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Hydantoin derivatives as non-peptidic inhibitors of Ras farnesyl transferase

Jinho Lee,[a,*] Jonghyun Kim,[b] Jong Sung Koh,[b] Hyun-Ho Chung[b] and Kyoung-Hee Kim[b]

[a]*Department of Chemistry, Keimyung University, 1000 Sindang-Dong, Dalseo-Gu, Daegu 704-701, Republic of Korea*
[b]*LG Life Sciences, Science Town, Taejon 305-380, Republic of Korea*

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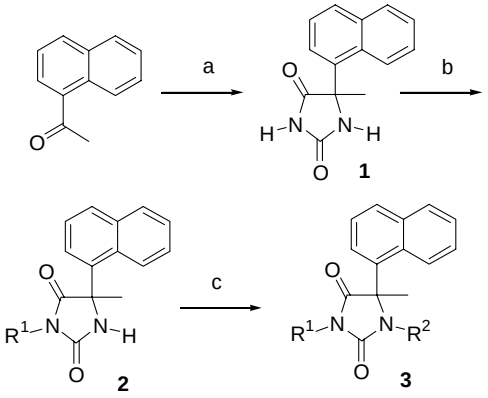
Abstract—1,3,5,5-Tetrasubstituted 2,4-imidazolinedione (hydantoin) derivatives were evaluated as Ftase inhibitors. Potent Ftase inhibitors without thiol or peptide were obtained in three steps.

Ras protein plays an important role in cell growth and differentiation. Oncogenic Ras has been found in 40% of human cancers including 90% of pancreatic and 50% of colon carcinomas.[1,2] Ras protein needs a series of post-translational modifications including the farnesylation catalyzed by farnesyl transferase (Ftase).[3] Inhibitors of Ftase are considered as potential anticancer agents.[4–6] The observation that Ftase can farnesylate C-terminal tetrapeptide of Ras (CAAX in which C is cysteine, A is aliphatic amino acid, and X is serine or methionine) triggers great efforts to develop peptidomimetic inhibitors. Substitution of aliphatic amino acids, AA of CAAX tetrapeptide, with benzodiazepine[7] 3-(aminomethyl)benzoic acid (3-AMBA),[8] piperazine,[9] and 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Tic)[10] had been tried. Conformational analysis of potent tetrapeptidic inhibitor CVFM (IC_{50} 25 nM)[11] renders us to design hydantoin scaffold.

Hydantoin was synthesized in one step using Sano's condition[12] in 90% yield (Scheme 1). 5-Methyl-5-(1-naphthyl)-2,4-imidazolidinedione (hydantoin **1**) was obtained as racemic mixtures from the reaction of 1′-acetonaphthone with potassium cyanide and ammonium carbonate and it was used without chiral resolution. Introduction of the substituent at N-3 position was performed with the corresponding alcohol under the Mitzunobu condition.[13] Normal alkylation at the N-1 position provided the desired compound **3**.



Scheme 1. Reagents: (a) KCN, $(NH_4)_2CO_3$, 90%; (b) R^1-OH, DEAD, Ph_3P; (c) NaH, R^2-Br.

Substitution of naphthalene at C-4 position of hydantoin improved inhibitory activity about one order of magnitude more than that of phenyl substituent (data not shown). However, conformational study showed that naphthalene could accommodate several spatial orientations relative to the hydantoin ring. Introduction of methyl group at C-4 position provided the steric restriction which forced the naphthalene to the desired orientation. Also, it removed the easy epimerizing character of hydantoin structure.

As a first step to remove the peptidic character, imidazole was used at the position of cysteine because it had

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* Corresponding author. Tel.: +82 53 580 5183; fax: +82 53 580 5164; e-mail: jinho@kmu.ac.kr

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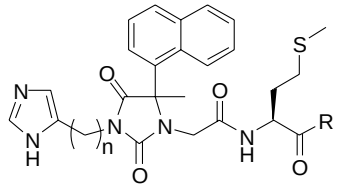
been used to replace cysteine for several non-peptidic small Ftase inhibitors and had been found to interact with the enzyme-bound Zn ion.[10] Introduction of imidazole with an optimal distance (3 methylene unit) from hydantoin provided high potency (IC_{50} 0.8 nM, Table 1).

