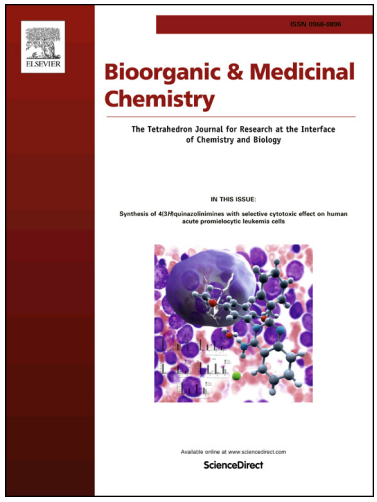

Discovery of Novel and Potent Stearoyl Coenzyme A Desaturase 1 (SCD1) Inhibitors as Anticancer Agents

Keisuke Imamura, Naoki Tomita, Youichi Kawakita, Yoshiteru Ito, Kouji Ono, Noriyuki Nii, Tohru Miyazaki, Kazuko Yonemori, Michiko Tawada, Hiroyuki Sumi, Yoshihiko Satoh, Yukiko Yamamoto, Ikuo Miyahisa, Masako Sasaki, Yoshinori Satomi, Megumi Hirayama, Ryuichi Nishigaki, Hironobu Maezaki

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Title

Discovery of Novel and Potent Stearoyl Coenzyme A Desaturase 1 (SCD1) Inhibitors as Anticancer Agents

Keisuke Imamura [a], Naoki Tomita [b], Youichi Kawakita [a], Yoshiteru Ito [a], Kouji Ono [a], Noriyuki Nii [a], Tohru Miyazaki [a], Kazuko Yonemori [a], Michiko Tawada [a], Hiroyuki Sumi [a], Yoshihiko Satoh [a], Yukiko Yamamoto [a], Ikuo Miyahisa [a], Masako Sasaki [a], Yoshinori Satomi [a], Megumi Hirayama [a], Ryuichi Nishigaki [a], Hironobu Maezaki [a]

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Abstract

A lead compound **A** was identified previously as an stearoyl coenzyme A desaturase (SCD) inhibitor during research on potential treatments for obesity. This compound showed high SCD1 binding affinity, but a poor pharmacokinetic (PK) profile and limited chemical accessibility, making it suboptimal for use in anticancer research. To identify potent SCD1 inhibitors with more promising PK profiles, we newly designed a series of 'non-spiro' 4, 4-disubstituted piperidine derivatives based on molecular modeling studies. As a result, we discovered compound **1a**, which retained moderate SCD1 binding affinity. Optimization around **1a** was accelerated by analyzing Hansch–Fujita and Hammett constants to obtain 4-phenyl-4-(trifluoromethyl)piperidine derivative **1n**. Fine-tuning of the azole moiety of **1n** led to compound **1o** (**T-3764518**), which retained nanomolar affinity and exhibited an excellent PK profile. Reflecting the good potency and PK profile, orally administrated compound **1o** showed significant pharmacodynamic (PD) marker reduction (at 0.3 mg/kg, bid) in HCT116 mouse xenograft model and tumor growth suppression (at 1 mg/kg, bid) in 786-O mouse xenograft model.

In conclusion, we identified a new series of SCD1 inhibitors, represented by compound **1o**, which represents a promising new chemical tool suitable for the study of SCD1 biology as well as the potential development of novel anticancer therapies.

Keywords

Stearoyl Coenzyme A desaturase 1, SCD1, 4, 4-disubstituted piperidine, cancer, antitumor efficacy, **T-3764518**

Abbreviations

Ac: acetyl, ADME: absorption, distribution, metabolism, and excretion, aq.: aqueous, AUC: total area under the blood concentration–time curve, bid: bis in die (twice per day), Boc: (*tert*-butoxy)carbonyl, Bu: butyl, Bz: benzoyl, cLogP: calculate logarithm of the octanol-water partition coefficient, Cl_{total}: total body clearance, CPME: cyclopentyl methyl ether, DI: desaturation index, DIPEA: *N,N*-diisopropylethylamine, DMA: *N,N*-dimethylacetamide, DMF: *N,N*-dimethylformamide, DMSO: dimethyl sulfoxide, Et: ethyl, ER: endoplasmic reticulum, Fu: unbound fraction, GI: growth inhibition, HBA: hydrogen bond acceptor, HBD: hydrogen bond donor, IPA: 2-propanol, IPE: diisopropyl ether, LMS: liver microsomal stability, *m*: *meta*, Me: methyl, MRT: mean residence time, MW: molecular weight, *o*: *ortho*, *p*: *para*, PC: phosphatidylcholine, PD: pharmacodynamic, Pd/C: palladium on carbon, PK: pharmacokinetic, PMB: *p*-methoxybenzyl, Pr: propyl, po: per os (oral administration), quant.: quantitative yield, rt: room temperature, SAR: structure-activity relationship, sat.: saturated aqueous, SCD: stearoyl coenzyme A desaturase, TEA: triethylamine, *tert*: tertiary, TFA: trifluoroacetic acid, THF: tetrahydrofuran, Ts: tosyl.

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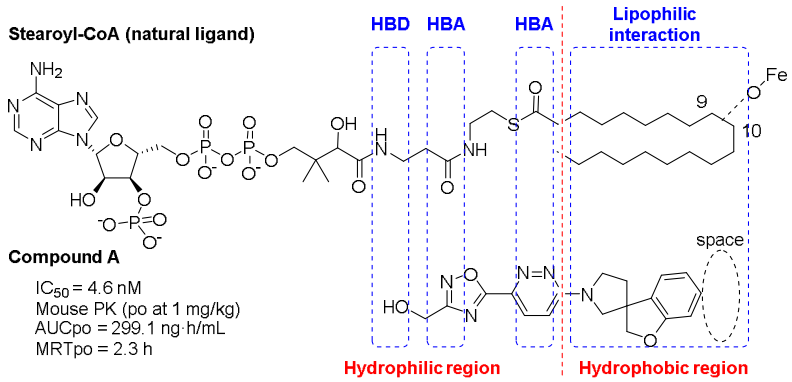
Introduction

Stearoyl coenzyme A desaturase 1 (SCD1) is an iron-containing endoplasmic reticulum (ER) enzyme, which controls the rate-limiting step in the synthesis of unsaturated fatty acids from saturated fatty acids.[1] Oleic and palmitoleic acids, which are the principal products of SCD1, are important components in lipid synthesis such as in the production of phospholipids, triglycerides, cholesterol esters, wax esters, and diacylglycerol.[1] Hence, SCD1 is responsible for a wide range of biological effects related to energy storage and signaling,[2a] as well as human physiology,[2b] and is regarded as a promising drug target for the treatment of lifestyle-related diseases such as obesity and metabolic disease.[3] Recently, it has been reported that SCD1 expression and activity are closely related to cancer pathogenesis and tumor malignancy,[4] and furthermore, it is believed that tumor cells obtain most of their required fatty acid from *de novo* synthesis.[5] Thus, inhibition of SCD1 function would be expected to induce an imbalance in fatty acid composition among the intracellular lipids, leading to cell death via lipotoxicity and ER stress responses in certain types of cancer.[6]

Spirocycle-based SCD inhibitor **A** was originally identified by Takeda for the treatment of metabolic disease.[7] This compound shows potent binding affinity (IC_{50} = 6.5 nM) to SCD1 and has promising druglike properties, such as low molecular weight (MW = 351.4), low lipophilicity (cLogP value = 0.38) and structural rigidity (number of rotatable bond = 3). Based on a structural similarity of compound **A** with stearoyl-CoA, the natural ligand of SCD1, and the reported binding mode of this ligand to SCD1,[8] we show an overlay model of their structures (Figure 1). According to this view, we hypothesized that these ligands could adopt a similar binding mode to each other and occupy the catalytic center of the enzyme. This model suggests the existence of an unoccupied space around the terminal fused phenyl ring of **A** and thus prompted new designs for increasing potency by filling this space. Compound **A** exhibited moderate oral exposure (AUC_{po} = 299.1 ng/mL) and duration (MRT_{po} = 2.3 h) in mouse cassette dosing (1 mg/kg po). However, we still sought to further increase exposure ad in vivo duration. In addition, the amenability of these compounds for chemical modification was considered suboptimal. Therefore, we sought to develop a non-spirocyclic series for use as a new lead series. We thus initiated synthetic studies aimed at the discovery of potent and orally available SCD1 inhibitors with long duration of PK, with the ultimate goal of using these compounds for anticancer therapy.

Figure 1. Structural similarity between stearoyl-CoA and compound **A**, and proposed key interactions

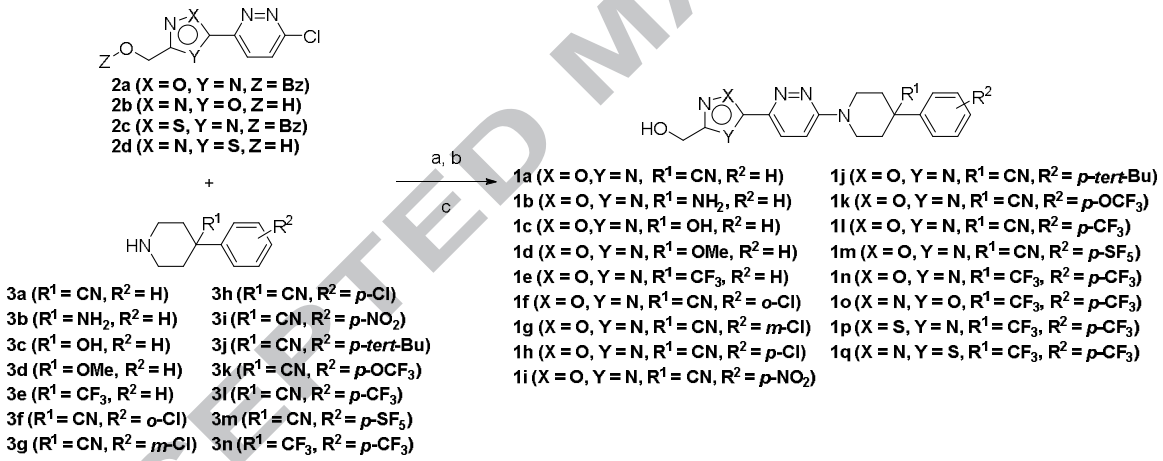


Chemistry

The compounds in this work were generally synthesized according to the route illustrated in Scheme 1. The preparation of chloropyridazines **2** and piperidines **3** are described in Schemes 2–4.

Compounds **1** were synthesized by nucleophilic substitution of chloropyridazines **2** with piperidines **3** directly or followed by deprotection of the *O*-Bz group by an alkaline hydrolysis.

