Inhibition of Restriction Enzymes *Eco*RI, *Bam*HI and *Hin*dIII by Phenethylphenylphthalimides Derived from Thalidomide

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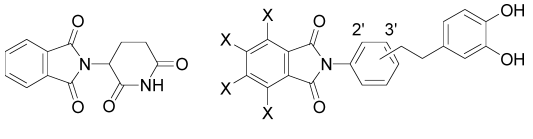
We discovered inhibitors of the restriction enzymes *Eco*RI, *Bam*HI and *Hin*dIII by screening our library of compounds with a phenethylphenylphthalimide skeleton, based on α-glucosidase inhibitors and liver X receptor antagonists derived from thalidomide. Structural development afforded the potent restriction enzyme inhibitors 25 and 26.

Key words restriction enzyme; endonuclease; enzyme inhibitor; phthalimide

We have been engaged in the creation of bioactive compounds based on the multi-template approach utilizing thalidomide (**1**).[1—6] The basic idea of the multi-template approach is that the number of three-dimensional spatial structures (fold structures) of human proteins is only approximately 1000, which is much smaller than the number of human proteins, estimated to be 50000—70000.[7—9] Therefore, ignoring physical/chemical interactions, a template/scaffold structure which is spatially complementary to one fold structure might serve as a multi-template for structural development of ligands that would interact specifically with at least 50—70 different human proteins. We have focused on thalidomide (**1**) as a candidate multi-template structure. Thalidomide (**1**) was launched as a hypnotic/sedative drug in the 1950's, but was withdrawn from the market in the 1960's because of severe teratogenicity. But, subsequently, thalidomide (**1**) has been established to be useful for the treatment of Hansen's disease and multiple myeloma. Further, many reports have appeared on its therapeutic potential for the treatment of a range of diseases, including cancers, rheumatoid arthritis and diabetes.[1—6,10] Using our multi-template approach, we have developed many biological response modifiers, including tumor necrosis factor-α (TNF-α) production regulators,[11,12] nitric oxide synthase (NOS) inhibitors,[13,14] cyclooxygenase (COX) inhibitors,[15—17] liver X receptor (LXR) antagonists,[18—21] α-glucosidase inhibitors[21—24] and glycogen phosphorylase inhibitors.[25] On the other hand, based on a result that thalidomide inhibits the replication of human immunodeficiency virus type 1 (HIV-1),[26] we speculated that thalidomide and its derivatives might inhibit the growth of other viruses. We focused on inhibitors of the influenza virus A, and phenethylphenylphthalimide derivatives **2**, **3** and **4,** which were derived from thalidomide-related α-glucosidase inhibitors and liver X receptors (Fig. 1), were found to inhibit the growth of influenza A.[27] We also investigated the mechanism of this growth inhibition and found that these compounds inhibit influenza virus PA endonuclease. So, we speculated that various inhibitors toward other DNA recognizing enzymes might be possible to generate by modification of phenethylphenylphthalimide derivatives. Thus, we screened our library of these derivatives for inhibitory activity towards restriction enzymes *Eco*RI, *Bam*HI and *Hin*dIII, which resemble endonucleases and are frequently utilized in biological experiments to proof our hypothesis. *Eco*RI, isolated from strains of *Escherichia* (*E.*) *coli*, recognizes the following palindromic DNA sequence: 5′-GAATTC-3′ 3′-CTTAAG-5′. *Bam*HI, derived from *Bacillus amyloliquefaciens*, recognizes the palindromic sequence: 5′-GGATCC-3′ 3′-CCTAGG-5′. *Hin*dIII, derived from *Haemophilus influenzae*, recognizes the palindromic sequence 5′-AAGCTT-3′ 3′-TTCGAA-5′.



Thalidomide (**1**)

Compound	X	position
2	H	2'
3	H	3'
4	Cl	2'

Fig. 1. Chemical Structures of Thalidomide (**1**) and Compounds **2**—**4**

Results and Discussion

*Eco*RI, *Bam*HI and *Hin*dIII were selected as target enzymes and pBR322 was selected as the assay substrate. At the initial screening of phenethylphenylphthalimide derivatives, compound **19** showed *Eco*RI-inhibitory activity with the IC_{50} value of 70 μM (Table 1). Compound **16** also showed weak *Eco*RI-inhibitory activity. Since compounds **16** and **19** possess a tetrachlorophthalimide skeletons and a dihydroxyphenyl group, these might be important for the *Eco*RI-inhibitory activity. Next, we tried to improve the inhibitory activity of compounds **16** and **19**. Based on the results of structure–activity relationship studies, phenethylphenylphthalimides bearing trihydroxy substituents were designed as candidates for more potent *Eco*RI inhibitors.

These analogs were synthesized as shown in Chart 1. 3,4,5-Trimethoxybenzyl alcohol was allowed to react with PBr_3 to generate 3,4,5-trimethoxybenzyl bromide. Reaction of the intermediate with triphenylphosphine provided triphenylphosphonium salt **30**. Diphenylethene derivatives **31a**—**c** were prepared as *E*/*Z* mixtures by Wittig reaction of nitrobenzaldehyde with **30**. After simultaneous reduction of the nitro group and olefin moiety of compounds **31a**—**c**, phenethylphenylphthalimides **33a**—**c** and **34a**—**c** were obtained by condensation with phthalic or tetrachlorophthalic anhydride, respectively. The conversion of the methoxy groups to hydroxyl groups using BBr_3 was conducted to give trihydroxy analogs **20**—**22** and **24**—**26**. Dihydroxyl analogs

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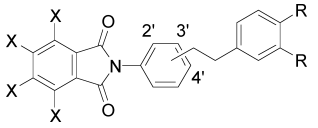
23 and **27**—**29** were also generated in the reaction and purified by silica gel column chromatography.

