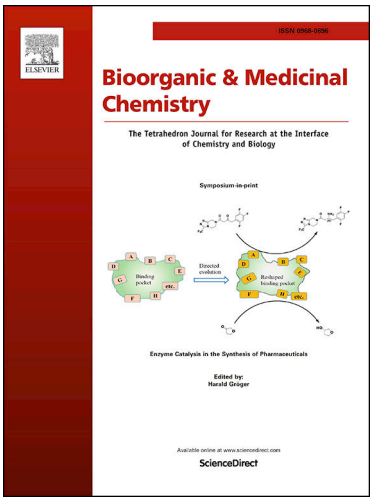
Journal Pre-proofs



Discovery of 1,8-Naphthyridin-2-one Derivative as a Potent and Selective Sphingomyelin Synthase 2 Inhibitor

Takafumi Yukawa, Takashi Nakahata, Rei Okamoto, Yuji Ishichi, Yasufumi Miyamoto, Satoshi Nishimura, Tatsuo Oikawa, Kazuki Kubo, Ryutaro Adachi, Yoshinori Satomi, Masanori Nakakariya, Nobuyuki Amano, Masahiro Kamaura, Nobuyuki Matsunaga

PII: S0968-0896(20)30172-3
DOI: https://doi.org/10.1016/j.bmc.2020.115376
Reference: BMC 115376

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 3 December 2019
Revised Date: 1 February 2020
Accepted Date: 6 February 2020

Please cite this article as: T. Yukawa, T. Nakahata, R. Okamoto, Y. Ishichi, Y. Miyamoto, S. Nishimura, T. Oikawa, K. Kubo, R. Adachi, Y. Satomi, M. Nakakariya, N. Amano, M. Kamaura, N. Matsunaga, Discovery of 1,8-Naphthyridin-2-one Derivative as a Potent and Selective Sphingomyelin Synthase 2 Inhibitor, *Bioorganic & Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.bmc.2020.115376

ELSEVIER

Bioorganic & Medicinal Chemistry
journal homepage: www.elsevier.com

Discovery of 1,8-Naphthyridin-2-one Derivative as a Potent and Selective Sphingomyelin Synthase 2 Inhibitor

Takafumi Yukawa*, Takashi Nakahata, Rei Okamoto, Yuji Ishichi, Yasufumi Miyamoto, Satoshi Nishimura, Tatsuo Oikawa, Kazuki Kubo, Ryutaro Adachi, Yoshinori Satomi, Masanori Nakakariya, Nobuyuki Amano, Masahiro Kamaura, and Nobuyuki Matsunaga

Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd., 26-1, Muraoka-higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

* Corresponding author. Tel.: +81-466-62-1111; fax: +81-466-29-4450; e-mail: takafumi.yukawa@takeda.com

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
sphingomyelin synthase 2
SMS2
2-quinolone
1,8-naphthyridin-2-one

ABSTRACT

Sphingomyelin synthase 2 (SMS2) has attracted attention as a drug target for the treatment of various cardiovascular and metabolic diseases. The modification of a high throughput screening hit, 2-quinolone **10**, enhanced SMS2 inhibition at nanomolar concentrations with good selectivity against SMS1. To improve the pharmaceutical properties such as passive membrane permeability and aqueous solubility, adjustment of lipophilicity was attempted and 1,8-naphthyridin-2-one **37** was identified as a potent and selective SMS2 inhibitor. A significant reduction in hepatic sphingomyelin levels following repeated treatment in mice suggested that compound **37** could be an effective in vivo tool for clarifying the role of SMS2 enzyme and developing the treatment for SMS2-related diseases.

