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Discovery of Novel TRPV1 Ligands through Rational Approach Based on Its Putative Endogenous Ligand, 12(*S*)-HPETE

Kyung Hoon Min,[*] Seul Lee, Hwa Soon Kim,[†] and Young-Ger Suh[‡,*]

College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea. [*]E-mail: khmin@cau.ac.kr
[†]Seoul Metropolitan Government Research Institute of Public Health and Environment, Seoul 130-864, Korea
[‡]College of Pharmacy, Seoul National University, Seoul 151-742, Korea. [*]E-mail: ygsuh@snu.ac.kr
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We report design and synthesis of the novel TRPV1 ligands through a rational approach. Simplified analogues of 12(*S*)-HPETE showing TRPV1 agonistic effect are disclosed. Biological evaluation revealed that substitution of functional groups without any change in conformation converted agonist into antagonist. Our work provided key information with regard to TRPV1 agonist/antagonist switching.

Key Words: TRPV1, 12(*S*)-HPETE, Vanilloid receptor, Antagonist

Introduction

Vanilloid receptor 1 (VR1), recently renamed TRPV1 (transient receptor potential channel, vanilloid subfamily member 1), plays a crucial role as a molecular integrator of multiple pain producing stimuli.[1] Therefore, it has been suggested that TRPV1 should be a useful therapeutic target for pain and inflammatory hyperalgesia. TRPV1 agonists, like capsaicin and resiniferatoxin (RTX), have been studied clinically as potential treatments for inflammatory and neuropathic pain.[2,3] However, direct TRPV1 antagonists have the same effect, but without the initial stimulatory and painful responses caused by TRPV1 agonists.[4] The cloning of TRPV1 initiated a massive effort to discover novel TRPV1 antagonists.[5] One of the most promising approaches to the development of antagonists involves altering conformation of agonist. Many TRPV1 agonists already have been developed, and the majority of these compounds with a vanilloid group show agonist activity with side effects like initial pungency. Intense structure-activity relationship (SAR) studies on capsaicin have yielded only capsazepine as a small molecule antagonist to date, which has poor metabolic and pharmacokinetic properties.[3] With regard to the discovery of novel lead molecules without vanilloid moiety, the identification of an endogenous ligand for TRPV1 could provide an opportunity to design new molecules. Thus, we decided to utilize a TRPV1 endogenous ligand instead of random screening or mimicking patented molecules. We have reported previously that 12(*S*)-HPETE is a potential ligand for TRPV1, because it has higher activity than endogenous lipids like eicosanoids and conformationally, is similar to capsaicin.[6] Accordingly, 12(*S*)-HPETE was utilized to develop antagonists or agonists with novel scaffold and functional groups rather than vanilloids. Herein, we describe the identification of new small molecule antagonists and alternatives to vanilloid based on a putative endogenous ligand, which support the conceptual developmental switch from TRPV1 agonist to antagonist.

Results and Discussion

Chemistry. Our previous modeling study revealed a high level of conformational similarity between capsaicin and 12(*S*)-HPETE.[6] Furthermore, the crystal structure of a capsaicin analogue with an agonistic effect was overlapped with that of a complex of capsaicin and 12(*S*)-HPETE to support this result (Figure 1). Accordingly, we attempted to synthesize small molecules with a carboxylic acid rather than a vanilloid group. If 12(*S*)-HPETE binds to TRPV1 in the same manner as capsaicin, the distance between the COOH group and the OOH group needs to be optimized. The B region was substituted with heteroatoms to mimic the hydrogen peroxide group of 12(*S*)-HPETE.