Introduction of a hydroxyl group at the 5″ position of **16** and **19**, *i.e.*, compounds **25** and **26**, resulted in a drastic increase of *Eco*RI-inhibitory activity (Table 2). On the other hand, the potency of analogs possessing a methoxy group instead of a hydroxyl group, *i.e.*, compounds **28** and **29**, was decreased compared with that of **25** and **26**, respectively. Hence, a hydroxyl group at the 5″ position might be important for *Eco*RI-inhibitory activity. Since compounds with a hydroxyl group at the 5″ position of the non-substituted phthalimide skeleton (**20**—**22**) had no apparent activity, the tetrachloro substitution of the phthalimide skeleton might also be important for *Eco*RI-inhibitory activity.

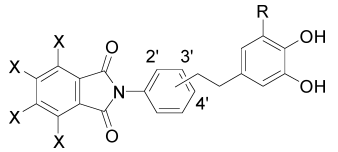
Subsequently, we evaluated the *Bam*HI- and *Hin*dIII-inhibitory activities of the phenethylphenylphthalimide derivatives possessing *Eco*RI-inhibitory activity (Table 3). Compounds **25** and **26** showed *Bam*HI- and *Hin*dIII-inhibitory activities. Compounds **16** and **23**, which possess weak *Eco*RI-inhibitory activity, also showed weak *Bam*HI-inhibitory ac-

Table 1. *Eco*RI-Inhibitory Activity of Phenethylphenylphthalimide Derivatives (**2**—**19**)

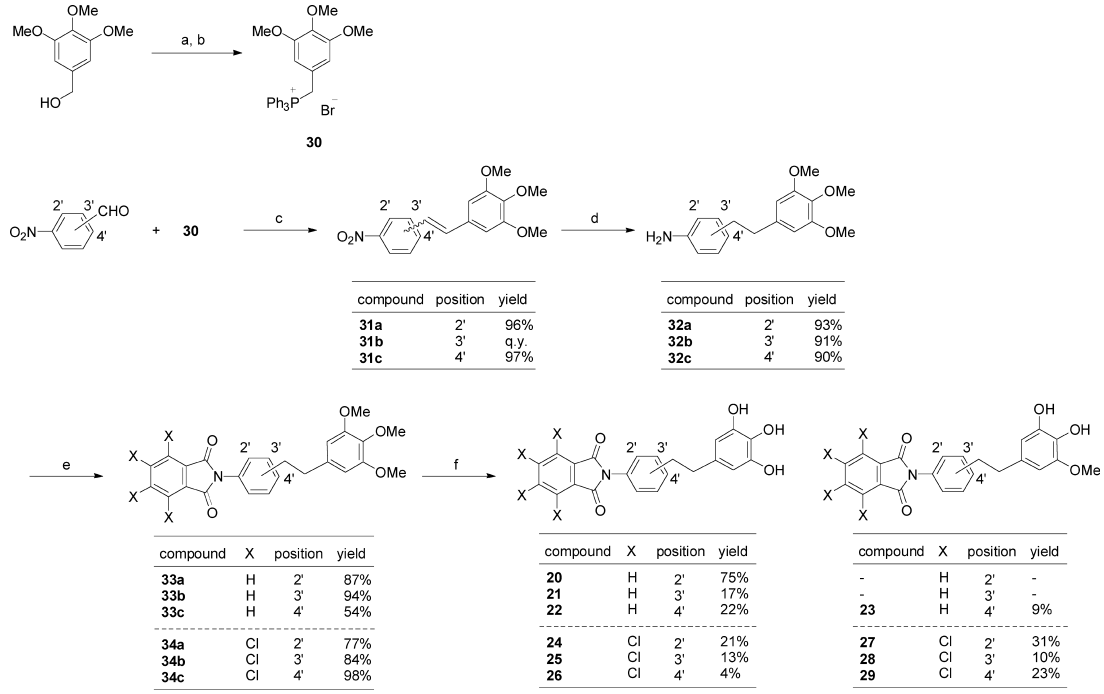


Compound	X	Position	R	Inhibitory ratio at 100 μM (IC_{50} [μM])
5	H	2′	H	0
6	H	2′	OMe	0
2	H	2′	OH	0
7	H	3′	H	0
8	H	3′	OMe	0
3	H	3′	OH	0
9	H	4′	H	0
10	H	4′	OMe	0
11	H	4′	OH	0
12	Cl	2′	H	0
13	Cl	2′	OMe	0
4	Cl	2′	OH	0
14	Cl	3′	H	0
15	Cl	3′	OMe	0
16	Cl	3′	OH	29%
17	Cl	4′	H	0
18	Cl	4′	OMe	0
19	Cl	4′	OH	68% (70)