The orientation of imidazole affected the inhibitory activities of compounds (Table 2). (1*H*-Imidazol-1-yl)propyl **9a**,**b** at N-3 position of hydantoin provided better activity than (1*H*-imidazol-4-yl)propyl **7a**,**b** or (1-methyl-1*H*-imidazol-5-yl)propyl **8** group.[15] The better potency of (1*H*-imidazol-1-yl) **9b** than (1-methyl-1*H*-imidazol-5-yl) **8** suggested that the increment of basicity was not the only factor of activity gain.

Since the modeling study showed that the naphthalene at C-4 position of hydantoin overlapped nicely with the phenyl ring of CVFM, the replacement of remaining peptidic character, *N*-acetylmethionine, of compound **6** had been tried. The variation of N-1 substituent was performed, while the N-3 position of hydantoin was fixed with (1*H*-imidazol-1-yl)propyl group. Among the hydrophobic substituents, benzyl group provided nM range inhibitory activity with substituent's dependency. Halogens **10a**–**c** were allowed at both *meta*- and *para*-positions, while phenoxy **10d**,**e** was a little bulky to fit in the enzyme pocket. Cyano group showed that *m*-position showed better activity up to 10 times than *p*-position (**10g** vs **10h**). X-ray crystallographic structure analysis showed that *m*-cyano group filled the narrow pocket created by Tyr93, Leu96, Leu103, Asp359, Phe360, and Tyr361 residues, while the *m*-carboxyl group could form H-bond interaction with the Tyr93 residue of the enzyme.[16] Cationic substituent at *p*-position was not favored so the protection of amine provided activity increase by 6 times (**10n** vs **10o**). Sulfide **10k**, **10m**, sulfone **10l**, and sulfonamide **10o**–**q** were tried as the surrogate of methionine residue, but they did not behave as expected. Combination of *p*-methylsulfide and *m*-cyano **10r** did not improve the inhibitory activity of *m*-cyano **10h** (Table 3).

Kinetic study showed that hydantoin derivatives were competitive with Ras protein. X-ray crystal structure showed that the hydantoin compound binds in the Ras binding region of enzyme and the nitrogen atom of imidazole interacts with Zn(II) metal.

Table 1. The effect of linker between hydantoin and imidazole on the inhibitory activity of the farnesyl transferase[a]

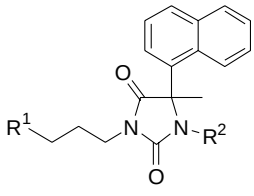


4,5,6

Compound	*n*	R	IC_{50}[b] (nM)
4a	1	OCH_3	19,000
4b	1	OH	61
5a	2	OCH_3	2500
5b	2	OH	4.8
6a	3	OCH_3	500
6b	3	OH	0.8

[a] The farnesyl transferase inhibitory assays were performed as described in Ref. 14.
[b] Values are means of three experiments.

Table 2. The effect of imidazole on the inhibitory activity of the farnesyl transferase

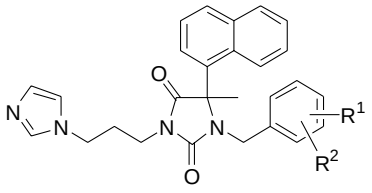


7,8,9

Compound	R^1	R^2	IC_{50}[a] (μM)
7a	1*H*-Imidazol-4-yl	H	40
9a	1*H*-Imidazol-1-yl	H	7.7
7b	1*H*-Imidazol-4-yl	$CH_2CO_2CH_2CH_3$	3.8
8	1-Methyl-imidazol-5-yl	$CH_2CO_2CH_2CH_3$	2.8
9b	1*H*-Imidazol-1-yl	$CH_2CO_2CH_2CH_3$	1.0

[a] Values are means of three experiments.

