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Design and synthesis of silicon-containing fatty acid amide derivatives as novel peroxisome proliferator-activated receptor (PPAR) agonists

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ABSTRACT

We recently reported that diphenylsilane structure can function as a *cis*-stilbene mimetic. Here, we investigate whether silyl functionality can also serve as a mimetic of aliphatic *cis*-olefin. We designed and synthesized various silyl derivatives of oleoylethanolamide (OEA: **8**), an endogenous *cis*-olefin-containing PPARα agonist, and evaluated their PPARα/δ/γ agonistic activity. We found that diethylsilyl derivative **20** exhibited PPARα/δ agonistic activity, and we also obtained a PPARδ-selective agonist, **32**. Our results suggest that incorporation of silyl functionality is a useful option for structural development of biologically active compounds.

In the field of medicinal chemistry, introduction of a silicon atom in place of a carbon atom (sila-substitution) is employed as a strategy to alter the activity, selectivity and pharmacokinetics of compounds, due to the different properties of carbon and silicon, such as atomic size, electronegativity and hydrophobicity.[1–4] In recent years, various silicon-containing bioactive compounds have been reported, such as BNP1350 (**1**), sila-haloperidol (**2**) and TAC101 (**3**) (Fig. 1),[5–7] and clinical studies have supported the idea that sila-substitution can improve the selectivity, potency and pharmacokinetics of drug candidates. We previously designed and synthesized diphenylsilane derivatives of the anticancer drug combretastatin A-4 (CA-4) (**4**) by replacing the *cis*-olefin with a silyl functionality (Fig. 2).[8] We found that silicon-containing **5** exhibited potent antitumor activity, together with much greater stability in aqueous solution compared with CA-4 (**4**), which isomerized to inactive form under the same conditions. Based on these findings, we planned to further investigate the utility of silyl functionality as an aliphatic *cis*-olefin mimetic. There are myriad *cis*-olefin-containing fatty acids and their derivatives in biological systems, and many of them function as biological factors or signal-transducing molecules. For instance, some endogenous fatty acids, such as leukotriene B4 (**6**), arachidonic acid (**7**), and an acid amide oleoylethanolamide (OEA: **8**), function as peroxisome proliferator-activated receptor α (PPARα) agonists (Fig. 3).[9–11]

PPAR is a member of the nuclear receptor superfamily of ligand-dependent transcriptional factors.[12–14] There are three subtypes of PPAR, namely PPARα, PPARγ and PPARδ (β). PPARα is activated by the endogenous agonists, and regulates various physiological processes, including lipid metabolism. Therefore, PPARα is an attractive target of drugs for metabolic disorders, and synthetic PPARα agonists such as fibrates have been used for treatment of hyperlipidemia.[15] Though the endogenous PPARα agonists can be considered as lead compounds for novel PPARα modulators, the chemical instability of the *cis*-olefinic bond is an undesirable feature for structural development. In this Letter, we described the design and synthesis of novel PPAR-modulating fatty acid derivatives containing a silyl functionality as a *cis*-olefin mimetic. In order to develop suitable derivatives, we focused on OEA (**8**). It was previously reported that administration of OEA caused a reduction of food intake in mice.[11] In addition to its agonistic activity toward PPARα, OEA promotes the secretion of GLP-1, a gut hormone controlling insulin secretion, insulin sensitivity, and appetite, by stimulating G-protein coupled receptor GPR119.[16] Furthermore, it was suggested that OEA activates TRPV1 (transient receptor potential cation channel subfamily V member 1), also

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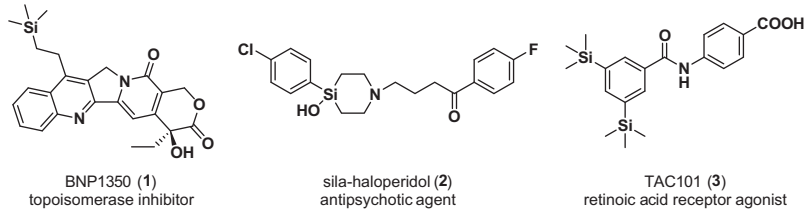


Figure 1. Examples of silicon-containing bioactive compounds.