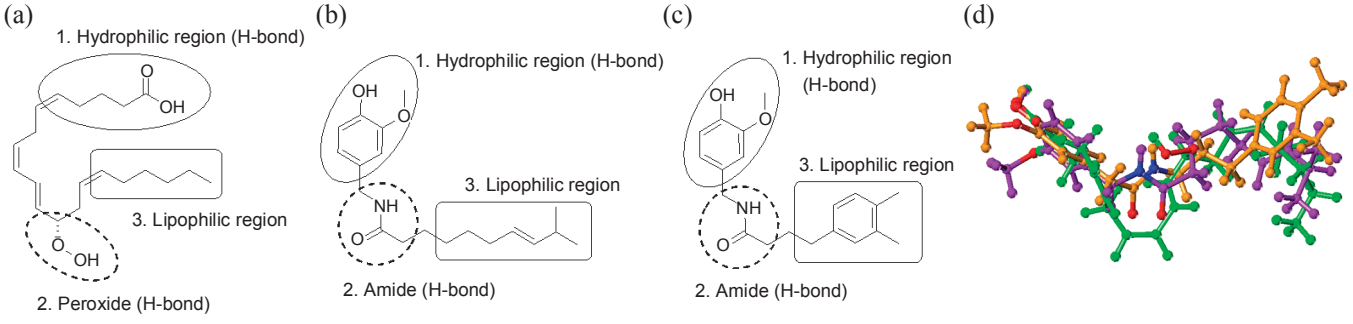


Figure 1. Structural feature of TRPV1 agonist. (a) 12(*S*)-HPETE (b) capsaicin (c) capsaicinoid. (d) Overlay of Ca^{2+}-influx agonists produced by GASP alignment. 12(*S*)-HPETE; green, Capsaicin; purple, and Capsaicinoid (crystal structure); orange.

The distance between the two functional groups in 12(*S*)-HPETE was controlled to about 7 Å based on the results of our modeling study. The lipophilic C region was fixed to the phenethyl group before optimization.

Initially, the eicosanoid 12(*S*)-HPETE was simplified to give compounds **5** and **6**, simplified aliphatic acids with central hetero atoms for hydrogen bonding. Starting material **1** was synthesized in two steps from 1,4-butanediol.[7] Intermediate **4** was synthesized in an eight-step sequence. Hydroxylamine was introduced as an equivalent of peroxide, and the rigid compound **5** and the flexible, saturated compound **6** were obtained. We next turned our attention to the synthesis of benzoic acid analogues. Aliphatic carboxylic acid was replaced with benzoic acid while maintaining the distance between pharmacophores. Compound **10** was designed as a key intermediate to modify the B region meaningfully. The straightforward reaction sequence (Swern oxidation,[8] Grignard addition, Pd catalyzed coupling reaction,[9] hydrogenation and PDC oxidation) provided ketone **10** in 50% overall yield from **7**. Treatment of **10** with semicarbazides or a hydrazine resulted in the formation of hydrazone, which on methyl ester hydrolysis gave the benzoic acid analogues **11**, **12** and **13**. Compound **17** with a vanilloid group was also synthesized to explore the effects of the vanilloid and carboxylic acid moieties. Carbamate or thiocarbamate was then introduced to the B region to provide compounds **24**-**27**.

Biological evaluation. Primary cultures of sensory neurons isolated from dorsal root ganglions of neonatal rats were prepared, and the patch-clamp technique was used to record single-channel currents as described previously.[6] As expected, the simplified 12(*S*)-HPETE's analogues **5** and **6** showed agonistic activity. On the other hand, semicarbazone **11** had an antagonistic effect. In contrast, compound **17** with a vanilloid moiety instead of a carboxylic acid had an agonistic effect. This observation indicates that modification of the vanilloid group can eliminate the agonistic effect. The thiosemicarbazone **12** had no agonistic activity, whereas the carbamate **24** and thiocarbamate **25** exhibited agonistic and antagonistic activities, respectively, revealing that agonist to antagonist switching was achieved by substituting oxygen with sulfur. The *p*-benzoic acid analogue **26** and the *m*-phenylacetic acid analogue **27** were inactive, indicating that an appropriate distance between the functional groups in A and B regions is important for maintaining activity. Interestingly, our findings indicate that electronic effect importantly determines the agonistic or antagonistic nature of a compound rather than its conformation. It was found that the substitution of a functional group, in a manner that does not induce a conformational change, can induce agonist/antagonist switching, which suggests that changing functional groups can convert strong agonists to strong antagonists. These findings led us back to the capsaicin scaffold because of its high affinity for TRPV1.

In the present study, we describe the developments of non-vanilloid agonists and antagonists achieved via conformational analysis and functional group screening. Although their activities are weak, we believe that this study provides useful information concerning the development of novel agonists and antagonists.