Table 2. *Eco*RI-Inhibitory Activity of Trisubstituted Phenethylphenylphthalimide Derivatives (**20**—**29**)



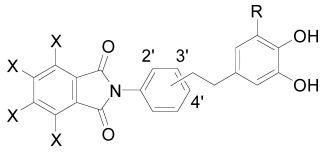
Compound	X	Position	R	Inhibitory ratio at 100 μM (IC_{50} [μM])
20	H	2′	OH	0
21	H	3′	OH	0
22	H	4′	OH	0
—	H	2′	OMe	—
—	H	3′	OMe	—
23	H	4′	OMe	17
24	Cl	2′	OH	0
25	Cl	3′	OH	100% (4.2)
26	Cl	4′	OH	97% (5.3)
27	Cl	2′	OMe	2
28	Cl	3′	OMe	4
29	Cl	4′	OMe	85% (45)



Reagents and conditions: (a) PBr_3, CH_2Cl_2, 0 °C; (b) PPh_3, toluene, reflux; (c) K_2CO_3, 18-crown-6, CH_2Cl_2, reflux; (d) H_2, Pd/C, EtOAc, rt; (e) phthalic or tetrachlorophthalic anhydride; (f) BBr_3, CH_2Cl_2, 0 °C.

Chart 1

Table 3. *Eco*RI-, *Bam*HI- and *Hin*dIII-Inhibitory Activities of Phenethylphenylphthalimide Derivatives (**16**, **19**, **23**, **25**, **26**, **29**)



Compound	X	Position	R	Inhibitory ratio at 100 μM (IC_{50} [μM])		
				*Eco*RI	*Bam*HI	*Hin*dIII
16	Cl	3′	H	29%	40%	51%
19	Cl	4′	H	68% (70)	41%	74%
23	H	4′	OMe	17%	23%	25%
25	Cl	3′	OH	100% (4.2)	56%	67%
26	Cl	4′	OH	97% (5.3)	64%	78%
29	Cl	4′	OMe	85% (45)	56%	41%

tivity and modest *Hin*dIII-inhibitory activity.

In conclusion, we discovered restriction enzyme inhibitors among our library of phenethylphenylphthalimide derivatives. Compounds **25** and **26** showed potent *Eco*RI-inhibitory activity with the IC_{50} values of 4.2 and 5.3 μM, respectively, together with modest *Bam*HI- and *Hin*dIII-inhibitory activities. These results indicate that the phenethylphenylphthalimide skeleton can be used as a multi-template for endonuclease inhibitors.

Experimental

General Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

General Procedure A (GP-A) To a solution of nitrobenzaldehyde in dehydrated CH_2Cl_2 were added benzyltriphenylphosphonium salt (1.0 eq), potassium carbonate (1.1 eq) and 18-crown-6 (0.18 eq) and the mixture was refluxed, then filtered and concentrated. The residue was purified by silica gel column chromatography to give the target compound as an *E*/*Z* mixture.

General Procedure B (GP-B) Substituted nitrobenzene was dissolved in EtOAc and hydrogenated with 10% Pd/C (catalytic amount). The mixture was filtered through a pad of Celite, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel columnn chromatography to give the target compound.

General Procedure C (GP-C) A mixture of phthalic anhydride and substituted aniline (1.0 eq) was heated at 160 or 200 °C for 1 h. After the reaction was completed, the residue was purified by silica gel column chromatography to give the target compound.

General Procedure D (GP-D) To a solution of substituted methoxybenzene in dehydrated dichloromethane was added dropwise boron tribromide (1.0 M solution in dichloromethane) (10 eq) at 0 °C under an argon atmosphere. The mixture was stirred for 5—60 min, then poured into water and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to give the target compound.

Triphenyl-(3,4,5-trimethoxybenzyl)phosphonium Bromide (30) To a solution of 3,4,5-trimethoxybenzyl alcohol (1.98 g, 10 mmol) in dichloromethane (15 ml) was added dropwise phosphorus tribromide (658 μl, 7 mmol) at 0 °C under an argon atmosphere. The mixture was stirred for 1 h, then poured into ice water (50 ml) and alkalized with sat. $NaHCO_3$ (50 ml). The aqueous mixture was extracted with CH_2Cl_2 (20 ml×3). The organic layer was washed with H_2O (20 ml×2) and then dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to give an intermediate. To a solution of the intermediate (2.48 g, 9.50 mmol) in toluene (30 ml) was added triphenylphosphine (3.74 g, 14.25 mmol). The mixture was refluxed for 30 min. The precipitate was filtered to give the target compound (4.52 g, 86% for 2 steps) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.79—7.75 (m, 9H), 7.65—7.61 (m, 6H), 6.47 (d, 2H, *J*=2.4 Hz), 5.40 (d, 2H, *J*=14.0 Hz), 3.77 (s, 3H), 3.52 (s, 6H). FAB-MS *m*/*z*: 443 $[M-Br]^+$.

