1688, 1593, 1511, 1463, 1398, 1350, 1275, 1175, 1105, 1002, 977, 947, 873, 802, 765, 677, 629, 588, 535, 499 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 2.36 (s, 3H, PhCH_3), 3.62 (s, 3H, N(1)CH_3), 6.24 (dd, J = 8.7, 2.4 Hz, 1H, Ph), 6.39 (d, J = 2.4 Hz, 1H, Ph), 7.05 (s, 1H, CH=C), 7.22 (d, J = 8.4 Hz, 2H, Ph), 7.30 (d, J = 8.4 Hz, 2H, Ph), 8.42 (d, J = 8.7 Hz, 1H, Ph), 10.00 (s, 1H, OH), 10.26 (s, 1H, OH); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 20.8 (PhCH_3), 30.6 (N(1)CH_3), 101.7, 106.9, 110.9, 117.2, 125.2, 128.6, 129.2, 131.3, 132.0, 138.1, 158.8, 161.4 (Ph, CH=C), 161.0 (C=O), 174.3 (C=S). MS m/z 340 $[M]^+$. HRMS (EI+) calcd for $C_{18}H_{16}N_2O_3S$ $[M]^+$: 340.0881; found 340.0878.

4.3.8. 3-(4-Methylphenyl)-5-(2,3-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3cy)

Use of thiohydantoin **11c** and aldehyde **8y** in the general procedure (80 °C, 9 h) afforded the title compound as a red solid: yield, 52 mg (78%); Mp 184–185 °C; R_f 0.60 (1:1 EtOAc/hexanes); IR (KBr) 3383, 3037, 2922, 2853, 2306, 2071, 1727, 1621, 1586, 1514, 1472, 1394, 1349, 1282, 1204, 1154, 1051, 1003, 942, 873, 764, 663, 523 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ: 2.41, 2.43 (2s, 3H, PhCH_3), 3.41, 3.76 (2s, 3H, N(1)CH_3), 6.73–7.10 (m, 3H, Ph, CH=C), 7.15–7.41 (m, 5H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 21.5 (PhCH_3), 31.4, 34.2 (N(1)CH_3), 110.9, 116.4, 116.6, 118.7, 120.0, 120.5, 122.1, 122.5, 122.6, 124.8, 128.1, 128.3, 130.1, 130.2, 130.5, 130.8, 139.6, 140.1, 142.9, 143.3, 144.1, 148.5 (Ph, CH=C, two peaks were not detected and believed to overlap with the observed peaks), 163.7, 164.8 (C=O), 175.9, 180.2 (C=S). MS m/z 340 $[M]^+$. HRMS (EI+) calcd for $C_{18}H_{16}N_2O_3S$ $[M]^+$: 340.0881; found 340.0878.

4.3.9. 3-(4-Methylphenyl)-5-(4-(*N*,*N*-dimethylamino) benzylidene)-1-methyl-2-thiohydantoin (3cz)

Use of thiohydantoin **11c** and aldehyde **8z** in the general procedure (65 °C, 11 h) afforded the title compound as a red solid: yield, 46 mg (66%); Mp 241–243 °C; R_f 0.83 (1:1 EtOAc/hexanes); IR (KBr) 3748, 2917, 2853, 2382, 2091, 1714, 1619, 1580, 1515, 1478, 1441, 1390, 1194, 1146, 1053, 952, 888, 819, 763, 662, 516 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ: 2.42 (s, 3H, PhCH_3), 3.06 (s, 6H, N$(CH_3)_2$), 3.51, 3.57 (2s, 3H, N(1)CH_3), 6.56, 7.03 (2s, 1H, CH=C), 6.65–6.77 (m, 3H, Ph), 7.23–7.36 (m, 3H, Ph), 8.15 (d, J = 9.0 Hz, 2H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 21.5 (PhCH_3), 31.1, 35.9 (N(1)CH_3), 40.3 (N$(CH_3)_2$), 111.6, 111.7, 118.3, 119.6, 120.3, 124.0, 125.2, 126.7, 128.2, 128.4, 129.9, 130.0, 131.2, 132.0, 133.8, 139.1, 139.3, 151.0, 151.8 (Ph, CH=C, one peak was not detected and believed to overlap with the observed peaks), 161.7, 163.9 (C=O), 174.9, 179.7 (C=S). MS m/z 351 $[M]^+$. HRMS (EI+) calcd for $C_{20}H_{21}N_3OS$ $[M]^+$: 351.1405; found 351.1407.