1. Introduction

Sphingomyelin (SM) is one of the most abundant component of sphingolipids in plasma membrane.[1–3] The high packing density of SM in the plasma membrane contributes to the formation of a rigid barrier against the extracellular environment, and has an important role in various cell functions, such as adhesion, migration, and proliferation.[4] The interaction of SM and cholesterol drives the formation of lipid raft microdomains and impacts the function of receptor-mediated signal transduction and membrane trafficking.[4–7]

Sphingomyelin synthase (SMS) is an enzyme that catalyzes the final step in the SM biosynthetic pathway and directly controls SM levels.[8,9] It catalyzes the transfer of the phosphorylcholine head group from phosphatidylcholine onto the primary hydroxy moiety of ceramide, producing SM and diacylglycerol via a catalytic process (Figure 1). Similar to other phosphatases of lipid phosphates, the de novo SM pathway is thought to be a reversible reaction that regulates the balance between phosphoglycerolipids and phosphosphingolipids.[9,10]

There are two isoforms of mammalian SMSs, SMS1 and SMS2, which has a 57% sequence similarity.[11,12] SMS-related protein (SMSr) is also recognized as the SMS family, however, SMSr does not exhibit SM synthetic behavior.[13] Despite their highly similar catalytic activity, SMS1 and SMS2 are distinguished from each other by the cellular localization. SMS1 is primarily localized at the *trans*-Golgi apparatus, whereas SMS2 predominantly appears in the exoplasmic surface of the plasma membrane.[11,12]

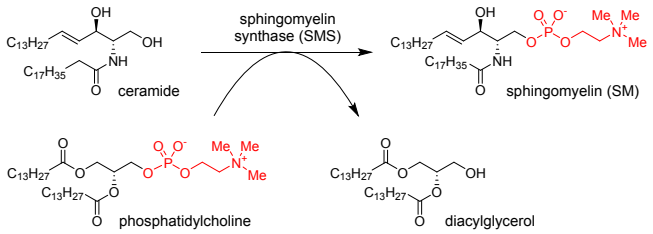


Figure 1. Biosynthesis of sphingomyelin catalyzed by SMS

Recent genetic analyses have revealed the essential in vivo functions of each SMS species. SMS2 deficiency in mice exhibits in reduced inflammatory response in macrophages[14] and amelioration of atherosclerosis.[15,16] It also results in the enhancement of insulin sensitivity[17] and resistance to high fat-induced obesity.[18] Thus, SMS2 inhibition appears to be beneficial in the treatment of cardiovascular and metabolic diseases. In contrast, SMS1 deficiency in mice led to lipid storage disorders[19] and severely impaired insulin secretion ability.[20] It has been reported that SMS1 knockout mice also exhibit hearing impairment[21] and T-cell dysfunction.[22] Therefore, the high selectivity for SMS2 against SMS1 should be essential for the development of SMS2 inhibitors without adverse events that arise from SMS1 inhibition.

Despite the attractive functions of SMS2 inhibitors, only a few studies have been conducted in this field. Potassium

tricy
reported as a cytotoxic anti-virus and anti-tumor compound.[23–28] However, D609 showed very weak SMS inhibition (IC_{50}: > 100 μM) and no information was available on the inhibition of SMS subtypes. Our group has previously reported 2-quinolones and 1,8-naphthyridin-2-ones with highly potent SMS2 inhibitory ability and selectivity against SMS1.[29] More recently, Zhou and Ye reported benzo[*d*]isoxazoles[30] and 2-benzyloxybenzamides[31] as potent SMS2 selective inhibitors, developed from α-aminonitriles[32–34] and oxazolopyridines[35] that inhibit SMS2 at micromolar concentrations. Through an in vivo study using a mouse model of T2DM, they revealed the biological relationship between SMS2 and expression of several cytokines and effects on the development of atherosclerosis.