Table 3. The effect of substituents on the inhibitory activity of the farnesyl transferase



10a-s

Compound	R^1	R^2	IC_{50}[a] (μM)
10a	H	*p*-Br	0.16
10b	H	*m*-Br	0.37
10c	H	*m*-Cl	0.24
10d	H	*p*-OPh	0.75
10e	H	*m*-OPh	0.72
10f	H	*p*-CO_2H	0.2
10g	H	*p*-CN	0.9
10h	H	*m*-CN	0.09
10i	H	*o*-CN	1.3
10j	H	*m*-CF_3	1.7
10k	H	*p*-SCH_3	0.7
10l	H	*p*-SO_2CH_3	1.1
10m	H	*p*-CH_2SCH_3	0.21
10n	H	*p*-CH_2NH_2	2.1
10o	H	*p*-$CH_2NHSO_2CH_3$	0.35
10p	H	*m*-$CH_2NHSO_2CH_3$	1.2
10q	H	*o*-$CH_2NHSO_2CH_3$	0.5
10r	*m*-CN	*p*-SCH_3	0.08
10s	*m*-CN	*p*-SO_2CH_3	0.73

[a] Values are means of three experiments.

In summary, hydantoin was found to be an effective scaffold that gives rise to a series of potent inhibitors of farnesyl transferase. Non-peptidic hydantoin derivatives were synthesized in 3 steps and they showed IC_{50} as low as 80 nM **10r**.

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14. The assay was done by a modification of the procedure described by Moores et al. Moores, S. L.; Schaber, M. D.; Mosser, S. D.; Rands, E.; O'Hara, M. B.; Garsky, V. M.; Marchall, M. S.; Pompliano, D. L.; Gibbs, J. B. *J. Biol. Chem.* **1991**, *266*, 14603, Briefly, Ras protein (20 μM) was incubated for 30 min at 37 °C with Ftase enzyme (16.6 nM), [^{3}H]farnesylpyrophosphate (FPP) (0.3 μM, 20 Ci/mmol, Dupont NEN) and serially diluted test compounds at a given concentration in the buffer containing 50 mM HEPES, pH 7.4, 250 mM $MgCl_2$, 25 mM KCl, 0.5% *N*-octylglucoside, 50 μM $ZnCl_2$, and 10 mM DTT. The enzyme reactions were then quenched by adding the solution containing 10% HCl in absolute ethanol. The [^{3}H]FPP-incorporated Ras was collected by filter binding (25 mm glass fiber filter, Whatman) and quantitated by scintillation counter. IC_{50}: the concentration of compound required to reduce the Ftase-catalyzed incorporation of [^{3}H]FPP into H-Ras protein by 50%.
15. (a) Synthesis of alcohol derivatives: 3-(1*H*-imidazol-4-yl)-1-propanol was obtained by reduction of urocanic acid with $LiAlH_4$ in quantitative yield. ^{1}H NMR ($CDCl_3$) 1.79 (p, 2H), 2.64 (t, J = 7.3 Hz, 2H), 3.56 (br, 1H), 3.60 (t, J = 6.1 Hz, 2H), 6.69 (s, 1H), 7.48 (s, 1H).; (b) 3-(1*H*-Iimidazol-1-yl)-1-propanol; imidazole 5 g (73.4 mmol) and methyl acrylate 12.6 g (148.6 mmol) were dissolved in 100 ml of acetonitrile. After refluxing for 8 h, acetonitrile and unreacted methyl acrylate were removed under reduced pressure. Ethyl acetate was added to the residue and washed with saturated NaCl solution. Removal of ethyl acetate gave methyl 3-(1*H*-imidazole-1-yl)-1-propionate 11.1 g in 90% yield. Reduction of methyl ester with $LiAlH_4$ provided desired alcohol in 93% yield. ^{1}H NMR ($CDCl_3$): 1.97 (p, 2H), 3.57 (t, J = 5.8 Hz, 2H), 4.06 (br, 1H), 4.11 (t, J = 6.7 Hz, 2H), 6.92 (s, 1H), 7.01 (s, 1H), 7.51 (s, 1H).
16. The coordinate has been deposited in the Protein Data Bank: PDB ID 2F0Y.