Scheme 1. General method for compound synthesis in this report[a]

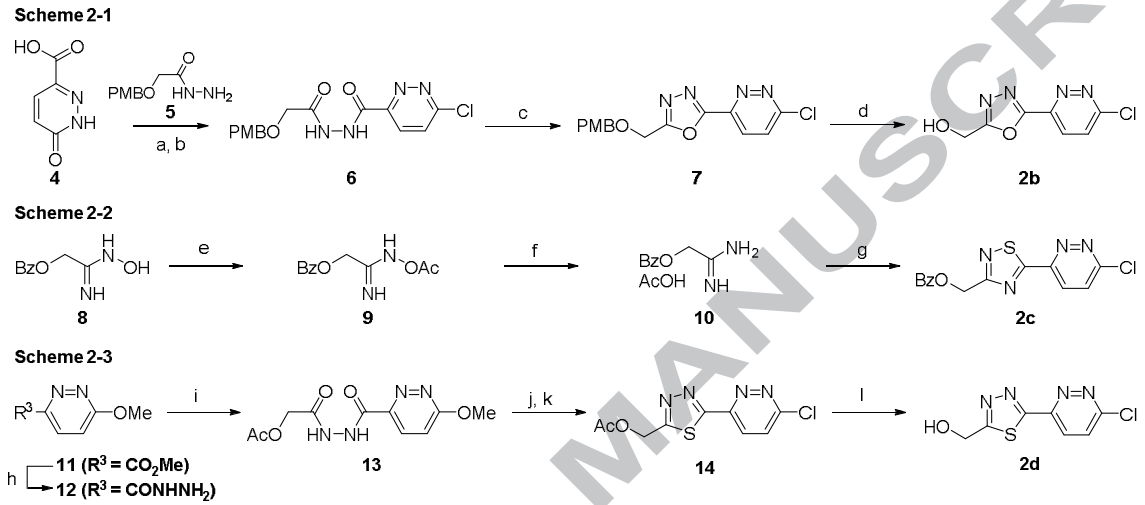


[a]Reagents and conditions: (a) **2a** or **2c**, **3**, DIPEA, IPA or DMA; (b) aq. NaOH, THF, MeOH, rt, 58–95% (2 steps from **2a** or **2c**); (c) **2b** or **2d**, **3n**, DIPEA, IPA, 150 °C, 1 h, microwave irradiation, 70–72%.

Chloropyridazine **2a** was prepared by the reported procedure,[7] and the synthesis of 1,3,4-oxadiazole intermediate **2b** is presented in Scheme 2-1. Condensation of pyridazine-4-one carboxylic acid **4** and *O*-PMB-protected hydrazide **5**[9] was performed via acyl chlorination of **4** to give diacyl hydrazide **6**, which was cyclized in the presence of *p*-TsCl and trimethylamine hydrochloride[10] to afford *O*-PMB-1,3,4-oxadiazole **7**. The PMB group was removed by treatment with TFA to give the desired intermediate **2b**. Synthesis of 1,2,4-thiadiazole intermediate **2c** is illustrated in Scheme 2-2. *O*-Bz-hydroxyamidine **8**[7] was predominantly acylated to **9**, which was reduced to amidine **10**. Construction of the 1,2,4-thiadiazole ring was carried out by

a modified method of the reported procedure using thionyl chloride, 3-chloro-6-methylpyridazine and **10** to give **2c**.[7] The synthesis of intermediate **2d** is illustrated in Scheme 2-3. Commercially available methyl ester **11** was converted to hydrazide **12** in the presence of hydrazine hydrate, which was acylated using acyl chloride to give diacyl hydrazide **13**. Hydrazide **13** was cyclized with phosphorus pentasulfide, followed by chlorination to give *O*-Ac-2-hydroxymethyl-1,3,4-thiadiazole **14**. The acetyl group was removed by alkaline hydrolysis to afford the desired intermediate **2d**.

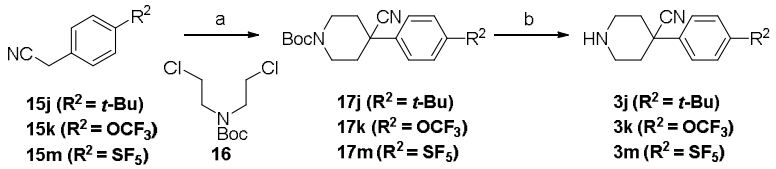
Scheme 2. Synthesis of key intermediate **2**[a]



[a]Reagents and conditions: (a) thionyl chloride, DMF, 80 °C, 1 h; (b) **5**, pyridine, THF, 0 °C to rt, overnight, 48% (2 steps from **4**); (c) TEA•HCl, TEA, *p*-TsCl, MeCN, 50 °C, 1 h, 90%; (d) TFA, anisole, rt, 1 h, 87%; (e) acetic anhydride, acetic acid, 0 °C to rt, overnight, 90%; (f) Pd/C, H_2 (balloon), EtOH, rt, overnight, 99%; (g) thionyl chloride, 3-chloro-6-methylpyridazine, reflux, overnight; then **10**, aq. NaOH, THF, −5 °C to 0 °C, 1 h, 71%; (h) hydrazine hydrate, MeOH, rt, overnight, 93%; (i) acetoxyacetyl chloride, dichloromethane, H_2O, 0 °C to rt, 1.5 h, 78%; (j) phosphorus pentasulfide, THF, 50 °C, overnight, 34%; (k) phosphoryl chloride, MeCN, 70 °C, 2 h, 34%; (l) aq. LiOH, THF, 0 °C to rt, 2 h, 85%.

Piperidines **3b**, **3e**, **3f**, **3g**, **3i** and **3l** were prepared by the reported procedures.[11] Synthesis of 4-cyano-4-phenylpiperidine intermediates **3j**, **3k** and **3m** are described in Scheme 3. The piperidine ring was cyclized via nucleophilic substitution of commercially available benzyl cyanides **15** with bis(2-chloroethyl)amine derivative **16** in the presence of sodium hydride. *N*-Boc deprotection under acidic conditions gave the desired **3** in moderate to high yield.

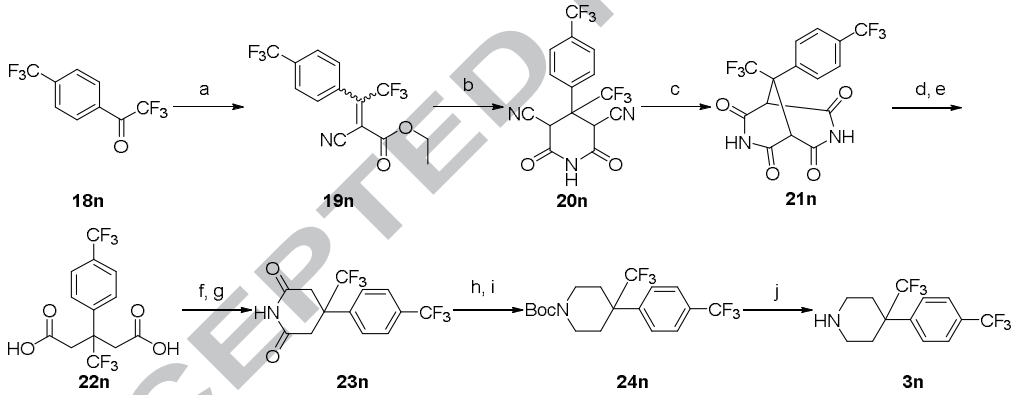
Scheme 3. Synthesis of 4-cyano-4-phenylpiperidine intermediates **3j**, **3k** and **3m**[a]



[a]Reagents and conditions: (a) **16**, NaH, DMF, 70 °C, overnight, 20–40%; (b) hydrogen chloride, CPME, EtOAc, 50 °C, overnight, 86–94%.

4-Trifluoromethyl-4-phenylpiperidine intermediate **3n** was synthesized in a manner similar to a reported procedure, with modifications as shown in Scheme 4.[11b] Commercially available acetophenone **18n** was converted to enone **19n** by Lewis acid-mediated Knoevenagel condensation. Further homologation of enone **19n** was achieved by sequential Michael addition and nucleophilic substitution reaction using 2-cyanoacetamide to lead to 2,6-dioxo-4-piperidine-3,5-dicarbonitrile **20n**. Removal of the cyano groups from **20n** was conducted by acid and alkaline hydrolysis followed by decarboxylation to give dicarboxylic acid **22n**. Introduction of an amino moiety was achieved using urea to give cyclic imide **23n** and it was converted to *N*-Boc-4-trifluoromethyl-4-phenylpiperidine **24n** via borane reduction followed by *N*-Boc protection. Finally, 4-trifluoromethyl-4-phenylpiperidine intermediate **3n** was obtained by deprotection of **24n** with acidic conditions.

Scheme 4. Synthesis of 4-trifluoromethyl-4-phenylpiperidine intermediate **3n**[a]



[a]Reagents and conditions: (a) ethyl cyanoacetate, $TiCl_4$, pyridine, dichloromethane, 0 °C to rt, overnight, 91%; (b) 2-cyanoacetamide, NaOAc, EtOH, rt, overnight; (c) H_2SO_4, H_2O, acetic acid, 130 °C, overnight; (d) aq. KOH, reflux, 4 h; (e) H_2SO_4, H_2O, reflux, 3 h; (f) Ac_2O, reflux, 3 h; (g) urea, 190 °C, 30 min, 41% (6 steps from **19n**); (h) BH_3•SMe_2, THF, 0 °C to reflux, overnight; (i) Boc_2O, DIPEA, THF, rt, 2 h, 77% (2 steps from **23n**); (j) aq. HCl, EtOAc, IPA, reflux, 5 h, 95%.

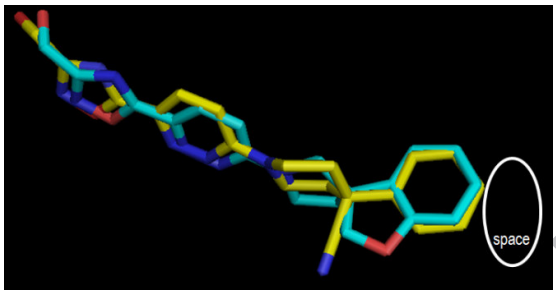
Result and discussion

In this work, newly synthesized compounds were evaluated for their binding affinity to SCD1 using (5-(6-(1'H-spiro(1-benzofuran-3,3'-pyrrolidin)-1'-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)[$^3H_2$]methanol

(^{3}H-**A**) as a radiolabeled ligand.[12] To measure the inhibitory activity in cells of our SCD1 inhibitors, we established growth inhibitory assay in HCT116 cells.

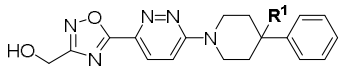
With the aim of discovering a new scaffold alternative to the spiro series, we conducted a ligand-based conformation study. As a result, we found 4-substituted 4-phenylpiperidine derivative **1a**, a ring-opened analogue of the spiro compound, which showed excellent overlay with compound **A** (Figure 2). As mentioned above, the conformation study and overlay study of compound **A** with stearoyl-CoA also suggested the existence of an unoccupied space around the 4-phenyl group that might accommodate an additional substituent and offer a good opportunity to increase affinity as well as to adjust the physicochemical properties. Therefore, we selected the corresponding 4-substituted 4-arylpiperidine as a candidate scaffold alternative to the spiro series.

Figure 2. Overlay study of spiro compound **A** and designed compound **1a** (compound **A** in cyan; designed compound **1a** in yellow).



Initially, we designed 4-cyano-4-phenylpiperidine derivative **1a** in consideration of synthetic tractability and evaluated compound potencies by SCD1 binding and cell growth assays. Thus, compound **1a** was found to exhibit moderate SCD1 binding affinity (IC_{50} = 190 nM) and GI activity (GI_{50} = 530 nM), indicating the potential of this new design as a starting point; this level of binding affinity was over 40-fold weaker than for compound **A**. Therefore, we initiated SAR studies around compound **1a** as a new lead to identify more potent inhibitors by utilizing the higher synthetic accessibility of this new series. First, SARs around the 4-substituted 4-aryl piperidine moiety were examined to identify the most suitable alternative scaffold to the spiro series in terms of potency, as well as physical properties. As for the 4-position of the piperidine ring, substituents with a variety of lipophilicities were introduced and the representative results are shown in Table 1. It was found that replacement of the cyano group with hydrophilic substituents such as amino **1b** (IC_{50} = 220 nM), hydroxy **1c** (IC_{50} = 130 nM), and methoxy **1d** (IC_{50} = 110 nM) groups was tolerated in terms of binding affinity. Remarkably, compound **1e** with a lipophilic trifluoromethyl group exhibited more than 5-fold enhancement in SCD1 binding affinity and 12-fold enhancement in growth inhibitory activity when compared with **1a**.