4.3.10. 3-(4-Ethylphenyl)-5-(2,4-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3dx)

Use of thiohydantoin **11d** and aldehyde **8x** in the general procedure (80 °C, 4 h) afforded the title compound as a red solid: yield, 43 mg (63%); Mp 235–239 °C; R_f 0.45 (1:1 EtOAc/hexanes); IR (KBr) 3434, 1627, 1513, 1391, 1348, 1155, 1105, 1053, 673, 497 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 1.22 (t, J = 7.6 Hz, 3H, PhCH$_2$CH_3), 2.66 (q, J = 7.6 Hz, PhCH_2), 3.61 (s, 3H, N(1)CH_3), 6.24 (dd, J = 8.7, 2.4 Hz, 1H, Ph), 6.39 (d, J = 2.4 Hz, 1H, Ph), 7.05 (s, 1H, CH=C), 7.24 (d, J = 8.4 Hz, 2H, Ph), 7.33 (d, J = 8.4 Hz, 2H, Ph), 8.41 (d, J = 8.7 Hz, 1H, Ph), 10.10 (br s, 2H, OH); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 15.4 (PhCH$_2$CH_3), 27.8 (PhCH_2), 30.6 (N(1)CH_3), 101.7, 106.9, 110.9, 117.2, 125.2, 128.6, 129.1, 131.5, 132.0, 144.2, 158.9, 161.4 (Ph, CH=C), 161.1 (C=O), 174.3 (C=S). MS m/z 354 $[M]^+$. HRMS (EI+) calcd for $C_{19}H_{18}N_2O_3S$ $[M]^+$: 354.1038; found 354.1035.

4.3.11. 3-(4-Ethylphenyl)-5-(2,3-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3dy)

Use of thiohydantoin **11d**, aldehyde **8y**, and additional $AlCl_3$ (0.040 mmol) and piperidine (0.60 mmol) in the general procedure (80 °C, 4 h and additionally, 4 h) afforded the title compound as a red solid: yield, 38 mg (53%); Mp 163–168 °C; R_f 0.51 (1:1 EtOAc/hexanes); IR (KBr) 3368, 2964, 1721, 1648, 1586, 1514, 1470, 1398, 1349, 1282, 1169, 1051, 1002, 939, 827, 763, 731, 692, 521 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ: 1.14–1.23 (m, 3H, PhCH$_2$-CH_3), 2.53–2.71 (m, 2H, PhCH_2), 3.31, 3.64 (2s, 3H, N(1)CH_3), 6.62–7.03 (m, 4H, Ph, CH=C), 7.12–7.32 (m, 4H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 15.2 (PhCH$_2$CH$_3$), 28.7 (PhCH_2), 31.3, 34.3 (N(1)CH_3), 111.4, 116.3, 116.6, 118.5, 119.8, 120.4, 121.9, 122.2, 122.3,

124.6, 128.1, 128.2, 128.8, 128.9, 130.2, 130.3, 130.8, 143.0, 143.3, 144.2, 145.6, 146.1, 148.1 (Ph, CH=C, one peak was not detected and believed to overlap with the observed peaks), 163.9, 164.6 (C=O), 175.9, 180.0 (C=S). MS *m*/*z* 354 $[M]^+$. HRMS (EI+) calcd for $C_{19}H_{18}N_2O_3S$ $[M]^+$: 354.1038; found 354.1041.