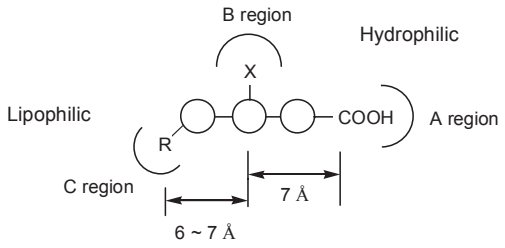
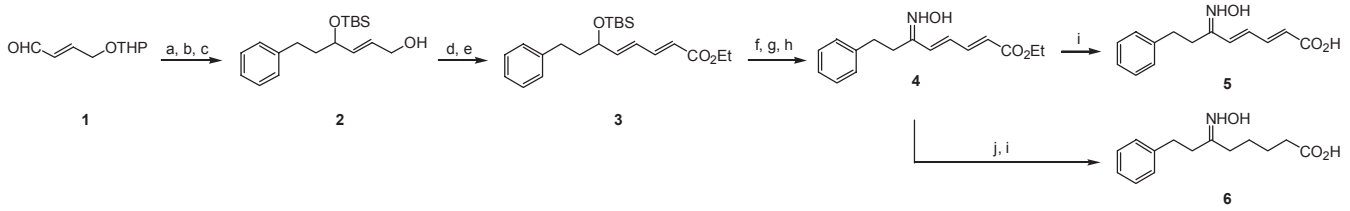


Figure 2. Schematic illustration on design of novel TRPV1 ligands.

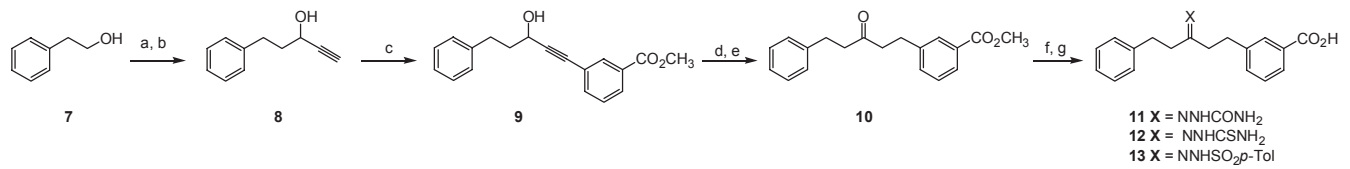
Materials and Methods

(*E*)-4-{[*tert*-Butyl(dimethyl)silyl]oxy}-6-phenyl-2-hexen-1-ol (2). To a solution of **1** (2,32 g, 10.9 mmol) in ether was added dropwise phenethyl magnesium bromide (2 M in ether,



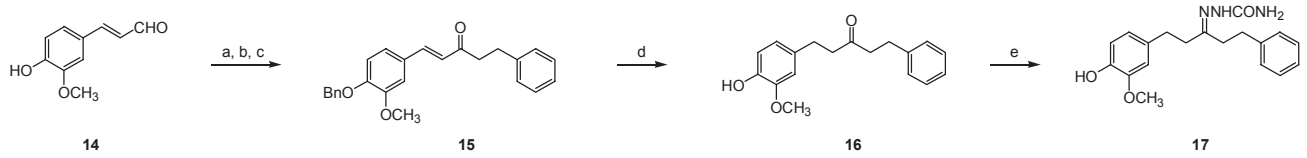
[a]Reaction conditions: (a) $Ph(CH_2)_2MgBr$, ether (b) TBSCl, imidazole, DMF (c) $MgBr_2$, ether (d) MnO_2, $CHCl_3$ (e) Ph_3PCHCO_2Et, CH_2Cl_2 (f)TsOH, MeOH (g) PDC, 4A MS, CH_2Cl_2 (h) NH_2OH (HCl), NaOAc, MeOH (i) LiOH, H_2O/THF(1:1) (j) Pd/C, H_2

Scheme 1[a]



[a]Reaction conditions: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C (b) ethynyl MgBr, ether (c) methyl 3-Iodobenzoate, CuI, $(PPh_3)_4Pd$, PPh_3, Et_3N (d)Pd/C, H_2, CH_3OH (e) PDC, 4A MS, CH_2Cl_2 (f) semicarbazide or thiosemicarbazide or *p*-toluenesulfonylhydrazine, NaOAc, CH_3OH, 50 °C (g) LiOH H_2O, THF, H_2O

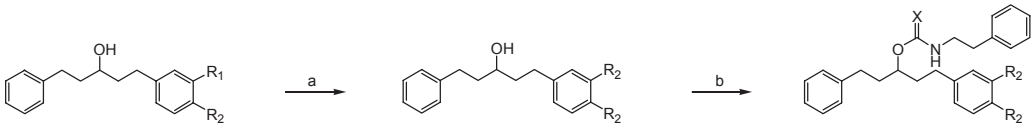
Scheme 2[a]