Table 1. SARs of 4-Substituted 4-Phenylpiperidines



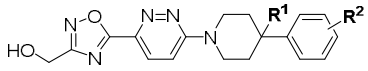
ID	R^1	IC_{50}[a]	GI_{50}[b]
1a	CN	190	530
1b	NH_2	220	530
1c	OH	130	>1000
1d	OMe	110	410
1e	CF_3	38	39

[a]hSCD1 Binding affinity (nM). [b]GI activity using HCT116 cells (nM).

Next, we examined substituent effects on the pendant phenyl group at the 4-position of the piperidine ring. With the aim of not only enhancing binding affinity but also modulating the resulting ADME-tox profiles, substituents with a wide range of size, lipophilicity, and electron effects were examined. As shown in Table 2, mono chlorination at any position of the pendant phenyl group increased the binding affinity and GI activity (**1f**–**h**). The potency increase was more than 4-fold larger for *m*-Cl **1g** (IC_{50} = 15 nM) and *p*-Cl **1h** (IC_{50} = 20 nM) than for *o*-Cl **1f** (IC_{50} = 80 nM). In contrast, effects on cell GI were larger for *p*-Cl **1h** (GI_{50} = 110 nM) than for *o*-Cl **1f** (GI_{50} = 240 nM) and *m*-Cl **1g** (GI_{50} = 200 nM). Thus, the *p*-position was indicated as the most favorable substitution position for obtaining both binding affinity and growth inhibitory activity. Next, in order to elucidate substituent effects at the *p*-position of the terminal benzene ring, we focused on substituents with a range of Hansch–Fujita π and Hammett σ values, which indicate the lipophilicity and electron-donating or electron-withdrawing nature of the substituents, respectively.[13] Replacement of *p*-Cl **1h** (π = +0.70) with less lipophilic groups such as *p*-H **1a** (π = ±0.00) and *p*-NO_2 **1i** (π = +0.24) decreased SCD1 binding affinity and GI activity (**1h** IC_{50} = 20 nM, GI_{50} = 110 nM; **1a** IC_{50} = 190 nM, GI_{50} = 530 nM; **1i** IC_{50} = 100 nM, GI_{50} = 170 nM), whereas replacement with more lipophilic groups such as *p*-*t*Bu **1j** (π = +1.68) and *p*-OCF_3 **1k** (π = +1.04) maintained or enhanced SCD1 binding affinity and GI activity (**1j** IC_{50} = 21 nM, GI_{50} = 37 nM; **1k** IC_{50} = 11 nM, GI_{50} = 32 nM). These results support the hypothesis that the enzyme has space around the pendant phenyl group and indicated the merit of occupying that space with lipophilic substituents at the *p*-position of the phenyl group for enhancing potency. On the other hand, liver microsomal stability (LMS) of **1k** with an electron-withdrawing *p*-OCF_3 group (σ = +0.35) increased more than 3-fold from that of **1j** with an electron-donating group *p*-*t*Bu (σ = –0.20). These results suggest that substitution with electron-withdrawing groups at the *p*-positon of the terminal benzene ring could improve the PK profile. Based on the results of binding affinity and metabolic stability studies, our focus was shifted to the more lipophilic (π > 0) and electron-withdrawing (σ > 0) region in the Craig plot,[14] which was expected to lead to highly potent and orally available SCD1 inhibitors with long PK duration. Indeed, derivatives with a *p*-CF_3 moiety (**1l** π = +1.07) and *p*-SF_5 moiety (**1m** π = +1.23) exhibited higher SCD1 binding affinity and GI activity (**1l** IC_{50} = 27 nM, GI_{50} = 39 nM; **1m** IC_{50} = 9.5 nM, GI_{50} = 8.4 nM) in proportion to their Hansch π values. Both compounds showed excellent LMS, as expected. Taken together with the SAR data obtained at the 4-position of the piperidine ring and the *p*-position of the terminal benzene ring, we identified

4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine (**1n**) as a new scaffold worthy of further study.

Table 2. SARs of 4-Substituted 4-Phenylpiperidines

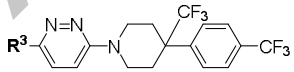


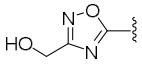
ID	R^1	R^2	IC_{50}[a]	GI_{50}[b]	hLMS[c]	mLMS[d]	π[e]	σ[f]
1a	CN	none	190	530	NS[g]	NS[g]	±0.00	±0.00
1f	CN	*o*-Cl	80	240	NS[g]	NS[g]	–	–
1g	CN	*m*-Cl	15	200	NS[g]	NS[g]	–	–
1h	CN	*p*-Cl	20	110	-14	39	+0.70	+0.23
1i	CN	*p*-NO_2	100	170	6	11	+0.24	+0.78
1j	CN	*p*-*t*Bu	21	37	0	119	+1.68	−0.20
1k	CN	*p*-OCF_3	11	32	-12	35	+1.04	+0.35
1l	CN	*p*-CF_3	27	39	2	33	+1.07	+0.55
1m	CN	*p*-SF_5	9.5	8.4	7	24	+1.23	+0.68
1n	CF_3	*p*-CF_3	2.3	1.3	20	19	–	–

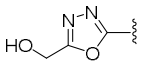
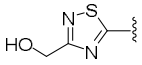
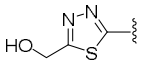
[a]hSCD1 Binding affinity (nM). [b]GI activity against HCT116 cells (nM). [c]LMS in human (μL/min/mg). [d]LMS in mouse (μL/min/mg). [e]Lipophilic parameter proposed by Hansch–Fujita. [f]Hammett electronic constant. [g]Not shown.

As shown in Table 3, compound **1n** showed good LMS (mLMS = 19 μL/min/mg; hLMS = 20 μL/min/mg) in vitro. However, compared to compound **A**, the improvement of oral exposure (AUC_{po} = 432.2 ng/mL) and plasma duration (MRT_{po} = 3.2 h) in a mouse cassette-dosing PK study (0.1 mg/kg iv; 1 mg/kg po) are limited. Final optimization of the oxadiazole moiety was conducted to improve the PK profile. All of the designed compounds **1o–q** showed excellent binding affinity, GI activity, and LMS. Among the regioisomers of oxadiazole and thiadiazole, 1,3,4-oxadiazole **1o** and 1,2,4- thiadiazole **1p** exhibited excellent PK profiles (**1o** AUC_{po} = 3756.5 ng/mL, MRT_{po} = 4.2 h; **1p** AUC_{po} = 1823.3 ng/mL, MRT_{po} = 4.2 h) represented by the reduced Cl_{total} values. In addition, compound **1o** demonstrated a higher fraction unbound in plasma (*F*u = 0.07) than for compound **1p** (*F*u = 0.01). Therefore, compound **1o** (**T-3764518**) was selected as a candidate for further in vivo PD testing and efficacy studies.

Table 3. SARs for Compounds Varied at the 1,2,4-Oxadiazole Moiety



ID	R^3	IC_{50}[a]	GI_{50}[b]	hLMS[c]	mLMS[d]	Cl_{total}[e]	AUC_{po}[f]	MRT_{po}[g]	Fu[h]
1n		2.3	1.3	20	19	926	432.2	3.2	0.05

1o		4.7	2.7	14	33	310	3756.5	4.2	0.07
1p		1.2	1.3	10	−11	494	1823.3	4.2	0.01
1q		2.5	1.1	−16	1	952	1191.1	4.1	0.03

[a]hSCD1 binding affinity (nM). [b]GI activity against HCT116 cells (nM). [c]LMS in human (μL/min/mg). [d]LMS in mouse (μL/min/mg). [e]Cl_{total} (mL/h/kg) in a mouse cassette-dosing PK study (0.1 mg/kg iv; 1 mg/kg po). [f]AUC (ng·h/mL) after oral administration in a mouse cassette-dosing PK study. [g]MRT (h) after oral administration in mouse cassette-dosing PK study. [h]Fu in plasma.

Compound **1o** (**T-3764518**) was evaluated for in vivo effects in mice bearing HCT116 xenograft tumors. The DI (desaturation index) of phosphatidylcholine (PC) was used as a PD marker for the evaluation of SCD1 inhibition in HCT116 xenograft tumors.[15] As shown in Figure 3, administration of compound **1o** decreased DI (ratio of PC C36:3/C36:0) in tumor tissue at all the oral doses down to the lowest dose tested in this study (0.3 mg/kg, po, bid). Reflecting these significant in vivo PD changes, compound **1o** also induced tumor growth inhibition.[6, 15]

Compound **1o** demonstrated tumor growth inhibition with a percent tumor growth inhibition (T/C) of 32.8% in a 786-O xenograft mouse model at an oral dose of 1 mg/kg, po, bid without severe toxicity (Figure 4). Overall, these data indicated **1o** to be an orally bioavailable SCD1 inhibitor with in vivo antitumor efficacy, demonstrating is to be a useful tool for assessing the pharmacology of SCD1 inhibition in medicine, particularly in terms of developing new cancer therapies.

Figure 3. Results of in vivo PD test using HCT116 xenograft model mice. Dose level 0.3, 3, 30 mg/kg, po, bid; ***P < 0.0005 by Williams' test.

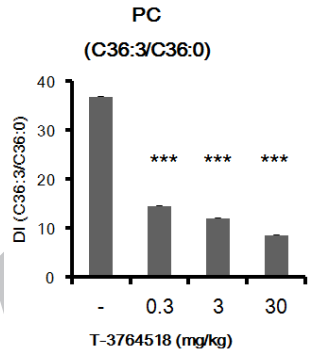
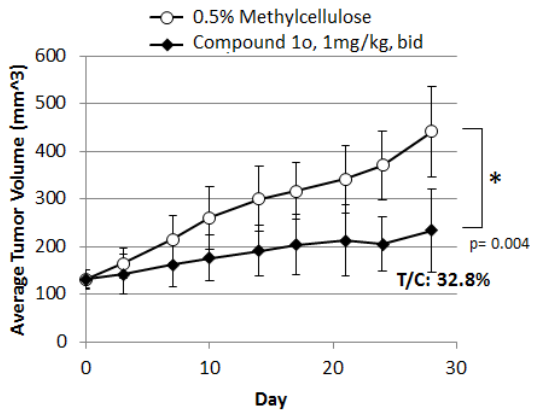


Figure 4. Result of in vivo efficacy test using 786-O xenograft model mice. Dose level 1 mg/kg, po, bid; *P < 0.005 by Student's t-test.



Conclusion

In conclusion, with the aim of discovering highly potent and orally available SCD1 inhibitors for cancer treatment, we designed novel 4-substituted-4-phenylpiperidine derivatives based on a ligand based overlay model of stearoyl CoA with spirocycle-based SCD inhibitor **A** and 3D conformational studies. The compound thus designed (**1a**) exhibited moderate binding affinity toward SCD1 as wells as moderate GI activity. Further substituent modification guided by Hansch–Fujita constants (π) and Hammett (σ) constants resulted in the identification of several inhibitors that showed high SCD1 binding affinity, potent GI activity in HCT116 cancer cell line, and an good PK profile. Notably, 1,3,4-oxadiazole analogue **1o** (**T-3764518**) showed potent in vivo PD response and tumor growth suppression without severe toxicity. These results indicate that our 4-substituted-4-phenylpiperidine-based SCD1 inhibitors, represented by compound **1o**, have potential use not only as chemical tools for basic pharmacology research into SCD1 function, but also as a new lead series of clinical candidates for therapy in oncology.