4.3.12. 3-(4-Ethylphenyl)-5-(4-(*N*,*N*-dimethylamino) benzylidene)-1-methyl-2-thiohydantoin (3dz)

Use of thiohydantoin **11d** and aldehyde **8z** in the general procedure (65 °C, 11 h) afforded the title compound as a red solid: yield, 145 mg (95%); Mp 160–162 °C; R_f 0.66 (1:1 EtOAc/hexanes); IR (KBr) 3435, 1710, 1621, 1581, 1514, 1477, 1388, 1344, 1193, 1145, 1052, 951, 887, 817, 515 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ: 1.27 (t, *J* = 7.6 Hz, 3H, $PhCH_2CH_3$), 2.71 (q, *J* = 7.6 Hz, 2H, $PhCH_2$), 3.03 (s, 6H, $N(CH_3)_2$), 3.68 (s, 3H, $N(1)CH_3$), 6.52 (s, 1H, CH=C), 6.65 (d, *J* = 9.0 Hz, 2H, Ph), 7.23–7.36 (m, 4H, Ph), 8.14 (d, *J* = 9.0 Hz, 2H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 15.3 ($PhCH_2CH_3$), 28.7 ($PhCH_2$), 30.1 ($N(1)CH_3$), 40.2 ($N(CH_3)_2$), 111.6, 120.2, 124.0, 125.1, 128.4, 128.7, 131.3, 133.7, 145.2, 151.8 (Ph, CH=C), 161.7 (C=O), 174.8 (C=S). MS *m*/*z* 365 $[M]^+$. HRMS (EI+) calcd for $C_{21}H_{23}N_3OS$ $[M]^+$: 365.1561; found 365.1564.

4.3.13. 3-(3-Chlorophenyl)-5-(2,4-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3ex)

Use of thiohydantoin **11e** and aldehyde **8x** in the general procedure (80 °C, 5 h) afforded the title compound as a red solid: yield, 51 mg (70%); Mp 225–228 °C; R_f 0.17 (1:2 EtOAc/hexanes); IR (KBr) 3748, 3610, 2916, 2382, 2307, 1832, 1700, 1593, 1481, 1390, 1275, 1163, 975, 852, 748, 617, 501 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 3.62 (s, 3H, $N(1)CH_3$), 6.25 (dd, *J* = 8.7, 1.8 Hz, 1H, Ph), 6.38 (d, *J* = 1.8 Hz, 1H, Ph), 7.06 (s, 1H, CH=C), 7.30–7.62 (m, 4H, Ph), 8.41 (d, *J* = 8.7 Hz, 1H, Ph), 10.03 (s, 1H, OH), 10.29 (s, 1H, OH); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 30.6 ($N(1)CH_3$), 101.7, 107.0, 110.8, 117.6, 125.1, 127.9, 128.7, 129.0, 130.2, 132.1, 132.7, 135.2, 158.9, 161.5 (Ph, CH=C), 160.6 (C=O), 173.7 (C=S). MS *m*/*z* 360 $[M]^+$. HRMS (EI+) calcd for $C_{17}H_{13}ClN_2O_3S$ $[M]^+$: 360.0335; found 360.0336.

4.3.14. 3-(3-Chlorophenyl)-5-(2,3-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3ey)

Use of thiohydantoin **11e** and aldehyde **8y** in the general procedure (80 °C, 3 h) with work-up and column chromatography afforded the title compound as a red solid: yield, 47 mg (65%); Mp 186–188 °C; R_f 0.23 (1:2 EtOAc/hexanes); IR (KBr) 3433, 1637, 1478, 1392, 1350, 1284, 1159, 1053 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ: 3.42, 3.76 (2s, 3H, $N(1)CH_3$), 6.77–7.09 (m, 4H, Ph, CH=C), 7.26–7.48 (m, 4H, Ph); ^{13}C NMR (75 MHz; $CDCl_3$) δ: 31.2, 34.1 ($N(1)CH_3$), 111.5, 116.2, 116.6, 118.6, 119.5, 120.2, 121.8, 121.9, 122.2, 124.4, 126.6, 126.7, 127.8, 128.6, 129.4, 129.8, 129.9, 130.0, 130.1, 133.5, 134.3, 134.6, 134.7, 143.0, 143.2, 143.8, 147.8, 161.1 (Ph, CH=C), 162.9, 163.9 (C=O), 174.9, 179.1 (C=S). MS *m*/*z* 360 $[M]^+$. HRMS (EI+) calcd for $C_{17}H_{13}ClN_2O_3S$ $[M]^+$: 360.0335; found 360.0333.