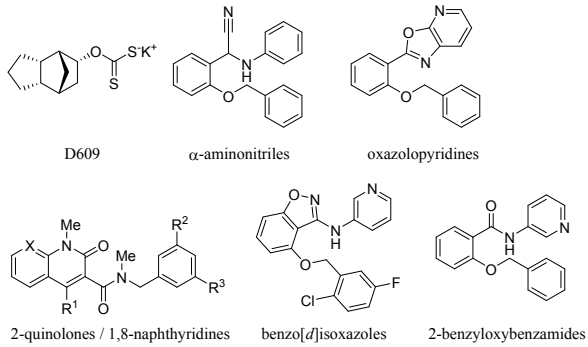


Figure 2. Chemical structures of SMS2 inhibitors

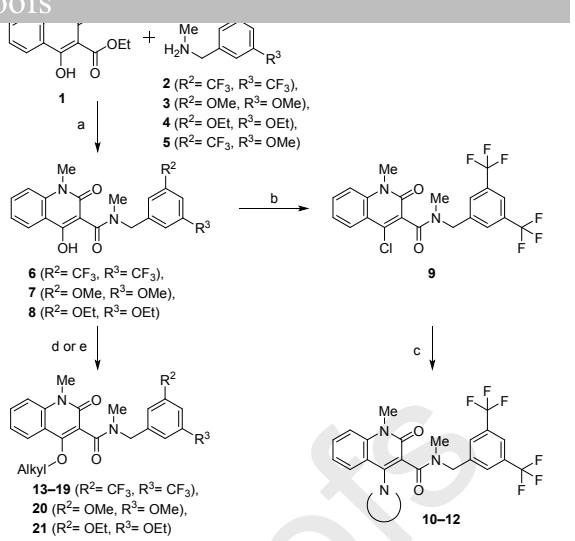
In this paper, we report the detailed design and synthesis of 1,8-naphthyridin-2-ones as novel, potent, and selective SMS2 inhibitors. In addition, we describe the reduction in hepatic SM levels as an in vivo biomarker in mice by treatment with 1,8-naphthyridin-2-one derivative.

2. Chemistry

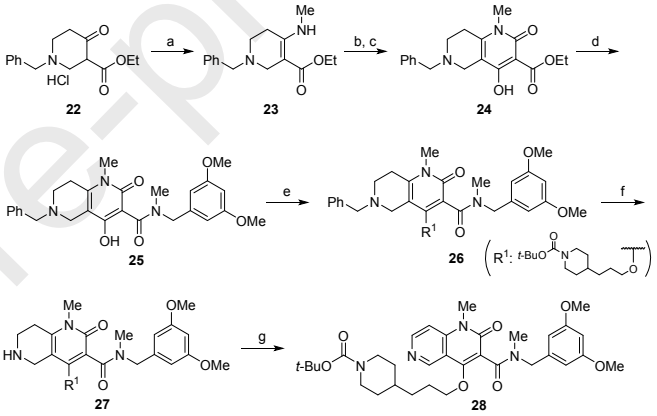
The synthesis of 2-quinolone derivatives **10**–**21** is shown in Scheme 1. Amide formation of ester **1** and benzylamines **2**–**4** gave *N*-benzyl-*N*-methylamides **6**–**8**. Compounds **6**–**8** were subjected to chlorination, followed by amination to afford cyclic amines **10**–**12**. Alkylation of **6**–**8** or Mitsunobu reaction using Tsunoda's reagent[36] produced alkoxy derivatives **13**–**21**.

The synthesis of naphthyridin-2-one derivatives **28** and **32**–**34** is shown in Schemes 2 and 3. Enamine formation of *β*-ketoester **22** with methylamine gave compound **23**. After acylation of **23**, pyridone derivative **24** was obtained by Dieckmann condensation under basic conditions. Compound **24** was subjected to amide formation, followed by alkylation to give *N*-benzyl-*N*-methylamide derivative **26**. After removal of the benzyl protection on piperidine by catalytic hydrogenation, the piperidine ring of **27** was oxidized by palladium on carbon under open-air conditions to afford 1,6-naphthyridin-2-one derivative **28**.

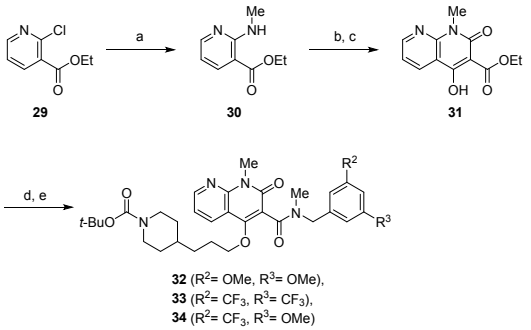
1,8-Naphthyridin-2-one derivative **31** was also synthesized from compound **29** through amination with methylamine, *N*-acylation, and Dieckmann condensation. *N*-Benzyl-*N*-methylamide derivatives **32**–**34** were synthesized via alkylation by a similar procedure used for the preparation of 2-quinolone derivatives, as shown in Scheme 1.