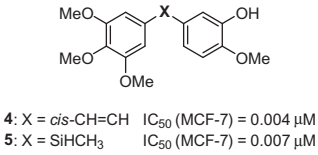


Figure 2. Structure of tubulin polymerization inhibitors. Silyl derivative **5**, in which *cis*-olefin is replaced by a silyl group, exhibited potent activity.

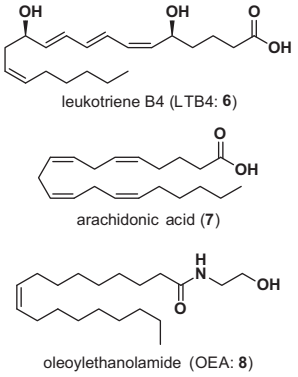


Figure 3. Structures of endogenous PPARα agonists containing a *cis*-olefin moiety.

known as capsaicin receptor.[17] Thus, the physiological roles of this endogenous fatty acid amide are interesting.

Initially, in order to examine the structural similarity of aliphatic *cis*-olefin and silyl group, we conducted molecular orbital calculations for *cis*-olefin (*Z*)-3-hexene (**9**), diethyldimethylsilane (**10**) and 3,3-dimethylpentane (**11**). As shown in Figure 4, the distance (*d*) in the silyl derivative **10** is similar to that of the *cis*-olefin **9**. On the other hand, the distance in the corresponding alkane **11** is significantly shorter than those in **9** and **10**. This result suggests that replacement of the *cis*-olefin of OEA with a silyl functionality would be a reasonable conversion, and therefore we designed silyl derivatives of OEA (Fig. 4).

Synthesis of silyl derivatives **19**–**22** bearing *n*-octyl and *n*-octanoyl moieties is illustrated in Scheme 1. Alkyne zipper reaction of 3-octyn-1-ol (**12**) gave the terminal alkyne **13**, then the hydroxyl group of **13** was protected with *p*-methoxybenzyl (PMB) group to afford compound **14**. Disubstitution of dichlorosilanes **15a**–**d** using compound **14** and 1-octyne in the presence of *n*-butyllithium afforded tetraalkylsilanes **16a**–**d**, respectively. Reduction of alkyne moieties and removal of the PMB group of **16a**–**d** by catalytic hydrogenation gave alcohol derivatives **17a**–**d**, respectively. The isolate yield of **17a** and **17d** was low because the alkylating step gave multiple products and there was difficulty in purification. Oxidation of the alcohols **17a**–**d** using Dess–Martin periodinane gave carboxylic acid derivatives **18a**–**d**, respectively. Finally, condensation of the carboxylic acids **18a**–**d** with ethanolamine via acid anhydride gave the designed OEA derivatives **19**–**22**, respectively (Scheme 1). Synthesis of silyl derivatives **28** and **32** is