1,2,3-Trimethoxy-5-[2-(2-nitrophenyl)vinyl]benzene (31a) This compound was prepared from 2-nitrobenzaldehyde and **30** by means of GP-A. Compound **31a** was obtained in 96% yield as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) **31a*Z*** δ: 8.09 (dd, 1H, *J*=8.5, 1.2 Hz), 7.46 (dd, 1H, *J*=7.3, 1.2 Hz), 7.40 (t, 2H, *J*=7.9 Hz), 6.87 (d, 1H, *J*=12.2 Hz), 6.67 (d, 1H, *J*=12.2 Hz), 6.25 (s, 2H), 3.92 (s, 3H), 3.59 (s, 6H). **31a*E*** δ: 7.97 (dd, 1H, *J*=8.5, 1.2 Hz), 7.75 (d, 1H, *J*=7.9 Hz), 7.60 (td, 1H, *J*=7.3, 1.2 Hz), 7.50 (d, 1H, *J*=15.9 Hz), 7.35 (d, 1H, *J*=7.9 Hz), 7.02 (d, 1H, *J*=15.9 Hz), 6.76 (s, 2H), 3.88 (s, 3H), 3.80 (s, 6H). FAB-MS *m*/*z*: 315 $[M]^+$, 316 $[M+H]^+$.

1,2,3-Trimethoxy-5-[2-(3-nitrophenyl)vinyl]benzene (31b) This compound was prepared from 3-nitrobenzaldehyde and **30** by means of GP-A. Compound **31b** was obtained in 100% yield as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) **31b*Z*** δ: 8.16 (s, 1H), 8.05 (dd, 1H, *J*=8.5, 2.4 Hz), 7.60 (d, 1H, *J*=7.9 Hz), 7.40 (t, 1H, *J*=7.9 Hz), 6.69 (d, 1H, *J*=12.2 Hz), 6.58 (d, 1H, *J*=11.6 Hz), 6.43 (s, 2H), 3.85 (s, 3H), 3.67 (s, 6H). **31b*E*** δ: 8.37 (t, 1H, *J*=1.8 Hz), 8.10 (dd, 1H, *J*=7.9, 1.2 Hz), 7.79 (d, 1H, *J*=7.9 Hz), 7.53 (t, 1H, *J*=7.9 Hz), 7.18 (d, 1H, *J*=16.5 Hz), 7.05 (d, 1H, *J*=15.9 Hz), 6.78 (s, 2H), 3.94 (s, 6H), 3.89 (s, 3H). FAB-MS *m*/*z*: 315 $[M]^+$, 316 $[M+H]^+$.

1,2,3-Trimethoxy-5-[2-(4-nitrophenyl)vinyl]benzene (31c) This compound was prepared from 4-nitrobenzaldehyde and **30** by means of GP-A. Compound **31c** was obtained in 97% yield as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) **31c*Z*** δ: 8.12 (dt, 2H, *J*=8.5, 2.4 Hz), 7.44 (d, 2H, *J*=8.5 Hz), 6.73 (d, 1H, *J*=12.2 Hz), 6.58 (d, 1H, *J*=11.6 Hz), 6.43 (s, 2H), 3.86 (s, 3H), 3.68 (s, 6H). **31c*E*** δ: 8.23 (d, 2H, *J*=9.2 Hz), 7.63 (d, 2H, *J*=9.2 Hz), 7.20 (d, 1H, *J*=16.5 Hz), 7.05 (d, 1H, *J*=15.9 Hz), 6.78 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H). FAB-MS *m*/*z*: 315 $[M]^+$, 316 $[M+H]^+$.

2-[2-(3,4,5-Trimethoxyphenyl)ethyl]phenylamine (32a) This compound was prepared from **31a** by means of GP-B. Compound **32a** was obtained in 93% yield as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.07—7.03 (m, 2H), 6.74 (t, 1H, *J*=7.3 Hz), 6.68 (d, 1H, *J*=7.3 Hz), 6.37 (s, 2H), 3.83 (s, 3H), 3.81 (s, 6H), 3.49 (br s, 2H), 2.89—2.86 (m, 2H), 2.80—2.77 (m, 2H). FAB-MS *m*/*z*: 287 $[M]^+$, 288 $[M+H]^+$.

3-[2-(3,4,5-Trimethoxyphenyl)ethyl]phenylamine (32b) This compound was prepared from **31b** by means of GP-B. Compound **32b** was obtained in 91% yield as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.08 (dt, 1H, *J*=7.9, 1.2 Hz), 6.61 (d, 1H, *J*=7.3 Hz), 6.54 (d, 1H, *J*=6.7 Hz), 6.54 (s, 1H), 6.38 (s, 2H), 3.83 (s, 6H), 3.83 (s, 3H), 2.86—2.83 (m, 2H), 2.83—2.80 (m, 2H). FAB-MS *m*/*z*: 287 $[M]^+$, 288 $[M+H]^+$.

4-[2-(3,4,5-Trimethoxyphenyl)ethyl]phenylamine (32c) This compound was prepared from **31c** by means of GP-B. Compound **32c** was obtained in 90% yield as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.98 (dt, 2H, *J*=8.5, 2.4 Hz), 6.64 (dt, 2H, *J*=8.5, 2.4 Hz), 6.37 (s, 2H), 3.83 (s, 6H), 2.80 (s, 3H). FAB-MS *m*/*z*: 287 $[M]^+$, 288 $[M+H]^+$.