Experimental section

General

All commercially available solvents and reagents for reactions were reagent-grade and were used as purchased. Analytical thin layer chromatography (TLC) was performed on silica gel $60F_{254}$ plates (Merck) or NH TLC plates (Fuji Silysia Chemical Ltd.). Flash column chromatography separations were performed on Purif-Pack (SI or NH, Fuji Silysia Chemical Ltd.). Melting points (mp), determined on a SRS OptiMelt automated melting point apparatus, are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker AVANCE II (300 MHz), Bruker AVANCE III (300 MHz, 400 MHz), or Varian mercury plus (300 MHz) spectrometers with tetramethylsilane (TMS) as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t= triplet, q = quartet, m = multiplet, br s = broad singlet. Coupling constants (*J* values) are given in hertz (Hz). Mass spectra (MS) were acquired using an Agilent LC/MS system (Agilent 1200SL/Agilent 6130MS), Agilent LC/MS system (Agilent 1200SL/Agilent 6110MS), Agilent LC/MS

system (Agilent 1200SL/Agilent 6120MS), Shimadzu LC/MS system (LC-10ADvp high pressure gradient system/LCMS-2010A) or Shimadzu UFLC/MS (Prominence UFLC high pressure gradient system/LCMS-2020) operating in electron spray ionization mode (ESI). The column used was an L-column 2 ODS (3.0 mm × 50 mm I.D., 3 μm, CERI, Japan) with a temperature of 40 °C and a flow rate of 1.2 or 1.5 mL/min. Mobile phase A was 0.05% TFA in ultrapure water. Mobile phase B was 0.05% TFA in MeCN, which was increased linearly from 5% to 90% over 2 min, 90% over the next 1.5 min, after which the column was equilibrated to 5% for 0.5 min. Elemental analyses were carried out by Sumika Chemical Analysis Service, Ltd. Each compound was confirmed to be ≥95% purity by either LC/MS or elemental analysis. Yields were not optimized.

6-Chloro-*N*'-(((4-methoxybenzyl)oxy)acetyl)pyridazine-3-carbohydrazide (6). To a suspension of 6-oxo-1,6-dihydropyridazine-3-carboxylic acid **4** (57.7 g, 412 mmol) in thionyl chloride (580 mL) was added DMF (5.32 mL) at room temperature. The mixture was stirred at 80 °C for 1 h. The mixture was concentrated in vacuo and diluted with THF (650 mL). To the mixture was added pyridine (55 mL) and a solution of 2-((4-methoxybenzyl)oxy)acetohydrazide **5** (72.1 g, 343 mmol) in THF (650 mL) at 0 °C. The mixture was stirred at room temperature under a dry atmosphere overnight. The mixture was poured into sat. $NaHCO_3$ at room temperature and extracted with THF–EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was triturated with EtOAc to give 6-chloro-*N*'-(((4-methoxybenzyl)oxy)acetyl)pyridazine-3-carbohydrazide **6** (57.0 g, 48%) as an off-white solid. This product was subjected to the next reaction without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.76 (3H, s), 4.02 (2H, s), 4.53 (2H, s), 6.93 (2H, d, J = 8.5 Hz), 7.34 (2H, d, J = 8.5 Hz), 8.10–8.18 (1H, m), 8.21–8.29 (1H, m), 10.12 (1H, d, J = 18.5 Hz), 11.01 (1H, br s).

3-Chloro-6-(5-(((4-methoxybenzyl)oxy)methyl)-1,3,4-oxadiazol-2-yl)pyridazine (7). To a suspension of 6-chloro-*N*'-(((4-methoxybenzyl)oxy)acetyl)pyridazine-3-carbohydrazide **6** (70.0 g, 0.199 mol) in MeCN (600 mL) was added trimethylamine hydrochloride salt (5.72 g, 59.8 mmol), Et_3N (83.4 mL, 0.598 mol) and *p*-TsCl (57.0 g, 0.299 mol) at room temperature. The mixture was stirred at 50 °C under N_2 for 1 h. The mixture was diluted with THF (300 mL), and the insoluble material was removed by filtration. The filtrate was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography to give 3-chloro-6-(5-(((4-methoxybenzyl)oxy)methyl)-1,3,4-oxadiazol-2-yl)pyridazine **7** (60.0 g, 90%) as a pale yellow solid. This product was subjected to the next reaction without further purification. MS(ESI+) *m/z*: 333.0 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.74 (3H, s), 4.59 (2H, s), 4.87 (2H, s), 6.92 (2H, d, J = 8.7 Hz), 7.30 (2H, d, J = 8.5 Hz), 8.20 (1H, d, J = 9.1 Hz), 8.47 (1H, d, J = 9.1 Hz).

(5-(6-Chloropyridazin-3-yl)-1,3,4-oxadiazol-2-yl)methanol (2b). A mixture of 3-chloro-6-(5-(((4-methoxybenzyl)oxy)methyl)-1,3,4-oxadiazol-2-yl)pyridazine **7** (50.0 g, 0.150 mol), TFA (150 mL) and anisole (50 mL) was stirred at room temperature for 1h. The reaction mixture was concentrated in

vacuo. The residue was poured into sat. $NaHCO_3$ at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was triturated with IPE to give (5-(6-chloropyridazin-3-yl)-1,3,4-oxadiazol-2-yl)methanol **2b** (27.6 g, 87%) as a light brown solid; MS(ESI+) *m/z*: 212.9 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.83 (2H, s), 6.10 (1H, br s), 8.14–8.27 (1H, m), 8.43–8.53 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 53.7, 129.1, 130.1, 146.6, 157.7, 161.6, 167.7.

2-(Acetoxyamino)-2-iminoethyl benzoate (9). To a solution of 2-amino-2-(hydroxyimino)ethyl benzoate **8** (68.0 g, 0.350 mol) in acetic acid (200 mL) was slowly added acetic anhydride (53.6 g, 0.525 mol) at 0 °C. The mixture was stirred at room temperature overnight. The mixture was concentrated to give 2-(acetoxyamino)-2-iminoethyl benzoate **9** (74.0 g, 0.313 mol, 90%) as colorless oil. This product was subjected to the next reaction without further purification. MS(ESI+) *m/z*: 237.7 $(M+H)^+$.

2-Amino-2-iminoethyl benzoate acetic acid salt (10). A mixture of 2-(acetoxyamino)-2-iminoethyl benzoate **9** (30.0 g, 0.127 mol) and 10% Pd/C (3.0 g) in EtOH (200 mL) was hydrogenated under balloon pressure at room temperature overnight. The catalyst was removed by filtration. The filtrate was concentrated in vacuo to give 2-amino-2-iminoethyl benzoate acetic acid salt **10** (30.0 g, 99%) as white solid. This product was subjected to the next reaction without further purification. MS(ESI+) *m/z*: 179.7 $(M+H)^+$.

(5-(6-Chloropyridazin-3-yl)-1,2,4-thiadiazol-3-yl)methyl benzoate (2c). A mixture of 3-chloro-6-methylpyridazine (20.0 g, 0.156 mol) and thionyl chloride (114 mL) was refluxed overnight. The mixture was cooled to room temperature, concentrated in vacuo and evaporated twice with toluene azeoptropically. The residue was suspended in THF (300 mL). To the suspension was added 2-amino-2-iminoethyl benzoate acetic acid salt **10** (18.6 g, 78.1 mmol) and stirred at −5 °C. Then sodium hydroxide (50% in water, 42 mL) was added drop wise to the mixture at −5 °C. After being stirred at 0 °C for 1 h, the reaction mixture was extracted with EtOAc and concentrated in vacuo. The residue was purified by column chromatography to give (5-(6-chloropyridazin-3-yl)-1,2,4-thiadiazol-3-yl)methyl benzoate **2c** (18.4 g, 71%) as a white solid; MS(ESI+) *m/z*: 333.0 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.74 (2H, s), 7.51–7.64 (2H, m), 7.66–7.78 (1H, m), 8.00–8.12 (2H, m), 8.15–8.25 (1H, m), 8.35–8.46 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 62.2, 127.1, 128.8, 129.0, 129.4, 130.7, 133.7, 151.7, 158.1, 165.2, 172.0, 185.2. Anal. Calcd for $C_{14}H_9ClN_4O_2S \cdot 0.1H_2O$: C, 50.26; H, 2.77; N, 16.75. Found: C, 50.43; H, 2.97; N, 16.74.

6-Methoxypyridazine-3-carbohydrazide (12). A solution of methyl 6-methoxypyridazine-3-carboxylate **11** (4.50 g, 26.8 mmol) and hydrazine hydrate (80% in water, 13.4 g, 214 mmol) in MeOH (50 mL) was stirred at room temperature for overnight. The mixture was added water (10 mL) and concentrated in vacuo to remove MeOH. The residue was freeze-dried and washed with petroleum ether to give 6-methoxypyridazine-3-carbohydrazide **12** (4.20 g, 93%) as a yellow solid. This product was subjected to the next reaction without further purification. MS(ESI+) *m/z*: 169.0 $(M+H)^+$.

2-(2-((6-Methoxypyridazin-3-yl)carbonyl)hydrazino)-2-oxoethyl acetate (13). Acetoxyacetyl chloride (16.3 g, 119 mmol) was added to a solution of 6-methoxypyridazine-3-carbohydrazide **12** (20.0 g, 119 mmol) in dichloromethane (250 mL)–water (10 mL) at 0 °C. The mixture was allowed to warm to room temperature for 1.5 h. The mixture was concentrated in vacuo at room temperature. The residue was added water (20 mL) and freeze-dried. The solid was washed with petroleum ether to give 2-(2-((6-methoxypyridazin-3-yl)carbonyl)hydrazino)-2-oxoethyl acetate **13** (25.0 g, 78%) as a light yellow solid. This product was subjected to the next reaction without further purification. MS(ESI+) *m/z*: 269.0 $(M+H)^+$.

(5-(6-Chloropyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methyl acetate (14). A solution of 2-(2-((6-methoxypyridazin-3-yl)carbonyl)hydrazino)-2-oxoethyl acetate **13** (25.0 g, 93.3 mmol) and phosphorus pentasulfide (21.4 g, 112 mmol) in THF (600 mL) was stirred at 50°C for overnight. The mixture was cooled to room temperature, quenched with sat. $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography to give (5-(6-hydroxypyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methyl acetate (7.90 g, 34%) as a yellow solid. A solution of (5-(6-hydroxypyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methyl acetate (7.90 g, 31.3 mmol) and phosphoryl chloride (9.60 g, 62.7 mmol) in MeCN (200 mL) was heated to 70 °C for 2 h. The mixture was cooled to room temperature, quenched with water at 0 °C and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography to give (5-(6-chloropyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methyl acetate **14** (2.90 g, 34%) as a yellow solid. This product was subjected to the next reaction without further purification. MS(ESI+) *m/z*: 271.0 $(M+H)^+$.

(5-(6-Chloropyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methanol (2d). To a solution of (5-(6-chloropyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methyl acetate **14** (1.60 g, 5.93 mmol) in THF (50 mL) was added lithium hydroxide (1 M in water, 14.8 mL) at 0°C. The mixture was stirred at 0°C for 1 h and the mixture was allowed to warm to room temperature for 1 h. After removing the solvent, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was crystallized from MeOH–dichloromethane to give (5-(6-chloropyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methanol **2d** (1.16 g, 85%) as a yellow solid; MS(ESI+) *m/z*: 229.2 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.88–5.05 (2H, m), 6.34–6.53 (1H, m), 8.11–8.21 (1H, m), 8.48–8.58 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 58.4, 127.0, 130.2, 152.1, 157.3, 165.3, 176.6.