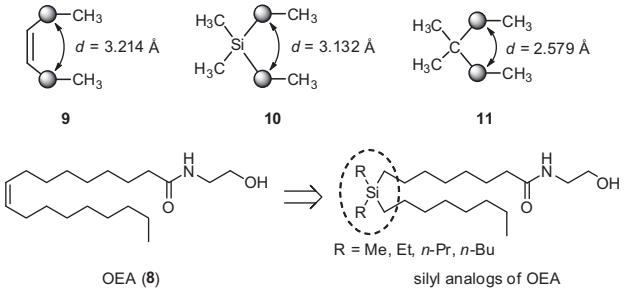
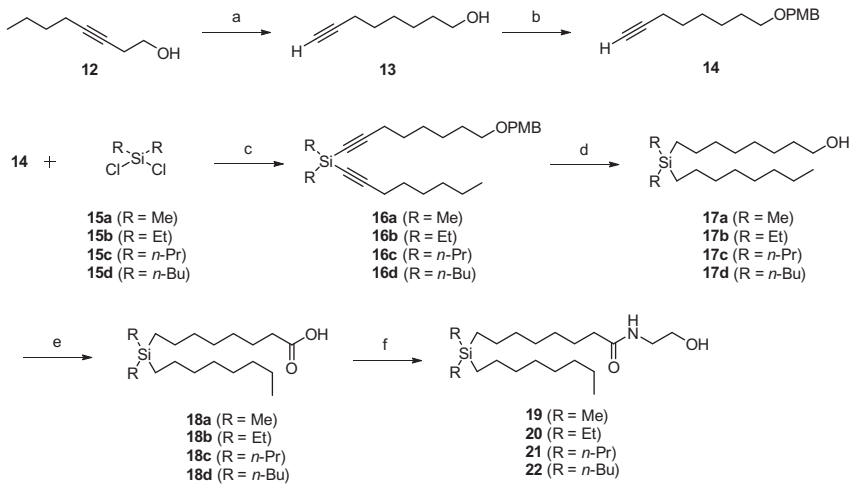


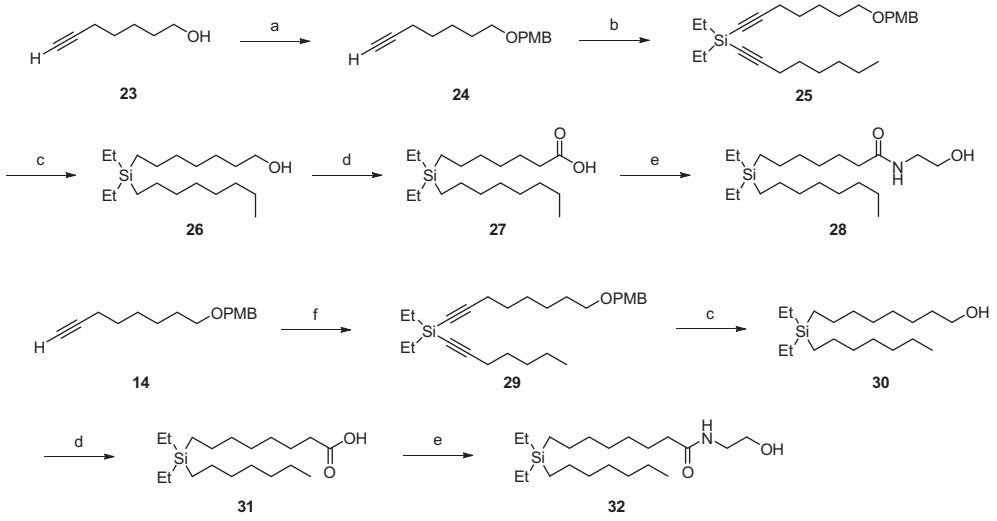
Figure 4. Design rationale of silicon-containing fatty acid derivatives. Calculated distances (*d*) between the two indicated carbon atoms in compounds **9**, **10** and **11** (top) are shown. Structures of the designed compounds (bottom).

summarized in Scheme 2. The hydroxyl group of **23** was protected with a PMB group to give compound **24**. Disubstitution of dichlorodiethylsilane (**15b**) using compound **24** and 1-octyne, or compound **14** and 1-heptyne afforded tetraalkylsilanes **25** and **29**, respectively. Catalytic hydrogenation of **25** and **29** gave alcohol derivatives **26** and **30**, respectively. Oxidation of alcohol **26** and **31** gave carboxylic acid derivatives **27** and **31**, and finally, condensation of the carboxylic acids **27** and **32** with ethanolamine gave compounds **28** and **32**, respectively (Scheme 2). *N*-(2-Hydroxyethyl) stearamide (**34**), a saturated fatty acid amide derivative of OEA, was also prepared from stearic acid (**33**) (Scheme 3).