2-{2-[2-(3,4,5-Trimethoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (33a) This compound was prepared from phthalic anhydride and **32a** by means of GP-C. Compound **33a** was obtained in 87% yield as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (dd, 2H, *J*=5.5, 2.4 Hz), 7.81 (dd, 2H, *J*=5.5, 2.4 Hz), 7.41 (td, 1H, *J*=7.9, 1.8 Hz), 7.36 (td, 1H, *J*=7.9, 1.8 Hz), 7.33 (dd, 1H, *J*=7.9, 1.8 Hz), 7.21 (dd, 1H, *J*=7.9, 1.8 Hz), 6.21 (s, 2H), 3.78 (s, 3H), 3.68 (s, 6H), 2.82—2.79 (m, 2H), 2.79—2.76 (m, 2H). FAB-MS *m*/*z*: 417 $[M]^+$, 418 $[M+H]^+$.

2-{3-[2-(3,4,5-Trimethoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (33b) This compound was prepared from phthalic anhydride and **32b** by means of GP-C. Compound **33b** was obtained in 94% yield as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (dd, 2H, *J*=5.5, 2.4 Hz), 7.80 (dd, 2H, *J*=5.5, 2.4 Hz), 7.42 (t, 1H, *J*=7.9 Hz), 7.27—7.26 (m, 2H), 7.21 (d, 1H, *J*=7.9 Hz), 6.37 (s, 2H), 3.82 (s, 6H), 3.82 (s, 3H), 3.00—2.97 (m, 2H), 2.92—2.89 (m, 2H). FAB-MS *m*/*z*: 417 $[M]^+$, 418 $[M+H]^+$.

2-{4-[2-(3,4,5-Trimethoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (33c) This compound was prepared from phthalic anhydride and **32c** by means of GP-C. Compound **33c** was obtained in 54% yield as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (dd, 2H, *J*=5.5, 2.4 Hz), 7.80 (dd, 2H, *J*=5.5, 2.4 Hz), 7.34 (d, 2H, *J*=8.5 Hz), 7.32 (d, 2H, *J*=8.5 Hz), 6.37 (s, 2H), 3.84 (s, 6H), 3.83 (s, 3H), 2.98—2.96 (m, 2H), 2.91—2.88 (m, 2H). FAB-MS *m*/*z*: 417 $[M]^+$, 418 $[M+H]^+$.

4,5,6,7-Tetrachloro-2-{2-[2-(3,4,5-trimethoxyphenyl)ethyl]phenyl}-isoindole-1,3-dione (34a) This compound was prepared from tetrachlorophthalic anhydride and **32a** by means of GP-C. Compound **34a** was obtained in 77% yield as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ:

7.42 (td, 1H, *J*=7.3, 1.2 Hz), 7.36 (td, 1H, *J*=7.3, 1.2 Hz), 7.31 (dd, 1H, *J*=7.3, 1.2 Hz), 7.17 (dd, 1H, *J*=7.3, 1.2 Hz), 6.26 (s, 2H), 3.78 (s, 3H), 3.75 (s, 6H), 2.82—2.80 (m, 2H), 2.79—2.77 (m, 2H). FAB-MS *m/z*: 553 $[M]^+$, 554 $[M+H]^+$, 555 $[M+2]^+$, 556 $[M+3]^+$, 557 $[M+4]^+$, 558 $[M+5]^+$.

4,5,6,7-Tetrachloro-2-{3-[2-(3,4,5-trimethoxyphenyl)ethyl]phenyl}-isoindole-1,3-dione (34b) This compound was prepared from tetrachlorophthalic anhydride and **32b** by means of GP-C. Compound **34b** was obtained in 84% yield as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.43 (m, 1H), 7.25—7.22 (m, 3H), 6.35 (s, 2H), 3.82 (s, 9H), 2.99—2.96 (m, 2H), 2.91—2.87 (m, 2H). FAB-MS *m/z*: 553 $[M]^+$, 554 $[M+H]^+$, 556 $[M+3]^+$, 558 $[M+5]^+$.

4,5,6,7-Tetrachloro-2-{4-[2-(3,4,5-trimethoxyphenyl)ethyl]phenyl}-isoindole-1,3-dione (35c) This compound was prepared from tetrachlorophthalic anhydride and **32c** by means of GP-C. Compound **35c** was obtained in 98% yield as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.31 (s, 4H), 6.36 (s, 2H), 3.83 (s, 3H), 3.83 (s, 6H), 2.99—2.96 (m, 2H), 2.91—2.88 (m, 2H). FAB-MS *m/z*: 553 $[M]^+$, 554 $[M+H]^+$, 555 $[M+2]^+$, 556 $[M+3]^+$, 557 $[M+4]^+$, 558 $[M+5]^+$.

2-{2-[2-(3,4,5-Trihydroxyphenyl)ethyl]phenyl}isoindole-1,3-dione (20) This compound was prepared from **33a** by means of GP-D. Compound **20** was obtained in 75% yield as a white solid. mp 104.0—108.0 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.94 (dd, 2H, *J*=5.5, 1.8 Hz), 7.80 (dd, 2H, *J*=5.5, 2.4 Hz), 7.41—7.38 (m, 1H), 7.35—7.32 (m, 2H), 7.20—7.17 (m, 1H), 6.15 (s, 2H), 5.17 (br s, 2H), 5.03 (br s, 1H), 2.78—2.75 (m, 2H), 2.73—2.70 (m, 2H). FAB-MS *m/z*: 375 $[M]^+$, 376 $[M+H]^+$. *Anal.* Calcd for $C_{22}H_{17}NO_5 \cdot 1/2H_2O$: C, 68.74, H, 4.72, N, 3.64. Found: C, 68.63, H, 4.88, N, 3.53.