Scheme 1. a) $NaHCO_3$, toluene, 120 °C, 68%–quantitative yield; b) $POCl_3$, MeCN, 90 °C, 99%; c) cyclic amine, Et_3N, DMSO, 80 °C, 87–94%; d) alkylhalide or alkylmethanesulfonate, K_2CO_3, DMF, 60–90 °C, 45%–quantative yield; e) alkylalcohol, Tsunoda's reagent, toluene, 90 °C, 38%.

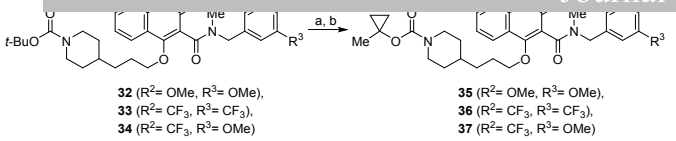


Scheme 2. a) $MeNH_2$, MeOH, 50 °C, quantative yield; b) ethyl malonylchloride, K_2CO_3, DMF, 100 °C, 44%; c) NaOEt, EtOH, 50 °C, 48%; d) **3**, toluene, 110 °C; e) *N*-Boc-piperidinylpropyl methanesulfonate, K_2CO_3, DMF, 100 °C, 78%; f) Pd/C, H_2, EtOH–THF, 60 °C, 86%; g) Pd/C, air, *n*-BuOH, 160 °C, 62%.



Scheme 3. a) $MeNH_2$, THF, 40 °C, quantative yield; b) ethyl malonylchloride, DMAP, DIPEA, THF, rt; c) KO*t*-Bu, THF, rt, 67% for two steps; d) **2**, **3** or **5**, toluene, 100–120 °C, 70–77%; e) *N*-Boc-piperidinylpropyl methanesulfonate, K_2CO_3, DMF, 90 °C, 82–83%.

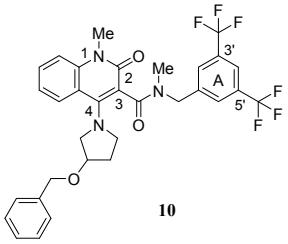
Furthermore, conversion of the carbamate moiety of **32**–**34** was performed as shown in Scheme 4. After the *tert*-butoxycarbonyl group was deprotected under acidic conditions, the 1-methylcyclopropyloxycarbonyl group was introduced to afford 1-methylcyclopropylcarbamate derivatives **35**–**37**.



Scheme 4. a) HCl, AcOEt–EtOH, rt or TFA, rt; b) 1-methylcyclopropyl 4-nitrophenyl carbonate, Et_3N, THF, rt, 67%–quantative yield for two steps.

3. Results and discussion

2-Quinolone derivative **10** was identified from a high-throughput screening campaign with an IC_{50} value of 610 nM for human SMS2 inhibition and 15000 nM for human SMS1 inhibition (Figure 3). The initial exploration of the structure-activity relationship (SAR) indicates that this scaffold has little tolerance for a change in substituents on the 2-quinolone and that the introduction of polar units mostly leads to a significant decrease in SMS2 inhibition. For example, a methyl group at the 1-position and an oxo group at the 2-position of quinolone are quite important because removal of these groups significantly dropped SMS2 inhibition. At the 3-position, the *N*-benzyl-*N*-methylamide moiety is also necessary for SMS2 inhibition to occur. This amide could not be changed by other functional moieties and the activity was diminished by the removal of either the benzyl group or the methyl group of the amide. In addition, 3',5'-disubstitution of the phenyl group (ring A) was found to be optimum and no substituents on the 2'-position or 4'-position of ring A were accepted.