14 **15** **16** **17**

[a]Reaction conditions: (a) BnBr, K_2CO_3, acetone (b) $PhCH_2CH_2MgBr$, ether (c) PDC, 4A MS, CH_2Cl_2 (d) Pd/C, H_2, MeOH (e) $H_2NHNCONH_2$, NaOAc, MeOH

Scheme 3[a]



18 $R_1=CO_2CH_3$, R_2=H
19 R_1=H, $R_2=CO_2CH_3$
20 $R_1=CH_2CO_2CH_3$, R_2=H

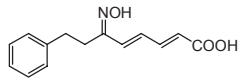
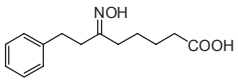
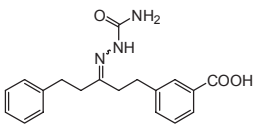
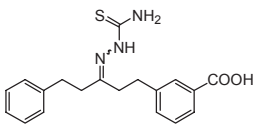
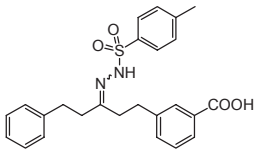
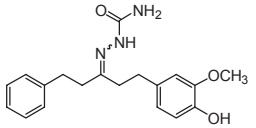
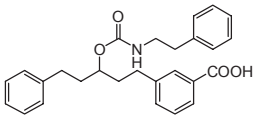
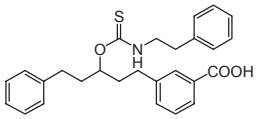
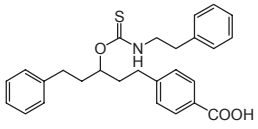
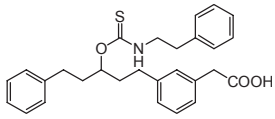
21 $R_1=CO_2H$, R_2=H
22 R_1=H, $R_2=CO_2H$
23 $R_1=CH_2CO_2H$, R_2=H

24 $R_1=CO_2H$, R_2=H, X=O
25 $R_1=CO_2H$, R_2=H, X=S
26 R_1=H, $R_2=CO_2H$, X=S
27 $R_1=CH_2CO_2H$, R_2=H, X=S

[a]Reaction conditions: (a) LiOH H_2O, THF/H_2O (b) Phenethylisothiocyanate, NaH, THF

Scheme 4[a]

Table 1. Channel activities of synthesized compounds

entry	structure	entry	structure	entry	structure	entry	structure
5		**6**					
Response	agonist (W)		agonist (W)				
11		**12**		**13**		**17**	
Response	antagonist (W)		NR		NR		agonist (W)
24		**25**		**26**		**27**	
Response	agonist (+)		antagonist (W)		NR		NR

Note: NR (no response), WW (very weak), W (weak), + (equal to capsaicin or capsazepine)

7 mL, 14 mmol) at 0 °C. The mixture was stirred for 30 min, and then quenched with aq. NH_4Cl, diluted with ether. The organic layer was washed with water and brine, dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo. Column chromatography (EtOAc : *n*-hexane = 1 : 3) of the residue gave yellow oil (1.89 g, 6.87 mmol, 63%). The alcohol (870 mg, 3.14 mmol) was dissolved with imidazole (280 mg, 4.11 mmol) in DMF (2 mL). After adding TBSCl (640 mg, 4.11 mmol) the mixture was stirred for 1 hour at room temperature, then diluted with ether, washed, dried, filtered, and concentrated in vacuo. Column chromatography (EtOAc : hexane = 1 : 10) of the residue gave a colorless oil (1.15 g, 2.95 mmol, 94%). The oil (1.02 g, 2.6 mmol) was dissolved in ether, then stirred with $MgBr_2$ (1.48 g, 7.8 mmol) at room temperature for 3 hours. The reaction mixture was quenched by water, diluted with ether, washed, dried, filtered, and concentrated in vacuo. Column chromatography (EtOAc : *n*-hexane = 1 : 6) of the residue gave **2** (518 mg, 1.69 mmol, 65%). ^{1}H-NMR (300MHz, $CDCl_3$) δ 7.11-7.26 (m, 5H), 5.63-5.80 (m, 2H), 4.10-4.13 (m, 1H), 4.11(d, 2H, J = 4.65 Hz), 2.53-2.71 (m, 2H), 1.75-1.87 (m, 2H), 0.88 (s, 9H), 0.28 (s, 6H).