***tert*-Butyl 4-(4-(*tert*-butyl)phenyl)-4-cyanopiperidine-1-carboxylate (17j)**. Sodium hydride (60% in oil, 3.46 g, 86.6 mmol) was added to a solution of *tert*-butyl bis(2-chloroethyl)carbamate **16** (10.0 g, 41.3 mmol) and 2-(4-(*tert*-butyl)phenyl)acetonitrile **15j** (5.00 g, 28.9 mmol) in DMF (300 mL) at 70 °C. The mixture was stirred at 70 °C under N_2 overnight. The mixture was poured into sat. NH_4Cl at room temperature and

extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography to give *tert*-butyl 4-(4-(*tert*-butyl)phenyl)-4-cyanopiperidine-1-carboxylate **17j** (3.50 g, 35 %) as a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (9H, s), 1.43–1.52 (9H, m), 1.85–2.19 (4H, m), 3.12–3.35 (2H, m), 4.17–4.41 (2H, m), 7.35–7.46 (4H, m).

4-(4-(*tert*-Butyl)phenyl)piperidine-4-carbonitrile hydrochloride salt (3j). A mixture of hydrogen chloride (4 M in CPME, 2.55 mL) and *tert*-butyl 4-(4-(*tert*-butyl)phenyl)-4-cyanopiperidine-1-carboxylate **16j** (3.50 g, 10.2 mmol) in EtOAc (10 mL) was stirred at 50 °C under N_2 overnight. The precipitate was collected by filtration to give 4-(4-(*tert*-butyl)phenyl)piperidine-4-carbonitrile hydrochloride salt **3j** (2.68 g, 9.61 mmol, 94 %) as a white solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (9H, s), 2.29–2.46 (4H, m), 3.00–3.18 (2H, m), 3.42–3.55 (2H, m), 7.38–7.56 (4H, m), 9.31 (2H, br s).

***tert*-Butyl 4-cyano-4-(4-(trifluoromethoxy)phenyl)piperidine-1-carboxylate (17k)**. *tert*-Butyl 4-cyano-4-(4-(trifluoromethoxy)phenyl)piperidine-1-carboxylate **17k** was prepared by a method similar to that described for *tert*-butyl 4-(4-(*tert*-butyl)phenyl)-4-cyanopiperidine-1-carboxylate **17j** using (4-(trifluoromethoxy)phenyl)acetonitrile **15k**. Yield: 40% as a white solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.32–2.47 (4H, m), 2.99–3.19 (2H, m), 3.42–3.59 (2H, m), 7.46–7.58 (2H, m), 7.63–7.76 (2H, m), 9.28 (2H, br s).

4-(4-Trifluoromethoxyphenyl)piperidine-4-carbonitrile hydrochloride salt (3k). 4-(4-Trifluoromethoxyphenyl)piperidine-4-carbonitrile hydrochloride salt **3k** was prepared by a method similar to that described for 4-(4-(*tert*-butyl)phenyl)piperidine-4-carbonitrile hydrochloride salt **3j** using *tert*-butyl 4-cyano-4-(4-(trifluoromethoxy)phenyl)piperidine-1-carboxylate **17k**. Yield: 86% as a white solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.32–2.47 (4H, m), 2.99–3.19 (2H, m), 3.42–3.59 (2H, m), 7.46–7.58 (2H, m), 7.63–7.76 (2H, m), 9.28 (2H, br s).

***tert*-Butyl 4-cyano-4-(4-(pentafluorosulfanyl)phenyl)piperidine-1-carboxylate (17m)**. *tert*-Butyl 4-cyano-4-(4-(pentafluorosulfanyl)phenyl)piperidine-1-carboxylate **17m** was prepared by a method similar to that described for *tert*-butyl 4-(4-(*tert*-butyl)phenyl)-4-cyanopiperidine-1-carboxylate **17j** using (4-(pentafluorosulfanyl)phenyl)acetonitrile **15m**. Yield: 20% as white solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (9H, s), 1.84–2.18 (4H, m), 3.01–3.40 (2H, m), 4.11–4.57 (2H, m), 7.53–7.64 (2H, m), 7.78–7.87 (2H, m).

4-(4-(Pentafluorosulfanyl)phenyl)piperidine-4-carbonitrile hydrochloride salt (3m). 4-(4-(Pentafluorosulfanyl)phenyl)piperidine-4-carbonitrile hydrochloride salt **3m** was prepared by a method similar to that described for 4-(4-(*tert*-butyl)phenyl)piperidine-4-carbonitrile hydrochloride salt **3j** using *tert*-butyl 4-cyano-4-(4-(pentafluorosulfanyl)phenyl)piperidine-1-carboxylate **17m**. Yield: 89% as a white

solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.26–2.47 (4H, m), 3.01–3.21 (2H, m), 3.45–3.62 (2H, m), 7.72–7.82 (2H, m), 8.03–8.13 (2H, m), 8.87–9.16 (2H, m).

Ethyl 2-cyano-4,4,4-trifluoro-3-(4-(trifluoromethyl)phenyl)but-2-enoate (19n). $TiCl_4$ (32.9 g, 174 mmol) was added drop wise to a solution of 2,2,2-trifluoro-1-(4-(trifluoromethyl)phenyl)ethanone **18n** (20 g, 82.6 mmol) and ethyl cyanoacetate (10.3 g, 90.9 mmol) in dichloromethane (400 mL) at 0 °C. The mixture was stirred for 20 min at 0 °C and then pyridine (6 mL) was added drop wise slowly, keeping the internal temperature below 5 °C. After the addition, the ice bath was removed. Then reaction was stirred for 30 min at room temperature. Then an additional pyridine (20 mL) was added and the mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane and washed with hydrogen chloride (2M in water), water and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography to give ethyl 2-cyano-4,4,4-trifluoro-3-(4-(trifluoromethyl)phenyl)but-2-enoate **19n** (25.2 g, 91%) as light yellow oil. This product was subjected to the next reaction without further purification as a mixture of *cis* and *trans* isomers. Major isomer: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.09 (3H, t, J = 7.2 Hz), 4.13 (2H, q, J = 7.2 Hz), 7.40 (2H, d, J = 8.0 Hz), 7.73 (2H, d, J = 8.0 Hz). Minor isomer: δ 1.42 (3H, t, J = 7.2 Hz), 4.44 (2H, q, J = 7.2 Hz), 7.60 (2H, d, J = 8.0 Hz), 7.80 (2H, d, J = 8.0 Hz).

2,6-Dioxo-4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine-3,5-dicarbonitrile (20n). To a solution of ethyl 2-cyano-4,4,4-trifluoro-3-(4-(trifluoromethyl)phenyl)but-2-enoate **19n** (116 g, 0.344 mol) in EtOH (1000 mL) was added 2-cyanoacetamide (50.7 g, 0.603 mol) and NaOAc (98.9 g, 1.21 mol) at room temperature. The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was treated with water. The aqueous layer was added hydrogen chloride (12M in water) to adjust the pH rose to 3, and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give 2,6-dioxo-4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine-3,5-dicarbonitrile **20n** (135 g, crude) as a yellow oil. This product was used for the next step without further purification. MS(ESI–) *m/z*: 374.0 $(M–H)^–$.

9-(Trifluoromethyl)-9-(4-(trifluoromethyl)phenyl)-3,7-diazabicyclo[3.3.1]nonane-2,4,6,8-tetraon (21n). A mixture of 2,6-dioxo-4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine-3,5-dicarbonitrile **20n** (135 g, crude) in H_2SO_4 (529.6 g, 5.40 mol)–water (400 mL)–acetic acid (400 mL) was stirred at 130 °C overnight. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give 9-(trifluoromethyl)-9-(4-(trifluoromethyl)phenyl)-3,7-diazabicyclo[3.3.1]nonane-2,4,6,8-tetraon **21n** (99.4 g, crude) as a grey solid. This product was used for the next step without further purification. MS(ESI–) *m/z*: 393.2 $(M–H)^–$.

3-(Trifluoromethyl)-3-(4-(trifluoromethyl)phenyl)pentanedioic acid (22n). A solution of 9-(trifluoromethyl)-9-(4-(trifluoromethyl)phenyl)-3,7-diazabicyclo[3.3.1]nonane-2,4,6,8-tetraon **21n** (99.4 g, crude) in potassium hydroxide (20% in water, 549 g) was refluxed for 4 h. After cooling to 0 °C, H_2SO_4 (220 g, 2.34 mol)–water (500 mL) was added drop wise to the reaction mixture. Then the resulting mixture was refluxed for additional 3 h. After cooling, the mixture was extracted with EtOAc. The organic layer was dried and concentrated in vacuo to give 3-(trifluoromethyl)-3-(4-(trifluoromethyl)phenyl)pentanedioic acid **22n** (79.6 g, crude) as a grey solid. This product was used for the next step without further purification. MS(ESI–) *m/z*: 343.2 $(M–H)^–$.

2,6-Dioxo-4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine (23n). A solution of 3-(trifluoromethyl)-3-(4-(trifluoromethyl)phenyl)pentanedioic acid **22n** (79.6 g, crude) in acetic anhydride (507.9 g, 4.98 mol) was refluxed 3 h. The reaction mixture was concentrated in vacuo to give desired intermediate. The mixture of intermediate and urea (14.6 g, 243 mmol) was stirred at 190 °C under N_2 for 30 min. After cooling to room temperature, the reaction mixture was dissolved in dichloromethane and filtered off the resulting precipitate. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography followed by recrystallization to give 2,6-dioxo-4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine **23n** (45.5 g, 41% in 6 steps from **19n**). This product was used for the next step without further purification. MS(ESI–) *m/z*: 342.2 $(M–H)^–$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.38 (2H, d, J = 16.4 Hz), 3.51 (2H, d, J = 16.4 Hz), 7.83–7.89 (4H, m), 11.03 (1H, s).

***tert*-Butyl 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine-1-carboxylate (24n)**. The mixture of borane dimethyl sulfide complex (2M in THF, 360 mL, 720 mmol) and 2,6-dioxo-4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine **23n** (39.0 g, 120 mmol) was refluxed overnight. After cooling to 0 °C, hydrogen chloride (6M in water, 210 mL) was added drop wise to the mixture. This mixture was refluxed for 1 h, then cooled with ice bath and added sodium hydroxide (8 M in water, 190 mL). Then this mixture was extracted with EtOAc. The organic layer was dried and concentrated in vacuo to give desired intermediate. Boc_2O (26.7 g, 122 mmol) was added to a solution of above mentioned intermediate and DIPEA (18.6 g, 144 mmol) in THF (350 mL) at room temperature.The mixture was stirred at room temperature for 2 h and then poured in to sat. $NaHCO_3$. The mixture was extracted with EtOAc. The organic layer was dried and concentrated in vacuo. The residue was purified by silica gel column chromatography to give *tert*-butyl 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine-1-carboxylate **24n** (36.5 g, 77% in 2 steps from **23n**) as a white solid. This product was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s), 2.06–2.14 (2H, m), 2.45–2.49 (2H, m), 2.61–2.78(2H, m), 3.94–4.15 (2H, m), 7.58 (2H, d, J = 8.0 Hz), 7.69 (2H, d, J = 8.8 Hz).

4-(Trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt (3n). To a solution of *tert*-butyl 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine-1-carboxylate **24n** (6.40 g, 16.1 mmol)

in EtOAc (20 mL) was added hydrogen chloride (12.1M in water, 4.0 mL, 48.4 mmol) and IPA (8.0 mL). Then this mixture was refluxed for 5 h. The reaction mixture was concentrated in vacuo. The residue was crystallized from diethyl ether to give 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt **3o** (5.10 g, 95%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.32–2.38 (2H, m), 2.54–2.58 (2H, m), 2.78–2.82 (2H, m), 3.29–3.43 (2H, m), 7.86 (4H, d, J = 8.8 Hz), 9.09–9.31 (2H, m).