10

SMS2 IC_{50} (human / mouse): 610 nM / 660 nM
SMS1 IC_{50} (human): 15000 nM

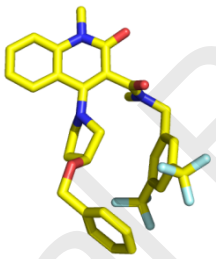


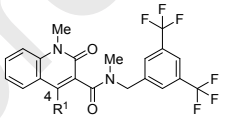
Figure 3. The structure of hit compound **10** (left) and the predicted stable conformation (right)

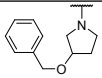
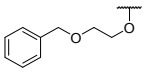
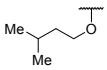
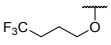
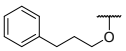
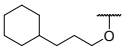
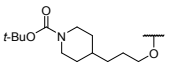
The three-dimensional information was obtained from the conformational search by Schrödinger Maestro, as shown in Figure 3. The calculated structure displays the characteristic twist conformation between the *N*-benzyl-*N*-methylamide group and the quinolone ring at an angle of approximately 90 degrees. This orthogonal structure was presumably caused by large steric repulsion of the *N*-benzyl-*N*-methylamide moiety and the oxo group at the 2-position and the pyrrolidine group at the 4-position on the quinolone. On the basis of the initial SAR and the conformational information of **10**, it was thought that the immobilized conformation of the scaffold might be essential for SMS2 inhibitory activity. Thus, for the reinforcement of SMS2 inhibitory activity, optimization of the lipophilic substituent at the 4-position on the quinolone was investigated, keeping the other substituents constant.

First, R^1 substituents at the 4-position of quinolone were explored as shown in Table 1. A slight decrease in SMS2 inhibition was observed by the removal of the benzyloxy group on pyrrolidine (compound **11**). Introduction of the piperidine group in the place of the pyrrolidine group led to a significant reduction in SMS2 inhibition (compound **12**). To evaluate the alkoxy substituents instead of rigid cyclic amines, compound **13** showed approximately two-fold more potent human SMS2 inhibition with the IC_{50} value of 380 nM, compared with compound **10**. From these findings, optimization of the alkoxy groups was explored to enhance SMS2 inhibition.

To explore the suitable substituents at R^1, several alkoxy groups were introduced, as in **14**–**19**. Compound **14** with the *n*-propoxy group showed decreased SMS2 inhibition, whereas compounds **15**–**18** with various lipophilic substituents maintained the potency of SMS2 inhibition. The SAR of these substituents indicated that a lipophilic bulkier moiety would be preferable at the terminal of the alkoxy group. Notably, SMS2 inhibition was boosted in compound **19** with the *N*-(*tert*-butoxycarbonyl)-piperidinylpropoxy group. This successful enhancement of SMS2 potency would be accomplished not only by its bulky lipophilic property but also by the specific interaction between the carbamate moiety and the SMS2 protein. The importance of carbamate moiety was also identified with the results that SMS2 inhibition was significantly reduced in the corresponding *N*-pivaloylpiperidines or *N*-alkylpiperidines (data not shown).

Table 1. In vitro SMS2 inhibition activity of **10**–**19**



cmpd	R^1	SMS2 IC_{50} (nM)		human SMS1 IC_{50} (nM)
		human	mouse	
10		610	660	15000
11		950	1100	>100000
12		11000	not tested	>100000
13		380	590	22000
14		2100	not tested	>100000
15		350	260	>100000
16		120	240	25000
17		210	120	14000
18		480	190	14000
19		30	7.7	3400

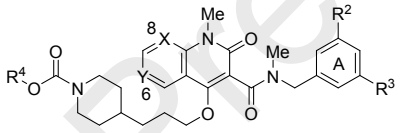
2-Quinolone **19** displayed strong SMS2 inhibition (human SMS2 IC_{50}: 30 nM, mouse SMS2 IC_{50}: 7.7 nM) and good selectivity against SMS1 (human SMS1 IC_{50}: 3400 nM). To confirm the in vivo effects of SMS2 inhibitors, there is a need to improving DMPK profiles, especially passive membrane permeability (PAMPA)[37] and aqueous solubility. With respect to PAMPA, high lipophilic compounds generally show good membrane permeability. In the present study, however, excess

lipo… 1.5, therefore planned to reduce the lipophilicity of **19**, although it appears to be challenging to effectively reduce lipophilicity from the initial SAR. Thus, we attempted three strategic approaches: replacement of the CF_3 groups with less lipophilic groups, introduction of an additional nitrogen atom in the quinolone ring, and modification of the *tert*-butyl group on the terminal carbamate moiety.