Ethyl (2*E*,4*E*)-6-{[*tert*-butyl(dimethyl)silyl]oxy}-8-phenyl-2,4-octadienoate (3). To a solution of 126 (133 mg, 0.434 mmol) in $CHCl_3$ (2 mL) was added MnO_2 (888 mg, 8.68 mmol). The reaction mixture was stirred at room temperature for 5 hours, then filtrated, and concentrated in vacuo. The resultant aldehyde was dissolved in methylene chloride (2 mL), and reacted with (carbethoxymethylene) triphenylphosphorane (239 mg, 0.651 mmol) at room temperature for 2 hours. After concentration, column chromatography (EtOAc : *n*-hexane = 1 : 15) of the residue gave ester **3** (113 mg, 0.304 mmol, 70%). ^{1}H-NMR

(300 MHz, $CDCl_3$) δ 7.09-7.26 (m, 7H), 6.02-6.31 (m, 1H), 5.57-5.98 (m, 1H), 4.09-4.25 (m, 3H), 2.55-2.63 (m, 2H), 1.76-1.81 (m, 2H), 1.23 (t, 3H, *J* = 7.05 Hz), 0.857 (s, 9H), 0.089 (s, 3H), 0.048 (s, 3H).

Ethyl (2*E*,4*E*)-6-(hydroxyimino)-8-phenyl-2,4-octadienoate (4). To a solution of **3** (271 mg, 0.723 mmol) in methanol (2 mL) was added TsOH (catalytic amount). The reaction mixture was stirred at room temperature for 2 hours, and then neutralized by aq. $NaHCO_3$. The organic layer was diluted with EtOAc, washed, dried, and concentrated in vacuo. Column chromatography of the residue gave alcohol (179 mg, 0.687 mmol, 95%). To a solution of the alcohol (17 mg, 0.651 mmol) in methylene chloride (1 mL) was added 4 Å MS and PDC (37 mg, 0.976 mmol). The mixture was stirred at room temperature for 1 hour, and then diluted with excessive ether, filtrated, and concentrated in vacuo. Column chromatography (EtOAc : *n*-hexane = 1 : 5) of the residue gave a ketone intermediate (14.4 mg, 0.056 mmol, 86%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.08-7.26 (m, 7H), 6.35 (d, 1H, *J* = 15.1 Hz), 6.35 (d, 1H, *J* = 15.1 Hz), 6.14 (d, 1H, *J* = 14.9 Hz), 4.16 (q, 2H, *J* = 7.05 Hz), 2.82-2.89 (m, 4H), 1.24 (t, 3H, *J* = 7.05 Hz).

To a solution of the ketone (12 mg, 0.0463 mmol) in methanol were added NaOAc (18 mg, 0.185 mmol) and NH_2OH·HCl (12 mg, 0.0926 mmol). The mixture was stirred at room temperature for 2 hours, and then diluted with EtOAc, washed with sat. NH_4Cl, dried, filtrated and concentrated in vacuo. Column chromatography (EtOAc : *n*-hexane = 1 : 2) of the residue gave oxime **4** (12 mg, 0.0440 mmol, 95%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.11-7.36 (m, 6H), 6.35-6.02 (m, 2H), 5.58-6.01 (m, 1H), 4.14 (q, 2H, *J* = 7.05 Hz), 2.63-2.85 (m, 4H), 1.24 (t, 3H, *J* = 7.05 Hz).

(2*E*,4*E*)-6-(Hydroxyimino)-8-phenyl-2,4-octadienoic acid (5). To a solution of **4** (7 mg, 0.0256 mmol) in THF-H_2O (1:1, 1 mL) was added LiOH·H_2O (3.2 mg, 0.0768 mmol). The mixture was stirred at room temperature for 4 hours, and then acidified with 1 N HCl, diluted with EtOAc, washed, dried, filtrated, and concentrated in vacuo. Column chromatography (EtOAc : *n*-hexane = 1 : 2 → CH_3OH : CH_2CH_2 = 1 : 10) of the residue gave **131** (6 mg, 0.0.0244 mmol, 95%) as a white solid. ^{1}H-NMR (300 MHz, CD_3OD) δ 7.12-7.45 (m, 6H), 6.43-6.66 (m, 2H), 5.87-6.03 (m, 1H), 2.65-2.86 (m, 4H).