1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile (**1a**). The mixture of 4-phenylpiperidine-4-carbonitrile hydrochloride salt **3a** (200 mg, 0.900 mmol), (5-(6-chloropyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methyl benzoate **2a** (200 mg, 0.630 mmol), DIPEA (0.650 mL, 3.73 mmol) and IPA (4.0 mL) was heated at 150°C for 1 h under microwave irradiation. After cooling to room temperature, the mixture was stirred at room temperature for 1h. The precipitate was collected and washed with IPA to give desired intermediate. Sodium hydroxide (2 M in water, 1.0 mL) was added to a solution of the above mentioned intermediate in THF (20 mL)–MeOH (10 mL) at room temperature. The mixture was stirred at room temperature under N_2 for 10 min. The mixture was poured into water at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from EtOAc–hexane to give 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** (200 mg, 0.552 mmol, 87 % in 2 steps from **2a**) as a colorless crystals; mp 195–199 °C. MS(ESI+) *m/z*: 363.2 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.07–2.22 (2H, m), 2.26–2.40 (2H, m), 3.28–3.45 (2H, m), 4.60–4.70 (2H, m), 4.74–4.90 (2H, m), 5.75–5.83 (1H, m), 7.34–7.65 (6H, m), 8.01–8.12 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 34.7, 42.0, 42.3, 54.7, 112.3, 121.7, 125.6, 127.7, 128.2, 129.0, 138.5, 139.5, 159.2, 170.5, 172.9. Anal. Calcd for $C_{19}H_{18}N_6O_2 \cdot 0.1H_2O$: C, 62.66; H, 5.04; N, 23.08. Found: C, 62.60; H, 5.27; N, 23.05.

(5-(6-(4-Amino-4-phenylpiperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol (**1b**). (5-(6-(4-Amino-4-phenylpiperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol **1b** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3b**. Yield: 94% (in 2 steps from **2a**) as a colorless crystals; mp 194–198 °C. MS(ESI+) *m/z*: 353.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64–1.81 (2H, m), 1.90–2.10 (4H, m), 3.55–3.76 (2H, m), 4.27–4.48 (2H, m), 4.55–4.75 (2H, m), 5.70–5.89 (1H, m), 7.16–7.24 (1H, m), 7.28–7.37 (2H, m), 7.41–7.47 (1H, m), 7.51–7.59 (2H, m), 7.93–8.05 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 37.4, 40.7, 52.3, 54.7, 111.6, 125.0, 126.0, 127.5, 127.9, 137.6, 150.6, 159.2, 170.4, 173.1. Anal. Calcd for $C_{18}H_{20}N_6O_2 \cdot 0.1H_2O$: C, 61.04; H, 5.75; N, 23.73. Found: C, 61.09; H, 5.98; N, 23.80.

1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidin-4-ol (**1c**). 1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidin-4-ol **1c** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3c**.

Yield: 68% (in 2 steps from **2a**) as a colorless crystals; mp 181–185 °C. MS(ESI+) *m/z*: 354.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70–1.84 (2H, m), 1.92–2.09 (2H, m), 3.41–3.58 (2H, m), 4.46–4.57 (2H, m), 4.62–4.69 (2H, m), 5.27 (1H, s), 5.73–5.83 (1H, m), 7.18–7.27 (1H, m), 7.29–7.38 (2H, m), 7.42–7.56 (3H, m), 7.95–8.04 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 37.3, 40.7, 54.8, 70.0, 111.7, 124.7, 126.4, 127.5, 127.9, 137.8, 149.2, 159.3, 170.5, 173.1. Anal. Calcd for $C_{18}H_{19}N_5O_3 \cdot 0.1H_2O$: C, 60.87; H, 5.45; N, 19.72. Found: C, 60.95; H, 5.72; N, 19.66.

(5-(6-(4-Methoxy-4-phenylpiperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol (**1d**). (5-(6-(4-Methoxy-4-phenylpiperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol **1d** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3d**. Yield: 95% (in 2 steps from **2a**) as a colorless crystals; mp 187–190 °C. MS(ESI+) *m/z*: 368.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.87–2.03 (2H, m), 2.08–2.21 (2H, m), 2.97 (3H, s), 3.32–3.47 (2H, m), 4.39–4.56 (2H, m), 4.60–4.69 (2H, m), 5.72–5.84 (1H, m), 7.24–7.51 (6H, m), 7.96–8.05 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 33.7, 40.4, 49.5, 54.8, 75.2, 111.8, 125.8, 127.3, 127.6, 128.4, 138.0, 143.7, 159.4, 170.5, 173.1. Anal. Calcd for $C_{19}H_{21}N_5O_3$: C, 62.11; H, 5.76; N, 19.06. Found: C, 61.90; H, 5.90; N, 19.21.

(5-(6-(4-Phenyl-4-(trifluoromethyl)piperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol (**1e**). (5-(6-(4-Phenyl-4-(trifluoromethyl)piperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol **1e** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3e**. Yield: 79% (in 2 steps from **2a**) as a colorless crystals; mp 221–225 °C. MS(ESI+) *m/z*: 406.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.00–2.19 (2H, m), 2.68–2.81 (2H, m), 2.84–3.01 (2H, m), 4.46–4.60 (2H, m), 4.61–4.69 (2H, m), 5.73–5.82 (1H, m), 7.36–7.57 (4H, m), 7.64–7.74 (2H, m), 7.96–8.07 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 34.7, 42.0, 42.3, 54.7, 112.3, 121.7, 125.6, 127.7, 128.2, 129.0, 138.5, 139.5, 159.2, 170.5, 172.9. Anal. Calcd for $C_{19}H_{18}F_3N_5O_2 \cdot 0.1H_2O$: C, 56.05; H, 4.51; N, 17.20. Found: C, 56.22; H, 4.81; N, 17.16.

4-(2-Chlorophenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile (**1f**). 4-(2-chlorophenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile **1f** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3f**. Yield: 63% (in 2 steps from **2a**) as a colorless crystals; mp 187–190 °C. MS(ESI+) *m/z*: 397.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.05–2.25 (2H, m), 2.59–2.75 (2H, m), 3.34–3.48 (2H, m), 4.60–4.72 (2H, m), 4.75–4.93 (2H, m), 5.69–5.88 (1H, m), 7.39–7.66 (5H, m), 8.03–8.13 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 32.8, 40.4, 41.6, 54.8, 112.3, 119.9, 127.8, 127.9, 128.1, 130.2, 131.6, 132.5, 135.1, 138.7, 159.3, 170.6, 172.9. Anal. Calcd for $C_{19}H_{17}ClN_6O_2 \cdot 0.1H_2O$: C, 57.25; H, 4.35; N, 21.08. Found: C, 57.30; H, 4.57; N,

21.15.

4-(3-Chlorophenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile (1g). 4-(3-Chlorophenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile **1g** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3g**. Yield: 74% (in 2 steps from **2a**) as a colorless crystals; mp 194–197 °C. MS(ESI+) *m/z*: 397.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.10–2.24 (2H, m), 2.28–2.40 (2H, m), 3.26–3.43 (2H, m), 4.59–4.70 (2H, m), 4.74–4.90 (2H, m), 5.72–5.83 (1H, m), 7.41–7.62 (4H, m), 7.64–7.68 (1H, m), 8.02–8.11 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 34.5, 42.0, 42.4, 54.8, 112.3, 121.3, 124.7, 125.8, 127.7, 128.3, 130.9, 133.8, 138.6, 141.9, 159.2, 170.6, 173.0. Anal. Calcd for $C_{19}H_{17}ClN_6O_2$: C, 57.51; H, 4.32; N, 21.18. Found: C, 57.50; H, 4.62; N, 21.33.

4-(4-Chlorophenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile (1h). 4-(4-chlorophenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile **1h** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3h**. Yield: 76% (in 2 steps from **2a**) as a colorless crystals; mp 194–195 °C. MS(ESI+) *m/z*: 397.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.07–2.22 (2H, m), 2.26–2.37 (2H, m), 3.27–3.42 (2H, m), 4.60–4.70 (2H, m), 4.74–4.88 (2H, m), 5.73–5.83 (1H, m), 7.49–7.57 (3H, m), 7.59–7.67 (2H, m), 8.01–8.12 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 34.6, 42.0, 42.1, 54.8, 112.3, 121.4, 127.7, 127.8, 129.0, 132.9, 138.6, 138.6, 159.2, 170.6, 173.0. Anal. Calcd for $C_{19}H_{17}ClN_6O_2$: C, 57.51; H, 4.32; N, 21.18. Found: C, 57.49; H, 4.60; N, 21.35.

1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-nitrophenyl)piperidine-4-carbonitrile (1i). 1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-nitrophenyl)piperidine-4-carbonitrile **1i** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3i**. Yield: 89% (in 2 steps from **2a**) as a colorless crystals; mp 211–214 °C. MS(ESI+) *m/z*: 408.1 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.17–2.29 (2H, m), 2.31–2.41 (2H, m), 3.31–3.45 (2H, m), 4.62–4.69 (2H, m), 4.80–4.91 (2H, m), 5.75–5.82 (1H, m), 7.51–7.60 (1H, m), 7.87–7.96 (2H, m), 8.04–8.11 (1H, m), 8.27–8.34 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 34.3, 41.9, 42.8, 54.7, 112.3, 120.9, 124.0, 127.5, 127.7, 138.6, 146.4, 147.2, 159.2, 170.5, 172.9. Anal. Calcd for $C_{19}H_{17}N_7O_4 \cdot 0.8H_2O$: C, 54.10; H, 4.44; N, 23.25. Found: C, 53.97; H, 4.35; N, 23.19.

4-(4-(*tert*-Butyl)phenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitrile (1j).

4-(4-(*tert*-Butyl)phenyl)-1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)piperidine-4-carbonitril e **1j** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3j**. Yield: 83% (in 2 steps from **2a**) as a colorless crystals; mp 227–231 °C. MS(ESI+) *m/z*: 419.2 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28 (9H, s), 2.05–2.21 (2H, m), 2.26–2.37 (2H, m), 3.29–3.41 (2H, m), 4.60–4.70 (2H, m), 4.75–4.88 (2H, m), 5.74–5.84 (1H, m), 7.42–7.59 (5H, m), 8.01–8.12 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 30.9, 34.2, 34.7, 41.8, 42.0, 54.7, 112.3, 121.8, 125.3, 125.7, 127.7, 136.6, 138.5, 150.6, 159.2, 170.5, 172.9. Anal. Calcd for $C_{23}H_{26}N_6O_2$: C, 66.01; H, 6.26; N, 20.08. Found: C, 65.86; H, 6.45; N, 20.29.

1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-(trifluoromethoxy)phenyl)piperidine-4-carbonitrile (**1k**).
1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-(trifluoromethoxy)phenyl)piperidine-4-ca rbonitrile **1k** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3k**. Yield: 78% (in 2 steps from **2a**) as a colorless crystals; mp 190–193 °C. MS(ESI+) *m/z*: 447.0 $(M+H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.10–2.25 (2H, m), 2.29–2.40 (2H, m), 3.27–3.42 (2H, m), 4.61–4.70 (2H, m), 4.77–4.92 (2H, m), 5.75–5.83 (1H, m), 7.43–7.50 (2H, m), 7.52–7.59 (1H, m), 7.70–7.79 (2H, m), 8.02–8.12 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 34.6, 42.0, 42.1, 54.8, 112.3, 119.9, 121.4, 121.5, 127.7, 128.0, 138.6, 138.9, 147.9, 159.2, 170.5, 172.9. Anal. Calcd for $C_{20}H_{17}F_3N_6O_3$: C, 53.81; H, 3.84; N, 18.83. Found: C, 53.91; H, 4.14; N, 18.96.