2-{3-[2-(3,4,5-Trihydroxyphenyl)ethyl]phenyl}isoindole-1,3-dione (21) This compound was prepared from **21** by means of GP-D. Compound **33b** was obtained in 17% yield as a white powder after recrystallization from EtOAc/*n*-hexane. mp 155.5—158.5 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.64 (br s, 2H), 7.96 (dd, 2H, *J*=5.5, 2.4 Hz), 7.90 (dd, 2H, *J*=5.5, 2.4 Hz), 7.84 (br s, 1H), 7.41 (t, 1H, *J*=7.9 Hz), 7.33 (s, 1H), 7.29 (d, 1H, *J*=7.9 Hz), 7.25 (d, 1H, *J*=7.9 Hz), 6.16 (s, 2H), 2.83—2.80 (m, 2H), 2.65—2.62 (m, 2H). FAB-MS *m/z*: 375 $[M]^+$, 376 $[M+H]^+$. *Anal.* Calcd for $C_{22}H_{17}NO_5 \cdot 1/2H_2O$: C, 68.74, H, 4.72, N, 3.64. Found: C, 68.45, H, 4.82, N, 3.60.

2-{4-[2-(3,4,5-Trihydroxyphenyl)ethyl]phenyl}isoindole-1,3-dione (22) and 2-{4-[2-(3,4-Dihydroxy-5-methoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (23) These compounds were prepared from **33c** by means of GP-D. Compounds **22** and **23** was obtained in 22% yield as a yellow powder after recrystallization from EtOAc/*n*-hexane and in 9% yield as colorless needles after recrystallization from EtOAc/*n*-hexane, respectively.

22: mp 218.5—221.0 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.65 (s, 2H), 7.96 (dd, 2H, *J*=5.5, 2.4 Hz), 7.90 (dd, 2H, *J*=5.5, 2.4 Hz), 7.84 (s, 1H), 7.35 (d, 2H, *J*=8.5 Hz), 7.34 (d, 2H, *J*=8.5 Hz), 6.17 (s, 2H), 2.86—2.82 (m, 2H), 2.68—2.65 (m, 2H). FAB-MS *m/z*: 375 $[M]^+$, 376 $[M+H]^+$. *Anal.* Calcd for $C_{23}H_{19}NO_5 \cdot 1/3H_2O$: C, 69.28, H, 4.67, N, 3.67. Found: C, 68.52, H, 4.59, N, 3.57.

23: mp 215.0—216.5 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.68 (s, 1H), 7.98 (s, 1H), 7.96 (dd, 2H, *J*=5.5, 2.4 Hz), 7.90 (dd, 2H, *J*=5.5, 2.4 Hz), 7.36 (d, 2H, *J*=8.5 Hz), 7.34 (d, 2H, *J*=8.5 Hz), 6.32 (d, 1H, *J*=1.8 Hz), 6.29 (d, 1H, *J*=1.8 Hz), 2.90—2.86 (m, 2H), 2.76—2.72 (m, 2H). FAB-MS *m/z*: 389 $[M]^+$, 390 $[M+H]^+$. *Anal.* Calcd for $C_{23}H_{19}NO_5 \cdot 1/3H_2O$: C, 68.96, H, 5.01, N, 3.54. Found: C, 69.81, H, 5.02, N, 3.39.

4,5,6,7-Tetrachloro-2-{2-[2-(3,4,5-trihydroxyphenyl)ethyl]phenyl}-isoindole-1,3-dione (24) and 4,5,6,7-Tetrachloro-2-{2-[2-(3,4-dihydroxy-5-methoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (27) These compounds were prepared from **34a** by means of GP-D. Compounds **24** and **27** was obtained in 21% yield as a yellow powder after recrystallization from EtOAc/*n*-hexane and in 31% yield as a yellow solid, respectively.

24: mp 263.0—265.5 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.44—7.41 (m, 1H), 7.35—7.32 (m, 2H), 7.13 (d, 1H, *J*=7.9 Hz), 6.14 (s, 2H), 5.02 (br s, 2H), 4.98 (br s, 1H), 2.79—2.76 (m, 2H), 2.72—2.70 (m, 2H). FAB-MS *m/z*: 511 $[M]^+$, 512 $[M+H]^+$, 513 $[M+2]^+$, 514 $[M+3]^+$, 515 $[M+4]^+$, 516 $[M+5]^+$. *Anal.* Calcd for $C_{22}H_{13}Cl_4NO_5 \cdot 1/3H_2O$: C, 51.04, H, 2.63, N, 2.71. Found: C, 51.13, H, 2.91, N, 2.59.