6-(Hydroxyimino)-8-phenyloctadienoic acid (6). To a solution of **4** (8 mg, 0.0296 mmol) in absolute MeOH (1 mL) was added 10% Pd/C (catalytic amount). The mixture was stirred under H_2 at room temperature for 1 hour, and then diluted with ether, filtrated through celite pad, and concentrated in vacuo. The residue was dissolved in THF-H_2O (1:1, 1 mL) with LiOH·H_2O (4.30 mg, 0888 mmol). The mixture was stirred at room temperature for 4 hours, and then acidified with 1 N HCl, diluted with CH_2Cl_2, washed, dried, filtrated and concentrated in vacuo. Column chromatography of the residue (EtOAc : *n*-hexane = 1 : 2 → CH_3OH : CH_2CH_2 = 1 : 10) gave acid **6** (3 mg, 0.120 mmol, 42%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.10-7.24 (m, 3H), 2.71-2.81 (m, 2H), 2.55-2.68 (m, 2H), 2.28-2.45 (m, 4H), 1.97-2.05 (m, 2H), 1.54 (m, 2H).

5-Phenyl-pent-1-yn-3-ol (8). To a solution of oxalyl chloride (0.38 mL, 4.4 mmol) in methylene chloride (14 mL) was added dropwise DMSO (0.44 mL, 6.24 mmol) at −78 °C. After 20 min, a solution of 3-phenyl-1-propanol (500 mg, 3.67 mmol) in methylene chloride (4 mL) was added. After 30 min, triethylamine (1.5 mL, 10.65 mmol) was added dropwise at room temperature. The reaction mixture was stirred at room temperature and then quenched by aq. NH_4Cl, diluted, washed, dried and concentrated in vacuo. Column chromatography (EtOAc : Hexane = 1 : 3) of the residue gave aldehyde (490 mg, 3.65 mmol, 99%). To a solution of the aldehyde in ether (10 mL) was added dropwise ethynyl magnesium bromide (0.5 M solution in THF, 8.8 mL, 4.33 mmol) at 0 °C. The mixture was stirred at room temperature for 2 hours, and then quenched by aq. NH_4Cl, diluted with ether, washed, dried, and concentrated in vacuo. Column chromatography (EtOAc : Hexane = 1 : 4) of the residue gave alcohol **8** (410 mg, 70%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.19-7.33 (m, 5H), 4.38 (dt, 1H, *J* = 6.6 Hz), 2.82 (t, 2H, *J* = 8 Hz), 2.51 (d, 1H, *J* = 2.2 Hz), 2.01-2.09 (m, 2H).

3-(3-Hydroxy-5-phenyl-pent-1-ynyl)-benzoic acid methyl ester (9). To a mixture of alcohol **8** (200 mg, 1.25 mmol) and iodobenzoic acid methyl ester (327 mg, 1.25 mmol) in THF were added triethylamine (0.35 mL, 2.5 mmol), CuI (20 mg, 0.125 mmol), $Pd(PPh_3)_2Cl$ (44 mg, 0.063 mmol). The mixture was stirred at room temperature for 40 min, and then diluted with ether, filtrated through celite pad, and concentrated in vacuo. Column chromatography (EtOAc : *n*-Hexane = 1 : 6 → 1 : 4) of the residue gave **9** (360 mg, 98%). IR (KBr) 3444, 2950, 1729, 1436, 1299, 1044, 754; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.09 (t, 1H, *J* = 1.44 Hz), 7.97 (dt, 1H, *J* = 1.44, 7.8 Hz), 7.58 (dt, 1H, *J* = 1.47, 7.56 Hz), 7.38 (t, 1H, *J* = 7.8 Hz), 7.16-7.31 (m, 5H), 4.59 (t, 1H, *J* = 6.6 Hz), 3.91 (s, 3H), 2.85 (t, 2H, *J* = 7.8 Hz), 2.03-2.20 (m, 2H), 1.99 (d, 1H, *J* = 4.89 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) 166.34, 141.11, 135.76, 132.75, 130.29, 029.35, 128.46, 128.41, 125.98, 122.97, 90.80, 84.09, 62.05, 52.26, 39.14, 31.39.; LRMS (LC/MS) 294 (M).