1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-(trifluoromethyl)phenyl)piperidine-4-carbonitrile (**1l**).
1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-(trifluoromethyl)phenyl)piperidine-4-carb onitrile **1l** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3l**. Yield: 79% (in 2 steps from **2a**) as a colorless crystals; mp 186–189 °C. MS(ESI+) *m/z*: 431.0 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.13–2.29 (2H, m), 2.29–2.43 (2H, m), 3.29–3.44 (2H, m), 4.62–4.70 (2H, m), 4.79–4.91 (2H, m), 5.73–5.84 (1H, m), 7.50–7.59 (1H, m), 7.78–7.91 (4H, m), 8.03–8.12 (1H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 35.0, 42.5, 43.2, 55.3, 112.9, 121.7, 124.5, 126.5, 127.4, 128.3, 129.4, 139.1, 144.6, 159.8, 171.1, 173.5. Anal. Calcd for $C_{20}H_{17}F_3N_6O_2 \cdot 0.1H_2O$: C, 55.58; H, 4.01; N, 19.45. Found: C, 55.69; H, 4.16; N, 19.45.

1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-(pentafluorosulfanyl)phenyl)piperidine-4-carbonitrile (**1m**).
1-(6-(3-(Hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-(4-(pentafluorosulfanyl)phenyl)piperidine-4-carbonitrile **1m** was prepared by a method similar to that described for

1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3m**. Yield: 58% (in 2 steps from **2a**) as a colorless crystals; mp 228–232 °C. MS(ESI+) *m/z*: 489.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.12–2.27 (2H, m), 2.29–2.40 (2H, m), 3.28–3.44 (2H, m), 4.61–4.68 (2H, m), 4.78–4.91 (2H, m), 5.73–5.81 (1H, m), 7.49–7.60 (1H, m), 7.79–7.91 (2H, m), 7.96–8.12 (3H, m). ^{13}C NMR (76 MHz, DMSO-d_6) δ 34.9, 42.5, 43.1, 55.3, 112.9, 121.5, 127.0, 127.1, 127.8, 128.3, 139.1, 144.4, 159.7, 171.1, 173.5. Anal. Calcd for $C_{19}H_{17}F_5N_6O_2S \cdot 0.2H_2O$: C, 46.38; H, 3.56; N, 17.08. Found: C, 46.31; H, 3.85; N, 16.90.

(5-(6-(4-(Trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol (**1n**). (5-(6-(4-(Trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)methanol **1n** was prepared by a method similar to that described for 1-(6-(3-(hydroxymethyl)-1,2,4-oxadiazol-5-yl)pyridazin-3-yl)-4-phenylpiperidine-4-carbonitrile **1a** using **3n**. Yield: 80% (in 2 steps from **2a**) as a colorless crystals; mp 189–192 °C. MS(ESI+) *m/z*: 474.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.03–2.28 (2H, m), 2.73–3.00 (4H, m), 4.48–4.60 (2H, m), 4.62–4.69 (2H, m), 5.71–5.83 (1H, m), 7.36–7.49 (1H, m), 7.82–7.91 (2H, m), 7.92–7.99 (2H, m), 8.00–8.08 (1H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.2, 39.9, 46.7, 54.8, 112.2, 122.2, 125.6, 129.1, 127.6, 128.9, 130.3, 138.2, 138.5, 159.4, 170.5, 172.9. Anal. Calcd for $C_{20}H_{17}F_6N_5O_2$: C, 50.75; H, 3.62; N, 14.79. Found: C, 50.82; H, 3.91; N, 14.88.

(5-(6-(4-(Trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,3,4-oxadiazol-2-yl)methanol (**1o**). The mixture of 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt **3n** (12.00 g, 36.0 mmol), (5-(6-chloropyridazin-3-yl)-1,3,4-oxadiazol-2-yl)methanol **2b** (7.50 g, 35.3 mmol), DIPEA (12 mL, 68.9 mmol) and IPA (110 mL) was heated at 150°C for 1 h under microwave irradiation. After cooling to room temperature, the mixture was stirred at room temperature overnight. The precipitate was crystallized from EtOH (300 mL)–water (500 mL) to give (5-(6-(4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,3,4-oxadiazol-2-yl)methanol **1o** (12.00 g, 72%) as colorless crystals; mp 208–211 °C. MS(ESI+) *m/z*: 474.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.06–2.28 (2H, m), 2.70–3.04 (4H, m), 4.43–4.59 (2H, m), 4.70–4.81 (2H, m), 5.88–6.10 (1H, m), 7.35–7.52 (1H, m), 7.78–8.10 (5H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.6, 39.4, 46.1, 53.1, 112.3, 121.7, 125.1, 125.3, 126.2, 128.3, 129.8, 137.6, 137.8, 158.9, 162.1, 165.9. Anal. Calcd for $C_{20}H_{17}F_6N_5O_2$: C, 50.75; H, 3.62; N, 14.79. Found: C, 50.70; H, 3.91; N, 14.93.

(5-(6-(4-(Trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,2,4-thiadiazol-3-yl)methanol (**1p**). The mixture of DIPEA (0.472 mL, 2.70 mmol), (5-(6-chloropyridazin-3-yl)-1,2,4-thiadiazol-3-yl)methyl benzoate **2c** (300 mg, 0.90 mmol), 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt **3n** (331 mg, 0.99 mmol) in DMA (5 mL) was stirred at 80 °C for 16 h. The mixture was poured into hydrogen chloride (0.1M in water) and

extracted with EtOAc. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give desired intermediate. Sodium hydroxide (2M in water, 1 mL, 2.0 mmol) was added to a solution of the above mentioned intermediate in THF (20 mL)–MeOH (20 mL) at room temperature. The mixture was stirred at room temperature under N_2 for 30 min. The mixture was poured into hydrogen chloride (0.1M in water) at room temperature and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from EtOAc-hexane to give (5-(6-(4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,2,4-thiadiazol-3-yl)methanol **1p** (335 mg, 76%) as a colorless crystals; mp 199–201 °C. MS(ESI+) *m/z*: 490.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.05–2.24 (2H, m), 2.73–2.99 (4H, m), 4.44–4.61 (2H, m), 4.67–4.79 (2H, m), 5.58–5.73 (1H, m), 7.41–7.51 (1H, m), 7.80–8.05 (5H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.7, 40.5, 47.2, 60.8, 113.5, 122.7, 125.3, 126.1, 126.3, 129.4, 130.9, 138.7, 144.7, 160.4, 177.5, 186.7. Anal. Calcd for $C_{20}H_{17}F_6N_5OS$: C, 49.08; H, 3.50; N, 14.31. Found: C, 49.04; H, 3.76; N, 14.39.

(5-(6-(4-(Trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methanol (1q). The mixture of 4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidine hydrochloride salt **3n** (50 mg, 0.15 mmol), (5-(6-chloropyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methanol **2d** (30 mg, 0.13 mmol), DIPEA (0.100 mL, 0.57 mmol) and IPA (4 mL) was heated at 150 °C for 1 h under microwave irradiation. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography to give (5-(6-(4-(trifluoromethyl)-4-(4-(trifluoromethyl)phenyl)piperidin-1-yl)pyridazin-3-yl)-1,3,4-thiadiazol-2-yl)methanol **1q** (45 mg, 0.092 mmol, 70%) as a colorless crystals; mp 199–202 °C. MS(ESI+) *m/z*: 490.1 $(M+H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.02–2.26 (2H, m), 2.73–2.97 (4H, m), 4.43–4.57 (2H, m), 4.83–4.95 (2H, m), 6.21–6.33 (1H, m), 7.41–7.50 (1H, m), 7.81–7.91 (2H, m), 7.93–8.01 (2H, m), 8.06–8.16 (1H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.6, 40.5, 47.2, 58.9, 113.8, 122.7, 125.3, 126.1, 126.3, 129.4, 130.9, 138.7, 144.7, 160.1, 167.7, 174.3. Anal. Calcd for $C_{20}H_{17}F_6N_5OS$: C, 49.08; H, 3.50; N, 14.31. Found: C, 49.08; H, 3.75; N, 14.51.

Human SCD1 binding assay using radiolabeled ligand

Radioligand binding assays were performed in a total volume of 200 µl in 96-well plates in a buffer containing 10 mM Tris-HCl (pH 7.5), 100 mM KCl, 3 mM $MgCl_2$, 10 mM NaF, 0.005% Tween-20 and 1 mM glutathione. The liver microsomes and various concentrations of test compounds were incubated with 3.0 nM (5-(6-(1'H-spiro(1-benzofuran-3,3'-pyrrolidin)-1'-yl)pyridazin-3-yl)-1,2,4-oxadiazol-3-yl)[$^3H_2$]methanol (^{3}H-**A**) for 120 min. Bound ligands on microsomes were captured on GF/B filter plates (PerkinElmer) pretreated with 0.3% polyethyleneimine and were separated from free ligands using rapid filtration with a Filtermate Harvester (PerkinElmer) followed by five washes in 300 µL of 10 mM Tris–HCl buffer (pH 7.5). Filter plates were then dried and radioactivity was measured after adding 25 µL of Microscint-O (PerkinElmer) using a TopCount liquid scintillation counter (PerkinElmer). DMSO and 10 µM nonlabeled

compound **A** were used as 100% and 0% controls, respectively. IC_{50} values of test compounds were determined by fitting the following three-parameter logistic equation to the data; % control = bottom + (top − bottom)/(1 + $10^{(\log[??]-\log IC_{50})}$).

Cell viability (GI) assay

HTC116 cells were seeded at 9 × 10^2 cells/well in 384-well cell culture plates (Corning, Corning, NY) and cultured 24 h. Then, test compounds were diluted in growth medium to the desired final concentration and added to the cells. After 72 h of incubation, cell proliferation was measured with a CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corporation, Madison, WI). GI_{50} values of test compounds were determined by fitting the Hill equation to the data, where bottom was fixed to 0, by using GraphPad Prism software (GraphPad, San Diego, CA, USA).

***In vivo* pharmacodynamics (PD) testing and lipidomics**

Mice were housed and maintained within the facility at Takeda Pharmaceutical Company Shonan Research Center in accordance with the Takeda Experimental Animal Care and Use Committee approved protocol. Athymic nude mice were injected with 5 × 10^6 HCT116 cells. When the tumor volumes reached approximately 200 mm^3, mice were randomly assigned to control or treatment groups. The compounds were orally administered to the mice in 0.5% methylcellulose (Shin-Etsu Chemical Co., Ltd., Tokyo, Japan). Tumors were collected 16 hours after twice administration of compound **1o**. Tumors were homogenized in isopropanol (100 mg/mL) for lipidomics, using TissueLyzerII (Qiagen), and centrifuged at 21500 g for 5 min. The supernatants were applied to lipidomics analysis.

Each 5 μL sample was injected onto a XBridge BEH C18 column (2.1 ×30 mm, 2.5 μm, Shiseido, Kyoto, Japan) maintained at 60°C, and the metabolites were separated by gradient elution of mobile phase A, 0.01% acetic acid, 1 mM NH_3 and 10 μM EDTA-2Na in MilliQ water, and of mobile phase B, 0.001% acetic acid and 0.2 mM NH_3 in ethanol/isopropanol (1:1), with the flow rate set to 0.5 mL/min. The eluate was introduced to an Orbitrap XL Mass Spectrometer (Thermo Fisher Scientific inc., San Jose, CA, USA), and the raw LC/MS data were processed by Expressionist Refiner MS software (ver.8.2, Genedata AG, Basel, Switzerland). Desaturation Index (DI) was calculated as the ratio of the unsaturated lipid molecule (PC C36:3) to the saturated lipid molecule (PC C36:0).