27: mp 106.0—109.0 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.44—7.41 (m, 1H), 7.36—7.33 (m, 2H), 7.13 (d, 1H, *J*=7.9 Hz), 6.30 (d, 1H, *J*=1.8 Hz), 6.08 (d, 1H, *J*=1.8 Hz), 5.14 (s, 1H), 5.12 (s, 1H), 3.74 (s, 3H), 2.81—2.78 (m, 2H), 2.76—2.73 (m, 2H). FAB-MS *m/z*: 525 $[M]^+$, 526 $[M+H]^+$, 527 $[M+2]^+$, 528 $[M+3]^+$, 529 $[M+4]^+$, 530 $[M+5]^+$. *Anal.* Calcd for $C_{23}H_{15}Cl_4NO_5 \cdot 1/6H_2O$: C, 52.15, H, 2.91, N, 2.64. Found: C, 52.02, H, 3.00, N, 2.65.

4,5,6,7-Tetrachloro-2-{3-[2-(3,4,5-trihydroxyphenyl)ethyl]phenyl}-isoindole-1,3-dione (25) and 4,5,6,7-Tetrachloro-2-{3-[2-(3,4-dihydroxy-5-methoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (28) These compounds were prepared from **34b** by means of GP-D. Compounds **25** and **28** was obtained in 13% yield as colorless needles after recrystallization from EtOAc/*n*-hexane and in 10% yield as yellow needles after recrystallization from EtOAc/*n*-hexane, respectively.

25: mp 249.0—250.5 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.64 (br s, 2H), 7.85 (br s, 1H), 7.44 (t, 1H, *J*=7.9 Hz), 7.33—7.30 (m, 2H), 7.23 (dt, 1H, *J*=8.5, 1.8 Hz), 6.15 (s, 2H), 2.84—2.81 (m, 2H), 2.65—2.62 (m, 2H). FAB-MS *m/z*: 511 $[M]^+$, 512 $[M+H]^+$, 513 $[M+2]^+$, 514 $[M+3]^+$, 515 $[M+4]^+$, 516 $[M+5]^+$. *Anal.* Calcd for $C_{22}H_{13}Cl_4NO_5$: C, 51.49, H, 2.55, N, 2.73. Found: C, 51.31, H, 2.73, N, 2.66.

28: mp 208.5—210.0 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.67 (s, 1H), 7.98 (s, 1H), 7.44 (t, 1H, *J*=7.9 Hz), 7.32 (d, 2H, *J*=1.8 Hz), 7.32—7.31 (m, 1H), 7.23 (d, 1H, *J*=7.9 Hz), 6.30—6.29 (m, 2H), 3.69 (s, 3H), 2.89—2.85 (m, 2H), 2.73—2.70 (m, 2H). FAB-MS *m/z*: 525 $[M]^+$, 526 $[M+H]^+$, 527 $[M+2]^+$, 528 $[M+3]^+$, 529 $[M+4]^+$, 530 $[M+5]^+$. *Anal.* Calcd for $C_{23}H_{15}Cl_4NO_5$: C, 52.40, H, 2.87, N, 2.66. Found: C, 52.16, H, 2.97, N, 2.61.

4,5,6,7-Tetrachloro-2-{4-[2-(3,4,5-trihydroxyphenyl)ethyl]phenyl}-isoindole-1,3-dione (26) and 4,5,6,7-Tetrachloro-2-{4-[2-(3,4-dihydroxy-5-methoxyphenyl)ethyl]phenyl}isoindole-1,3-dione (29) These compounds were prepared from **34c** by means of GP-D. Compounds **26** and **29** was obtained in 4% yield as a white powder after recrystallization from EtOAc/*n*-hexane and in 23% yield as colorless needles after recrystallization from EtOAc/*n*-hexane, respectively.

26: mp 259.0—262.0 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.65 (s, 2H), 7.85 (s, 1H), 7.39 (d, 2H, *J*=7.9 Hz), 7.31 (d, 2H, *J*=8.5 Hz), 6.17 (s, 2H), 2.86—2.83 (m, 2H), 2.69—2.65 (m, 2H). FAB-MS *m/z*: 511 $[M]^+$, 512 $[M+H]^+$, 513 $[M+2]^+$, 514 $[M+3]^+$, 515 $[M+4]^+$, 516 $[M+5]^+$. *Anal.* Calcd for $C_{22}H_{13}Cl_4NO_5 \cdot 1/3H_2O$: C, 51.04, H, 2.63, N, 2.71. Found: C, 51.02, H, 2.80, N, 2.55.

29: mp 215.5—218.0 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.68 (s, 1H), 7.99 (s, 1H), 7.39 (d, 2H, *J*=7.9 Hz), 7.32 (d, 2H, *J*=7.9 Hz), 6.32 (d, 1H, *J*=1.8 Hz), 6.29 (d, 1H, *J*=1.8 Hz), 3.70 (s, 3H), 2.90—2.87 (m, 2H), 2.76—2.73 (m, 2H). FAB-MS *m/z*: 525 $[M]^+$, 526 $[M+H]^+$, 527 $[M+2]^+$, 528 $[M+3]^+$, 529 $[M+4]^+$, 530 $[M+5]^+$. *Anal.* Calcd for $C_{23}H_{15}Cl_4NO_5 \cdot 1/3H_2O$: C, 51.81, H, 2.96, N, 2.63. Found: C, 51.98, H, 2.94, N, 2.62.