Methyl 3-(3-oxo-5-phenyl-pentyl)-benzoate (10). To a solution of **9** (250 mg, 0.35 mmol) in methanol (4 mL) was added 10% Pd/C (catalytic amount). The mixture was stirred under H_2 at room temperature for 2 hours, and then diluted with ether, filtrated through celite pad. Concentration in vacuo gave saturated ester (253 mg, 99%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.77-7.80 (m, 2H), 7.09-7.32 (m, 7H), 3.83 (s, 3H), 3.58 (m, 1H), 2.54-2.82 (m, 4H), 1.66-1.79 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$) 167.26, 142.46, 141.96, 133.10, 130.18, 129.46, 128.41, 128.38, 128.35, 127.12, 125.81, 70.48, 52.04, 39.20, 38.99, 32.00, 31.76, 20.99, 14.15.

To a solution of the saturated ester (17 mg) in methylene chloride (1 mL) was added 4 Å MS and PDC (37 mg, 0.976 mmol). The mixture was stirred at room temperature for 1 hour, and then diluted with excessive ether, filtrated, and concentrated in vacuo. Column chromatography (EtOAc : *n*-hexane = 1 : 5) of the residue gave **10** (86%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.77-7.80 (m, 2H), 7.06-7.32 (m, 7H), 3.83 (s, 3H), 2.74-2.88 (m, 4H), 2.62-2.68 (m, 4H).

General procedure for preparation of hydrozono compounds. To a solution of Semicarbazide HCl (1 eq.) in MeOH were added NaOAc (2 eq.) and a ketone (1 eq.) at 0 °C. The mixture was heated slowly to 70 °C and then concentrated in vacuo. The residue was dissolved in THF/H_2O (1:1) with LiOH H_2O (2 eq.). The mixture was stirred at room temperature for 1 hour

and then acidified with 1 N HCl to be pH 5, diluted with EtOAc, dried and concentrated in vacuo. Column chromatography (MeOH : CH_2Cl_2 = 1 : 20) was carried out.

3-{3-[2-(Aminocarbonyl)hydrazono]-5-phenylpentyl} benzenecarboxylicacid (11). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.05 & 7.78-7.83 (m, 2H), 7.08-7.33 (m, 7H), 3.85 & 3.83 (s, 3H), 2.66-2.83 (m, 4H), 2.37-2.54 (m, 4H).

3-{3-[2-(Aminocarbothioyl)hydrazono]-5-phenylpentyl}-benzenecarboxylicacid (12). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.84-7.88 (m, 2H), 7.28-7.36 (m, 2H), 7.07-7.23 (m, 5H), 2.81-2.91 (m, 4H), 2.64-2.70 (m, 4H).

3-(3-{2-[(4-Methylphenyl)sulfonyl]hydrazono}-5-phenyl pentyl)benzene carboxylic acid (13). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.65-8.02 (m, 4H), 7.16-7.26 (m, 7H), 6.96-7.04 (m, 2H), 2.66-2.87 (m, 4H), 2.40-2.46 (m, 7H).

(*E*)-1-[4-(Benzyloxy)3-methoxyphenyl]-5-phenyl-1-penten-3-one (15). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.10-7.42 (m, 12H), 6.94-6.99 (m, 2H), 6.80 (d, 1H, J = 8.3 Hz), 6.53 (d, 1H, J = 16.0 Hz), 5.12 (s, 2H), 3.84 (s, 2H), 2.92 (s, 4H).

1-(4-Hydroxy-3-methoxyphenyl)-5-phenyl-3-pentanone (16). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.06-7.22 (m, 5H), 6.74 (m, 1H), 6.55-6.58 (m, 2H), 3.78 (s, 3H), 2.72-2.83 (m, 4H), 2.58-2.66 (m, 4H).

2-[1-(4-Hydroxy-3-methoxyphenethyl)-3-phenylpropylidene]-1-hydrazinecarboxamide (17). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.70 & 7.65 (s, 1H), 7.07-7.24 (m, 5H), 6.76 (m, 1H), 6.55-6.58 (m, 2H), 5.41 (br s, 1H), 3.80 & 3.79 (s, 3H), 2.62-2.80 (m, 4H), 2.32-2.44 (m, 4H).

3-(3-Hydroxy-5-phenyl-pentyl)-benzoic acid (21). To a solution of **18** (100 mg, 0.335 mmol) in THF/H_2O (1:1, 3 mL) was added LiOH·H_2O (70 mg, 1.68 mmol). The mixture was stirred at 35 °C for 6 hours, and then acidified with 1 N HCl, diluted with EtOAc, washed, dried and concentrated in vacuo. Column chromatography (MeOH : CH_2Cl_2 = 1 : 20) gave **21** (76 mg, 80%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.86-7.88 (m, 2H), 7.10-7.38 (m, 7H), 3.57-3.65 (m, 1H), 2.56-2.85 (m, 4H), 1.71-1.82 (m, 4H).