***In vivo* efficacy testing and statistical analysis**

All experimental procedures were approved by Shanghai Medicilon Inc., which was fully accredited by Association for Assessment and Accreditation of Laboratory Animal Care International. Athymic nude mice (BALB/cA Jcl-nu/nu) and 786-O Human renal cell adenocarcinoma cell line were purchased from Shanghai SINO-British SIPPR/BK Lab Animal Ltd (Shanghai, China) and ATCC (American Type Culture Collection), respectively. Seven weeks old female balb/c nu/nu mice were inoculated subcutaneously in the flank (cell suspension, 1:1 with matrigel) with 3.0 × 10^6 786-O cells. Tumor growth was monitored with vernier calipers. The mean tumor volume was calculated using the formula Volume = $Width^2$ × Length/2. When the mean

tumor volume reached approximately 130 mm^3, the animals were randomized into 8 treatment groups (n=8/group). Mice were then dosed with 0.5% methylcellulose or compound **1o** at 1 mg/kg over a 28 day period. The dosing volume for each mouse was 10 mL/kg. Tumor growth was measured twice per week. Tumor growth inhibition was calculated on Day 28 of treatment.

All statistical analyses were carried out using SAS System, Release 9.3 (SAS Institute Inc [Cary, NC, USA]).

References

1. a) Kim, Y.-C.; Ntambi, M. J. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 1. b) Zhang, Z.; Dales, A. N.; Winther, D. M. *J. Med. Chem.*, **2014**, *57*, 5039.
2. a) Dobrzyn, P.; Dobrzyn, A.; Miyazaki, M.; Cohen, P.; Asilmaz, E.; Hardie, D. G.; Friedman, M. J.; Ntambi, M. J. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 6409. b) Hulver, W. M.; Berggren, R. J.; Carper, J. M.; Miyazaki, M.; Ntambi, M. J.; Hoffman, P. E.; Thyfault, P. J.; Stevens. R.; Dohm, G. L.; Houmard. A. J.; Muoio, M. D. *Cell Metab.* **2005**, *2*, 251.
3. a) Liu, G. *Expert Opin. Ther. Patents* **2009** *19*, 1169. b) Powell, D. A. *Expert Opin. Ther. Patents* **2014**, *24*, 155. c) Uto, Y. *Chem. Phys. Lipids* **2016**, *197*, 3. d) Liu, G.; Lynch, J. K.; Freeman, J.; Liu, B.; Xin, Z.; Zhao, H.; Serby, M. D.; Kym, P. R.; Suhar, T. S.; Smith, H. T.; Cao, N.; Yang, R.; Janis, R. S.; Krauser, J. A.; Cepa, S. P.; Beno, D. W. A.; Sham, H. L.; Collins, C. A.; Teresa K. Surowy, T. K.; Camp, H, S. *J. Med. Chem.*, **2007**, *50*, 3086. e) Atkinson, A. K.; Beretta, E. E.; Brown, A. J.; Castrodad, M.; Chen, Y.; Cosgrove, M. J., Du, P.; Litchfield, J.; Makowski, M.; Martin, K.; McLellan, J. T.; Neagu, C.; Perry, A. D.; Piotrowski, W. D.; Steppan, M. C.; Trilles, R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1621. f) Renata M. Oballa, M. R.; Belair, L.; Black, C. W.; Bleasby, K.; Chan, C. C.; Desroches, C.; Du, X.; Gordon, R.; Guay, J.; Guiral, S.; Hafey, M. J.; Hamelin, E.; Huang, Z.; Kennedy, B.; Lachance, N.; Landry, F.; Li, S. C.; Mancini, J.; Normandin, D.; Pocai, A.; Powell, D. A.; Ramtohul, K. Y.; Skorey, K.; Sørensen, D.; Sturkenboom, W.; Styhler, A.; Waddleton, M. D. *J. Med. Chem.*, **2011**, *54*, 5082. g) Deng, Y.; Yang, Z.; Shipps Jr, W. G.; Lo, S-M.; West, R.; Hwa, J.; Zheng, S.; Farley, C.; Lachowicz, J.; Heek, M.; Bass, S. A.; Sinha, P. D.; Mahon, R. C.; Cartwright, E. M. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 791. h) Yang, S-M.; Tang, Y.; Rano, T.; Lu, H.; Kuo, G-H.; Gaul, D. M.; Li, Y.; Ho, G.; Lang, W.; Conway, G. J.; Liang, Y.; Lenhard, M. J.; Demarest, T. K.; Murray, V. W. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1437. i) Sun, S.; Zhang, Z.; Pokrovskaia, N.; Chowdhury, S.; Jia, Q.; Chang, E.; Khakh, K.; Kwan, R.; McLaren, G. D.; Radomski, C. C.; Ratkay, G. L.; Fu, J.; Dales, A. D.Winther, D. M. *Bioorg. Med. Chem.* **2015**, *23*, 455. j) Silvers, W. C.; Cai, H.; Öz, O. K.; Sun, X. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 924. and references cited therein.
4. a) Holder, M. A.; Gonzalez-Angulo, M. A.; Chen, H.; Akcakanat, A.; Do, K-A..; Symmans, W. Fraser.; Pusztai, L.; Hortobagyi, N. G.; Mills, B. G.; Meric-Bernstam, F. *Breast Cancer Res. Treat.* **2013**, *137*, 319. b) Roongta, V. U.; Pabalan, G. J.; Wang, X.; Ryseck, R.-P.; Fargnoli, J.; Henley, J. B.; Yang, W.-P.; Zhu, J.; Madireddi, T. M.; Lawrence, R. M.; Wong, W. T.; Rupnow, A. B. *Mol. Cancer Res.* **2011**, *9*, 1551. c) Ide,

Y.; Waki, M.; Hayasaka, T.; Nishio, T.; Morita, Y.; Tanaka, H.; Sasaki, T.; Koizumi K.; Matsumura, R.; Hosokowa, Y.; Ogura, H.; Shiiya, N.; Setou, M. *PLoS One* **2013**, *8*, e61204.

5. Mohammadzadeh, F.; Mosayebi, G.; Montazeri, V.; Darabi, M.; Fayezi, S.; Shaaker, M.; Rahmati, M.; Baradaran, B.; Mehdizadeh, A; Darabi M. *J. Breast Cancer* **2014**, *17*, 136.
6. a) Roemeling, A. C.; Marlow, A. L.; Wei, J. J.; Cooper, J. S.; Caulfield, R. T.; Wu, K.; Tan, W. W.; Tun, W. H.; Copland, A. J. *Clin Cancer Res* **2013**, *19*, 2368. b) Wang, H.; Zhang, Y.; Lu, Yongning.; Song, Jiajia.; Huang, M.; Zhang, J.; Huang, Yiran. *Tumor Biology* **2016**, *37*, 479.
7. Taniguchi, T.; Miyata, K.; Kubo, O. Preparation of pyridazine ring-containing spiro compounds as stearoyl-CoA desaturase inhibitors for treatment of obesity, diabetes, etc. WO2008096746 A1, Aug 14, 2008.
8. a) Bai, Y.; McCoy, G. J.; Levin J. E.; Sobrado, P.; Rajashankar, R. K.; Fox, G. B.; Zhou M. *Nature* **2015**, *524*, 252. b) Wang, H.; Klein, G. M.; Zou, H.; Lane, W.; Snell G.; Levin I.; Li, K.; Sang, B.-C. *Nat Struct Mol Biol.* **2015**, *22*, 581.
9. Amegadzie, A. K.; Gardinier, K. M.; Hembre, E. J.; Hong, J. E.; Jungheim, L. N.; Muehl, B. S.; Remick, D. M.; Robertson, M. A.; Savin, K. A. Preparation of triazole derivatives as tachykinin receptor antagonists. WO2003091226 A1, Nov 06, 2003.
10. Yoshida, Y.; Sakakura, Y.; Aso, N.; Okada, S.; Tanabe, Y. *Tetrahedron*, **1999**, *55*, 2183.
11. a) Matsuoka, N.; Iwashita, A.; Yamazaki, S.; Miyake, H.; Ohkubo, M.; Kamijo, K.; Nakanishi, I.; Hattori, K.; Kido, Y.; Ishida, J.; Yamamoto, H. Preparation of 2-substituted-4(3H)-quinazolinone derivatives as PARP inhibitors, WO2002048117 A1, Jun 20, 2002. b) Cid-Nunez, J. M.; Oehlrlch, D.; Trabanco-Suarez, A. A.; Tresadern, G. J.; Vega R., Juan A.; MacDonald, G. J. Preparation of 1,2,3-triazolo[4,3-a]pyridines for the treatment or prevention of neurological and psychiatric disorders. WO2010130424 A1, Nov 18 2010. c) Patane, M. A.; Bock, M. G.; Freidinger, R. M.; Ponticello, Rose A.; Newton, R. C. Preparation of N-[4-(arylpiperidino)alkyl]aralkanoamides and analogs as α1a-adrenergic receptor antagonist. US5977115 A, Nov 02 1999. d) Castelhano, A. L.; Mckibben, B.; Steinig, A. G. Preparation of pyrrolopyrimidine A2b selective antagonist compounds, method of synthesis and therapeutic use., WO 2003053361 A2, Jul 03 2003. e) Breslin, H. J.; Chatterjee, S.; Diebold, J. L.; Dorsey, B. D.; Dunn, D.; Gingrich, D. E.; Hostetler, G. A.; Hudkins, R. L.; Hunter, R.; Josef, K.; Lisko, J.; Mesaros, E. F.; Milkiewicz, K. L.; Ott, G. R.; Sundar, B. G.; Theroff, J. P.; Thieu, T.; Tripathy, R.; Underiner, T. L.; Weinberg, L.; Wells, G. J.; Zificsak, C. A. Preparation of pyrrolotriazines as inhibitors of ALK and JAK2 kinases for treating proliferative diseases., WO 2010071885 A1, Jun 24 2010. f) Howell, M. J.; Feng, K.; Clark, R. J.; Trzepkowski, J. L.; White, M. C. *J. Am. Chem. Soc.* **2015**, *137*, 14590.
12. a) Imamura, K.; Tomita, N.; Ito, Y.; Ono, K.; Maezaki, H.; Nii, N. Preparation of pyridazine compounds for prophylactic and therapeutic treatment of cancer. WO2015137385 A1, Sep 17, 2015. b) Miyahisa, I.; Suzuki, H.; Mizukami, A.; Tanaka, Y.; Ono, M.; Hixon, S. M.; Matsui, J. *ACS Med. Chem. Lett.* **2016** *7*, 868.
13. a) Hansch, C.; Leo, A.; Unger, H. S.; Kim, H. K.; Nikaitani, D.; Lien, J. E. *J. Med. Chem.*, **1973**, *16*, 1207. b) Ojha, K. P.; Roy, K. *Eur. J. Med. Chem.*, **2010**, *45*, 4645. and references cited therein.

14. Craig, P. N.; Guidelines for drug and analog design. Wolff, M. E., Ed. *The Basis of Medicinal Chemistry*. Wiley-Interscience, New York, pp 331-348.
15. Nishizawa, S.; Sumi, H.; Satoh, Y.; Yamamoto, Y.; Kitazawa, S.; Honda, K.; Araki, H.; Kakoi, K.; Imamura, K.; Sasaki, M.; Miyahisa, I.; Satomi, Y.; Nishigaki, R.; Hirayama, M.; Aoyama, K.; Maezaki, H.; Hara, T. *Eur. J. Pharmacol.*, **2017**, *807*, 21.

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Graphical Abstract