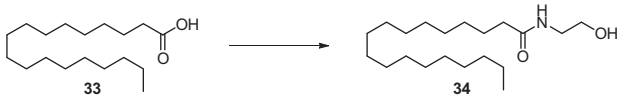
The PPAR-agonistic activities of the synthesized silyl derivatives of OEA were evaluated by means of PPAR reporter gene assays. Figure 5A shows the PPARα-agonistic activity of compounds **19**–**22** bearing different dialkylsilyl functionalities and saturated fatty acid amide **34**. As reported, OEA exhibited agonistic activity toward PPARα. On the other hand, compound **34** did not exhibit significant PPARα-agonistic activity. This result indicated that *cis*-olefin is a key substructure for PPARα-agonistic activity. Regarding silyl derivatives, compounds **20** bearing a diethylsilyl group showed moderate PPARα-agonistic activity. Dimethylsilyl derivative **19** and di-*n*-propyl derivative **21** also exhibited PPARα-agonistic activity somewhat more potent than that of saturated fatty acid amide **34**. Compound **22** bearing a di-*n*-butylsilyl moiety showed no activity. Figure 5B shows the agonistic activity of compounds **18**–**22** toward PPARδ. Diethylsilyl derivative **20** exhibited PPARδ-agonistic activity with similar potency to that of OEA. Compounds **19** and **21** also exhibited PPARδ-agonistic activity, whereas compound **22** exhibited no activity. No agonistic activity was observed toward PPARγ (data not shown). These results suggested that dialkylsilyl substitution of the *cis*-olefin of OEA at least partially retains the biological activity. Alkyl groups on the silicon atom considerably affected the activity (Fig. 5). Next, we investigated the structure–activity relationship of diethylsilyl derivatives. Compound **28** bearing heptanoyl structure and compound **32** bearing an *n*-heptyl group exhibited quite low activity toward PPARα. Modification of the chain length resulted in a decrease of PPARα-agonistic activity (Fig. 6A). Modification of the chain length also affected the agonistic activity toward



Scheme 1. Synthesis of compounds **19**–**22**. Reagents and conditions: (a) NaH, ethylenediamine, 65 °C, 72%; (b) CSA, 4-methoxybenzyl trichloroacetimidate, CH_2Cl_2, rt, 34%; (c) 1-octyne, *n*-BuLi, THF, −78 °C to rt, 44% or crude; (d) H_2, Pd/C, ethyl acetate, rt, 42% or 1.4–44% (2 steps); (e) DMP, CH_2Cl_2, rt, 56–70% or crude; (f) triethylamine, methyl chloroformate, ethanolamine, CH_2Cl_2, rt, 14–40% or 34% (2 steps).



Scheme 2. Synthesis of compounds **28** and **32**. Reagents and conditions: (a) CSA, 4-methoxybenzyl trichloroacetimidate, CH_2Cl_2, rt, 70%; (b) 1-octyne, *n*-BuLi, dichlorodiethylsilane, THF, −78 °C to rt, crude; (c) H_2, Pd/C, ethyl acetate, rt, 14–15% (2 steps); (d) DMP, CH_2Cl_2, rt, 19–50%; (e) triethylamine, methyl chloroformate, ethanolamine, CH_2Cl_2, rt, 40–68%; (f) 1-heptyne, *n*-BuLi, dichlorodiethylsilane, THF, −78 °C to rt, crude.

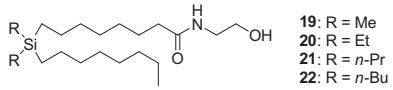


Scheme 3. Synthesis of compound **34**. Reagents and conditions: triethylamine, methyl chloroformate, ethanolamine, CH_2Cl_2, rt, 74%.