4.3.15. 3-(3-Chlorophenyl)-5-(4-(*N*,*N*-dimethylamino) benzylidene)-1-methyl-2-thiohydantoin (3ez)

Use of thiohydantoin **11e** and aldehyde **8z** in the general procedure (65 °C, 10 h) with work-up and column chromatography afforded the title compound as a red solid: yield, 61 mg (79%); Mp 148–150 °C; R_f 0.39 (1:2 EtOAc /hexanes); IR (KBr) 3435, 1725, 1631, 1594, 1480, 1386, 1370, 1193, 1154, 1052, 950, 823, 517 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 3.01 (s, 6H, $N(CH_3)_2$), 3.64 (s, 3H, $N(1)CH_3$), 6.75 (d, *J* = 9.0 Hz, 2H, Ph), 6.91 (s, 1H, CH=C), 7.32–7.45 (m, 1H, Ph), 7.50–7.61 (m, 3H, Ph), 8.17 (d, *J* = 9.0 Hz, 2H, Ph); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 30.6 ($N(1)CH_3$), 111.0, 119.6, 124.1, 124.3, 127.8, 128.5, 129.0, 130.1, 132.5, 133.3, 135.2, 151.3 (Ph, CH=C), 160.4, (C=O), 172.8 (C=S). MS *m*/*z* 371 $[M]^+$. HRMS (EI+) calcd for $C_{19}H_{18}ClN_3OS$ $[M]^+$: 371.0859; found 371.0857.

4.3.16. 3-(Indan-5-yl)-5-(2,4-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3fx)

Use of thiohydantoin **11f** and aldehyde **8x** in the general procedure (80 °C, 1 h) afforded the title compound as a yellow solid: yield, 47 mg (63%); Mp 228–229 °C; R_f 0.29 (1:2 EtOAc/hexanes); IR (KBr) 3735, 3434, 1633, 1396, 1348, 704, 611, 518 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 1.96–2.12 (m, 2H, CH_2-indanyl), 2.82–2.94 (m, 4H, CH_2-indanyl), 3.60 (s, 3H, $N(1)CH_3$), 6.23 (dd, *J* = 9.0, 2.1 Hz, 1H, Ph), 6.36 (d, *J* = 2.1 Hz, 1H, Ph), 7.03 (s, 1H, CH=C), 7.04 (d, *J* = 7.8 Hz, 1H, Ph-indanyl), 7.15 (s, 1H, Ph-indanyl), 7.31 (d, *J* = 7.8 Hz, 1H, Ph-indanyl), 8.38 (d, *J* = 9.0 Hz, 1H, Ph); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 25.4 (CH_2-indanyl), 30.8 ($N(1)CH_3$), 32.2, 32.4 (CH_2-indanyl), 101.9, 107.1, 111.1, 117.3, 124.4, 124.8, 125.4, 126.8, 132.0, 132.2, 144.5, 159.0, 161.5 (Ph, Ph-indanyl, CH=C, one peak was not detected and believed to overlap with the observed peaks), 161.3 (C=O), 174.6 (C=S). MS *m*/*z* 366 $[M]^+$. HRMS (EI+) calcd for $C_{20}H_{18}N_2O_3S$ $[M]^+$: 366.1038; found 366.1035.

4.3.17. 3-(Indan-5-yl)-5-(2,3-dihydroxybenzylidene)-1-methyl-2-thiohydantoin (3fy)

Use of thiohydantoin **11f** and aldehyde **8y** in the general procedure (80 °C, 5 h) afforded the title compound as a yellow solid: yield, 51 mg (83%); Mp 110–113 °C; R_f 0.45 (1:1 EtOAc/hexanes); IR (KBr) 3433, 2102, 1637, 1384, 1051, 531 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 1.98–2.13 (m, 2H, CH_2-indanyl), 2.82–2.95 (m, 4H, CH_2-indanyl), 3.33, 3.61 (2s, 3H, $N(1)CH_3$), 6.55–6.83 (m, 2H, Ph), 6.85, 7.01 (2s, 1H, CH=C), 7.02–7.67 (m, 4H, Ph, Ph-indanyl); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 25.4 (CH_2-indanyl), 30.8, 34.2 ($N(1)CH_3$), 32.2, 32.4 (CH_2-indanyl), 112.3, 116.4, 116.5, 117.0, 118.2, 119.1, 119.9, 120.0, 121.3, 121.4, 124.5, 124.6, 124.7, 124.8, 126.6, 126.8, 128.1, 129.1, 131.9, 132.0, 144.5, 144.6, 144.7, 144.8, 144.9, 145.1, 145.4, 145.5 (Ph, Ph-indanyl, CH=C), 161.1, 163.2 (C=O), 176.0, 179.7 (C=S). MS *m*/*z* 366 $[M]^+$. HRMS (EI+) calcd for $C_{20}H_{18}N_2O_3S$ $[M]^+$: 366.1038; found 366.1035.