Restriction Enzyme-Inhibitory Activity *Eco*RI, *Bam*HI and *Hin*dIII were purchased from Fermentas. pBR322 was purchased from Nippon Gene, Japan. Restriction enzyme (0.2 unit) in Milli Q water (16 μl) and 10×buffer (2 μl) in an Eppendorf tube were incubated for 3 min at 37 °C, then 2 μl pBR322 solution (final concentration 0.01 μg/ml) was added. The mixture was incubated at 37 °C for 30 min, 37 °C for 30 min or 37 °C for 15 min in the inhibitory assays for *Eco*RI, *Bam*HI or *Hin*dIII, respectively. Then *Eco*RI, *Bam*HI or *Hin*dIII was inactivated by incubation at 75 °C for 15 min, 85 °C for 20 min or 85 °C for 20 min. pBR322 was analyzed by agarose electrophoresis and stained with ethidium bromide. Quantitative analysis was performed by measuring the amount of pBR322 using FLA-7000 (GE Healthcare, U.K.).

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References

1) Hashimoto Y., *Arch. Pharm. Chem. Life Sci.*, **341**, 536—547 (2008).
2) Hashimoto Y., *Bioorg. Med. Chem.*, **10**, 461—479 (2002).
3) Hashimoto Y., *Cancer Chemother. Pharmacol.*, **52** (Suppl. 1), S16—S23 (2003).
4) Hashimoto Y., Tanatani A., Nagasawa K., Miyachi H., *Drugs Future*, **29**, 383—391 (2004).
5) Hashimoto Y., *Curr. Med. Chem.*, **5**, 163—178 (1998).
6) Hashimoto Y., *Mini Rev. Med. Chem.*, **2**, 543—551 (2002).
7) Koonin E. V., Wolf Y. I., Karev G. P., *Nature* (London), **420**, 218—223 (2002).
8) Grishin N. V., *J. Struct. Biol.*, **134**, 167—185 (2001).
9) Koch M. A., Wittenberg L.-O., Basu S., Jeyaraj D. A., Gourzoulidou E., Reinecke K., Odermatt A., Waldmann H., *Proc. Natl. Acad. Sci. U.S.A.*, **101**, 16721—16726 (2004).
10) Bartlett J. B., Dredge K., Dalgleish A. G., *Nat. Rev. Cancer*, **4**, 314—322 (2004).

11) Miyachi H., Azuma A., Ogasawara A., Uchimura E., Watanabe N., Kobayashi Y., Kato F., Kato M., Hashimoto Y., *J. Med. Chem.*, **40**, 2858—2865 (1997).
12) Miyachi H., Ogasawara A., Azuma A., Hashimoto Y., *Bioorg. Med. Chem.*, **5**, 2095—2102 (1997).
13) Shimazawa R., Sano H., Tanatani A., Miyachi H., Hashimoto Y., *Chem. Pharm. Bull.*, **52**, 498—499 (2004).
14) Noguchi T., Sano H., Shimazawa R., Tanatani A., Miyachi H., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **14**, 4141—4145 (2004).
15) Noguchi T., Shimazawa R., Nagasawa K., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **12**, 1043—1046 (2002).
16) Sano H., Noguchi T., Tanatani A., Miyachi H., Hashimoto Y., *Chem. Pharm. Bull.*, **52**, 1021—1022 (2004).
17) Sano H., Noguchi T., Tanatani A., Hashimoto Y., Miyachi H., *Bioorg. Med. Chem.*, **13**, 3079—3091 (2005).
18) Noguchi-Yachide T., Aoyama A., Makishima M., Miyachi H., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **17**, 3957—3961 (2007).
19) Noguchi-Yachide T., Miyachi H., Aoyama H., Aoyama A., Makishima M., Hashimoto Y., *Chem. Pharm. Bull.*, **55**, 1750—1754 (2007).
20) Dodo K., Aoyama A., Noguchi-Yachide T., Makishima M., Miyachi H., Hashimoto Y., *Bioorg. Med. Chem.*, **16**, 4272—4285 (2008).
21) Motoshima K., Noguchi-Yachide T., Sugita K., Hashimoto Y., Ishikawa M., *Bioorg. Med. Chem.*, **17**, 5001—5014 (2009).
22) Sou S., Mayumi S., Takahashi H., Yamasaki R., Kadoya S., Sodeoka M., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **10**, 1081—1084 (2000).
23) Sou S., Takahashi H., Yamasaki R., Kagechika H., Endo Y., Hashimoto Y., *Chem. Pharm. Bull.*, **49**, 791—793 (2001).
24) Takahashi H., Sou S., Yamasaki R., Sodeoka M., Hashimoto Y., *Chem. Pharm. Bull.*, **48**, 1494—1499 (2000).
25) Motoshima K., Ishikawa M., Sugita K., Hashimoto Y., *Biol. Pharm. Bull.*, **32**, 1618—1620 (2009).
26) Makonkawkeyoon S., Limson-Pobre R. N., Moreira A. L., Schauf V., Kaplan G., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 5974—5978 (1993).
27) Iwai Y., Takahashi H., Hatakeyama D., Motoshima K., Ishikawa M., Sugita K., Hashimoto Y., Harada Y., Itamura S., Odagiri T., Tashiro M., Sei Y., Yamaguchi K., Kuzuhara T., *Bioorg. Med. Chem.*, **18**, 5379—5390 (2010).