PPARδ. Interestingly, *n*-heptylsilyl derivative **32**, which had quite low PPARα-agonistic activity, exhibited potent PPARδ-agonistic activity, indicating that modification of the silyl moiety caused selectivity switching (Fig. 6B). These compounds showed no agonistic activity toward PPARγ (data not shown).

We performed docking studies of diethyl-*n*-heptylsilyl derivative **32** and diethyl-*n*-octylsilyl derivative **20** with the reported crystal structure of PPARδ (PDB ID: 3SP9),[18] using the AutoDock 4.2 docking program.[19,20] Figure 7A shows the docking form of compound **32** in the PPARδ ligand-binding domain (LBD). In the docking structure, the fatty acid chain of compound **32** occupies the hydrophobic pocket of the LBD, and ethanolamide moiety shows a polar interaction. It was also suggested that the diethylsilyl group improves hydrophobic interaction with the LBD. On the other hand, it was suggested that the diethylsilyl group of compound **20** could sterically interfere with the receptor surface (Fig. 7B). This might be a possible reason why compound **32** exhibited potent PPARδ agonistic activity in comparison with compound **20**.

In conclusion, based on the hypothesis that an alkylsilyl group can function as a mimic of aliphatic *cis*-olefin, we designed and synthesized a series of silicon-containing fatty acid amide derivatives. In PPAR reporter gene assays, several compounds showed PPARα- and/or PPARδ-agonistic activity. Among them, diethyl-*n*-octyl derivative **20** exhibited PPARα/δ-dual agonistic activity, and diethyl-*n*-heptyl derivative **32** exhibited potent and selective PPARδ-agonistic activity. Thus, introduction of a dialkylsilyl group in place of aliphatic *cis*-olefin is an effective strategy to modify biological activity and selectivity. In addition, tetraalkylsilyl structure is more stable to isomerization and oxidation than *cis*-olefin. These



19: R = Me
20: R = Et
21: R = *n*-Pr
22: R = *n*-Bu

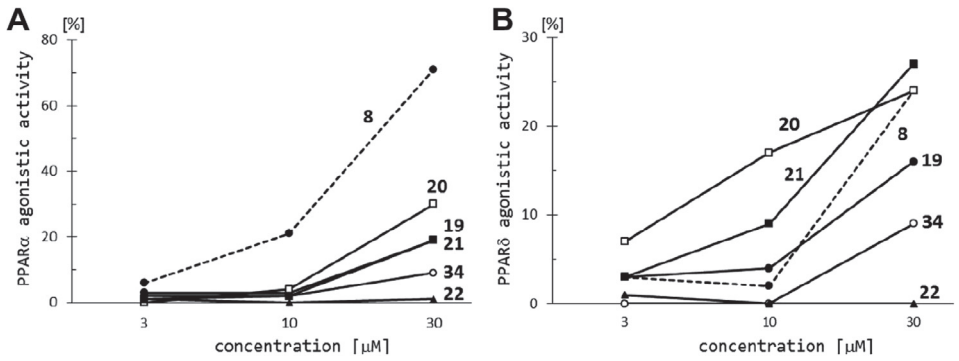
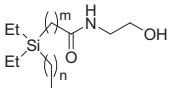


Figure 5. Biological evaluation of OEA (**8**) and compounds **19**–**22** and **34**. (A) Agonistic activity toward PPARα. Activities are indicated as percent of maximum activity of GW7647. (B) Agonistic activity toward PPARδ. Activities are indicated as percent of maximum activity of GW501516.