4.3.18. 3-(Indan-5-yl)-5-(4-(*N*,*N*-dimethylamino)benzylidene)-1-methyl-2-thiohydantoin (3fz)[23]

Use of thiohydantoin **11f** and aldehyde **8z** in the general procedure (65 °C, 8 h) afforded the title compound as a yellow solid: yield, 46 mg (61%); Mp 129–131 °C; R_f 0.67 (1:1 EtOAc /hexanes); IR (KBr) 3455, 1715, 1635, 1599, 1489, 1387, 1353, 1159, 1122, 1050, 951, 822, 512 cm^{-1}; ^1H NMR (300 MHz; DMSO-d_6) δ: 1.98–2.13 (m, 2H, CH_2-indanyl), 2.80–2.95 (m, 4H, CH_2-indanyl), 3.00 (s, 6H, $N(CH_3)_2$), 3.63 (s, 3H, $N(1)CH_3$), 6.72 (d, *J* = 9.0 Hz, 2H, Ph), 6.88 (s, 1H, CH=C), 7.05 (d, *J* = 7.9 Hz, 1H, Ph-indanyl), 7.16 (s, 1H, Ph-indanyl), 7.32 (d, *J* = 7.9 Hz, 1H, Ph-indanyl), 8.17 (d, *J* = 9.0 Hz, 2H, Ph); ^{13}C NMR (75 MHz; DMSO-d_6) δ: 25.1 (CH_2-indanyl), 30.6 ($N(1)CH_3$), 31.9, 32.1 (CH_2-indanyl), 111.0, 119.8, 123.9, 124.1, 124.3, 124.5, 126.5, 131.9, 133.2, 144.2, 151.2 (Ph, Ph-indanyl, CH=C, one peak was not detected and believed to overlap with the observed peaks), 160.9 (C=O), 173.6 (C=S). MS *m*/*z* 377 $[M]^+$. HRMS (EI+) calcd for $C_{22}H_{23}N_3OS$ $[M]^+$: 377.1561; found 377.1565.

4.4. General procedure for evaluation of NOX 1 and 4 inhibitory activities

We established transgenic fly lines overexpressing human NOX 1 or human NOX 4 in a *Drosophila* DUOX (dDUOX) knockdown condition according to the previous procedure.[33] The genotypes of lines used in this study were human NOX 1 (*UAS-hNOX1/UAS-dDUOX-RNAi; Da-GAL4/+*) and human NOX 4 transgenic fly (*UAS-hNOX4/UAS-dDUOX-RNAi; Da-GAL4/+*). We detected human NOX 1 or human NOX 4 isozyme in each transgenic fly using real-

time quantitative PCR (qPCR). Transgenic flies were homogenated with ice-cold PBS containing protease inhibitors and membranes enriched human NOX 1 or NOX 4 were harvested. Membranes containing human NOX 1 or NOX 4 served to monitor ROS production by lucigenin chemiluminescence in the absence or presence of chemical compound. The reaction medium consisted in Hepes-buffered salt solution (128 mM NaCl, 2.5 mM KCl, 1.2 mM $MgSO_4$, 1.0 mM KH_2PO_4, 1.75 μM $CaCl_2$, 0.03 Na_2EDTA, 5.5 mM glucose, and 20 mM HEPES, pH 7.4), 400 μM lucigenin (10,10-dimethyl-bis-9,9-bisacridinium nitrate) and 500 μM NADPH.[34]

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grants (2012R1A5A1048236, 2012R1A1A2001033 and 2011-0028885), by Redoxomics grant (2012M3A9C5048708), and by Priority Research Centers Program (2016R1A6A1A03007648) through NRF funded by the Ministry of Education, Science and Technology (MEST) – Republic of Korea.

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