Initially, CF_3 groups at R^2 and R^3 were converted to alkoxy groups as in compound **20** and **21** (Table 2). As expected, dimethoxy derivative **20** significantly improved membrane permeability and aqueous solubility by lowering the CLogP value, although SMS2 inhibition was decreased. The diethoxygroup in **21** also resulted in a reduction of activity. To more balanced profiles and stronger inhibition, the introduction of a nitrogen atom in the quinolone was investigated. Although aqueous solubility was remarkably improved by the lower lipophilicity as expected, 1,6-naphthyridin-2-one **28** displayed a significant loss of SMS2 inhibition. The dropped SMS2 inhibition was also observed in 1,7-naphthyridin-2-ones analogues (data not shown). These results indicated that a nitrogen atom at the 6-position or 7-position of naphthyridine was not accepted, presumably owing to its basicity. In contrast, 1,8-naphthyridin-2-one **32** successfully exhibited almost equipotent inhibition to 2-quinolone **20** and greater aqueous solubility. It was thought that the nitrogen at the 8-position might be accepted owing to its lower basicity than the …and that of **28** is 4.4, as shown in Figure 4).[38] The difference of the predicted pKa value would be caused by the electron density of the nitrogen in each naphthyridine. Compared to **28**, the electron density of the nitrogen in **32** is thought to be reduced due to the inductive effect of the neighbor *N*-methylcarbonyl moiety. From these findings, we decided to continue further optimization based on the 1,8-naphthyridin-2-one scaffold.

The 1-methylcyclopropylcarbamate group is known as an attractive substituent, not only because of its acid-resistant properties, but also because of its lower lipophilicity than *tert*-butylcarbamate.[39] The introduction of the 1-methylcyclopropylcarbamate group was investigated for 1,8-naphthyridines instead of *tert*-butylcarbamate (compounds **35**–**37**). Although **35** with dimethoxy groups at R^2 and R^3, was less lipophilic than **33**, human SMS2 inhibition (300 nM) was insufficient. In contrast, **36** with trifluoromethyl groups at R^2 and R^3, showed potent SMS2 inhibition, although the aqueous solubility was low despite conversions at two key parts of **19**: introduction of a nitrogen on the fused ring and conversion of the *tert*-butyl moiety. The combination of a methoxy group and a trifluoromethyl group as for compound **37** successfully led to strong SMS2 inhibition as for **36**, but with slightly better solubility. Therefore, **37** with excellent SMS2 inhibition and good selectivity against SMS1 was selected for further biological evaluation.

Table 2. Biological properties of 2-quinolones **19**–**21** and naphthyridin-2-ones **28**, **32**, **35**–**37**.



cmpd	X	Y	R^2	R^3	R^4	CLogP	SMS2 IC_{50} (nM)		human SMS1 IC_{50} (nM)	PAMPA[a] (nm/sec)	solubility[b] (μg/mL)
							human	mouse			
19	CH	CH	CF_3	CF_3	*t*-Bu	7.3	30	7.7	3400	11	< 0.16
20	CH	CH	OMe	OMe	*t*-Bu	5.6	91	19	19000	295	1.8
21	CH	CH	OEt	OEt	*t*-Bu	6.6	380	51	15000	275	< 0.080
28	CH	N	OMe	OMe	*t*-Bu	4.6	1600	340	>100000	260	33
32	N	CH	OMe	OMe	*t*-Bu	4.6	130	32	10000	241	7.3
35	N	CH	OMe	OMe		4.3	300	14	18000	200	16
36	N	CH	CF_3	CF_3		6.0	47	3.0	850	721	< 0.30
37	N	CH	CF_3	OMe		5.3	45	2.2	2400	424	0.79

[a] Membrane permeability was measured by pH 7.4 solution. [b] Aqueous solubility was measured by pH 6.8 solution.