3-(3-Phenethylthiocarbamoyloxy-5-phenyl-pentyl)-benzoic acid (24). To a suspension of 95% NaH (10 mg, 0.424 mmol) in THF was added a solution of **21** (30 mg, 0.106 mmol) in THF (2 mL) at 0 °C. After 10 min, $Ph(CH_2)_2NCS$ (50 μL, 0.306 mmol) was added. The reaction mixture was stirred at room temperature for 2 hours, and then quenched by aq. NH_4Cl, diluted with EtOAc, washed, dried and concentrated in vacuo. Column chromatography (EtOAc : Hexane = 1 : 2 → MeOH : CH_2Cl_2 = 1 : 20) gave **24** (33 mg, 70%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.93 (d, 2H, J = 8.1 Hz), 7.09-7.23 (m, 11H), 6.58 & 6.02 (t, 1H), 3.66-3.78 (m, 2H), 2.57-2.89 (m, 6H), 1.78-2.06 (m, 4H).

3-(3-Phenethylcarbamoyloxy-5-phenyl-pentyl)-benzoic acid (25). To a solution of 18 (21 mg, 0.0703 mmol) in benzene was added $Ph(CH_2)_2NCO$ (40 μL, 0.0.281 mmol). The mixture was refluxed for 5 hours, and then concentrated in vacou. After column chromatography (EtOAc : Hexane = 1 : 2 → MeOH : CH_2Cl_2 = 1 : 20) of the residue, the resulting ester was hydrolyzed by LiOH H_2O same as the above described. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.85-7.87 (m, 2H), 7.08-7.34 (m, 12H), 4.81 (m, 1H), 4.59 (m, 1H), 3.29-3.41 (m, 2H), 2.69-2.79 (m, 2H), 2.49-2.64 (m, 4H), 1.80-1.83 (m, 4H).

Methyl 4-(3-hydroxy-5-phenylpentyl)benzenecarboxylate (19). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.93 (dd, 2H, J = 1.6, 6.6 Hz), 7.14-7.32 (m, 7H), 3.88 (s, 3H), 3.63 (m, 1H), 2.59-2.87 (m, 4H), 1.71-1.82 (m, 4H).

4-(3-Hydroxy-5-phenylpentyl)benzenecarboxylic acid (22). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.95 (d, 2H, J = 8.2 Hz), 7.10-7.23 (m, 7H), 3.60 (m, 1H), 2.55-2.85 (m, 4H), 1.70-1.78 (m, 4H).

4-(3-{[(Phenethylamino)carbothioyl]oxy}-5-phenylpentyl) benzenecarboxylic acid (26). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.93 (d, 2H, J = 8.0 Hz), 7.08-7.24 (m, 12H), 6.57 & 6.02 (m, 1H), 5.55 (m, 1H), 3.74 (m, 1H), 3.38 (m, 2H), 2.26-2.89 (m, 5H), 1.78-2.05 (m, 4H).

Methyl 2-[3-(3-hydroxy-5-phenylpentyl)phenyl]acetate (20). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.00-7.22 (m, 9H), 3.60 (s, 3H), 3.55 (m, 1H), 3.51 (s, 2H), 2.52-2.75 (m, 4H), 1.64-1.80 (m, 4H).

2-[3-(3-Hydroxy-5-phenylpentyl)phenyl]acetic acid (23). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.02-7.23 (m, 9H), 3.60 (m, 1H), 3.50 (s, 2H), 2.48-2.76 (m, 4H), 1.62-1.78 (m, 4H).

2-[3-(3-{[(Phenethylamino)carbothioyl]oxy}-5-phenylpentyl)phenyl]acetic acid (27). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.00-7.25 (m, 9H), 6.74 & 6.05 (br t, 1H, J = 5.8 Hz), 5.52 (m, 1H), 3.69 & 3.33 (q, 2H, J = 6.6 Hz), 3.52 (s, 2H), 2.84 & 2.72 (t, 2H, J = 7.0 Hz), 2.54-2.59 (m, 4H), 1.84-2.00 (m, 4H).

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