20: m = 7, n = 7
28: m = 6, n = 7
32: m = 7, n = 6

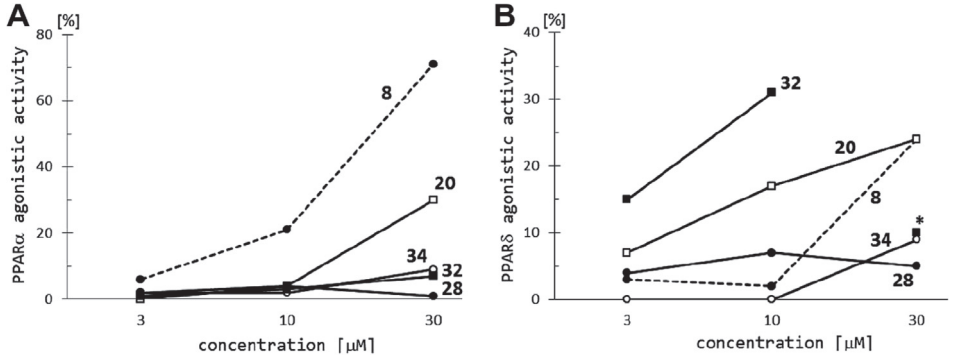


Figure 6. Biological evaluation of OEA (**8**) and compounds **20**, **28**, **32** and **34**. (A) Agonistic activity toward PPARα. Activities are indicated as percent of maximum activity of GW7647. (B) Agonistic activity toward PPARδ. Activities are indicated as percent of maximum activity of GW501516. *Compound **32** exhibited cytotoxicity at 30 μM.

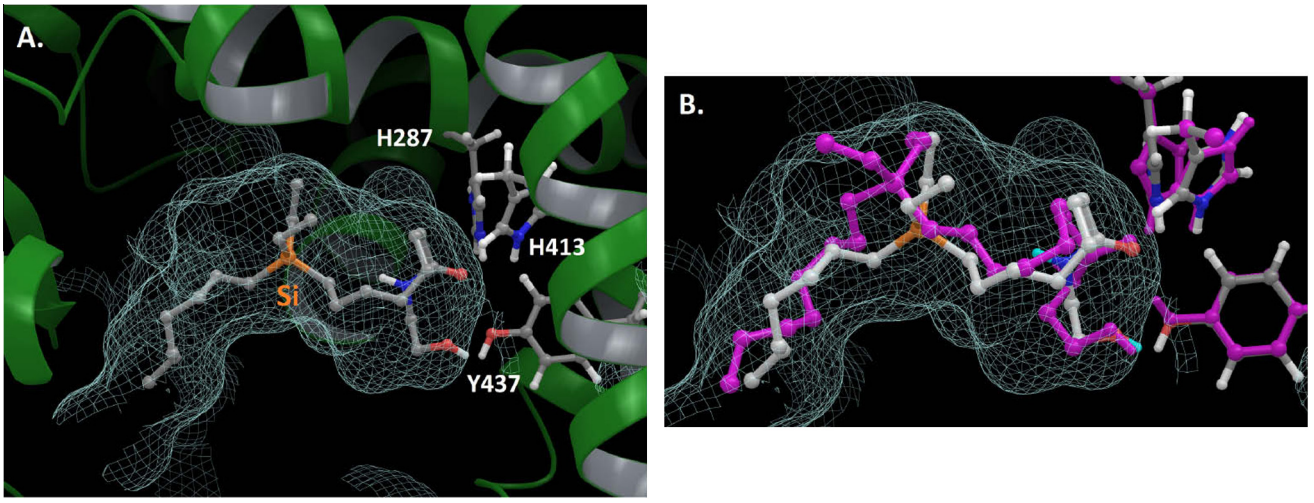


Figure 7. (A) Binding model of compound **32** at the LBD of PPARδ. The protein surface is indicated by light blue mesh. (B) Superimposition of binding models of compound **20** (magenta) and compound **32** (atom color) at the PPARδ-LBD.

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results indicate that incorporation of silyl functionality is a flexible option for structural development of biologically active compounds.

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Supplementary data

Supplementary data (analytical data and experimental procedures for synthetic preparation, biological evaluation and computational studies) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.05.045.

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