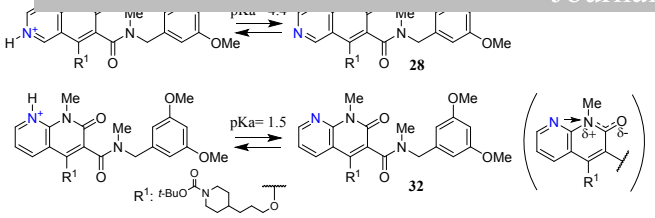


Figure 4. pKa value of 1,6-naphthylidine-2-one **28** and 1,8-naphthylidine-2-one **32** predicted by Schrödinger Jaguar.[38]

The X-ray single crystal structure of **36** contributes to the understanding of the stable conformation of the 1,8-naphthyridine series (Figure 5). The twist conformation was observed between 1,8-naphthyridine and the amide at the 3-position of 1,8-naphthyridine, similar to the predicted stable structure of hit compound **10**. The restricted movement of this conformation may effect a certain increase in SMS2 inhibition owing to immobilization of the piperidine ring, which will direct the carbamate group toward the key residues of the SMS2 protein.

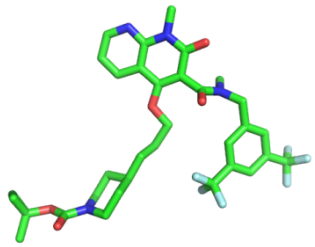
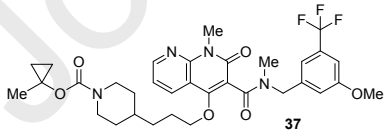


Figure 5. The X-ray single crystal structure of compound **36**.

The results of pharmacokinetic (PK) profiles of **37** are shown in Table 3 and Figure 6. A certain level of plasma drug exposure in KK-A^y/Ta mice was observed at a dose of 100 mg/kg in both oral and subcutaneous administration with different PK patterns. The PK profiles after oral administration displayed high C_{max} but a short duration of plasma drug concentration, whereas those after subcutaneous administration showed a lower C_{max} and an extended duration of the drug concentration. In particular, subcutaneous administration resulted in a much higher drug concentration at 24 h after dosing ($C_{24\ h}$= 0.232 μg/mL), compared with oral administration ($C_{24\ h}$= 0.007 μg/mL). As different patterns of PK were observed, we attempted to evaluate the effect of **37** on hepatic SM levels in in vivo studies following both oral and subcutaneous administrations.

Table 3. Pharmacokinetic parameters for **37** in KK-A^y/Ta mice after a single treatment.



cmpd[a]	C_{max} (μg/mL)	T_{max} (h)	$C_{24\ h}$ (μg/mL)	$AUC_{0\text{-}24h}$[b] (μg·h/mL)	MRT[c] (h)
37 (p.o.)	6.5	0.83	0.007	18	3.0
37 (s.c.)	0.70	8.0	0.23	12	9.4

[a]Compound was administered by 100 mg/kg, [b]AUC (μg・h/mL) denotes area under the curve after oral administration (0−24 h). [c]MRT (h) denotes mean residence time.

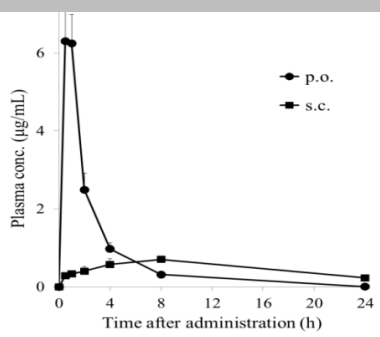


Figure 6. Pharmacokinetic data of **37** in KK-A^y/Ta mice after a single treatment.

Reduction in SM (22:0) levels in KK-A^y/Ta mice was tested after 7 days of repeated treatment of **37**, as shown in Figure 7. The change in SM (22:0) levels was not observed in oral administration. On the other hand, SM (22:0) levels were significantly reduced after subcutaneous treatment with **37** at both 100 mg/kg/day, q.d. and 200 mg/kg/day, b.i.d. Especially, a strong reduction of SM (22:0) level was observed at 200 mg/kg/day, b.i.d. compared with that at 100 mg/kg/day, q.d. The superiority of subcutaneous administration compared with oral administration suggests that a higher trough concentration after the treatment would be an important factor for the reduction in SM (22:0) levels.

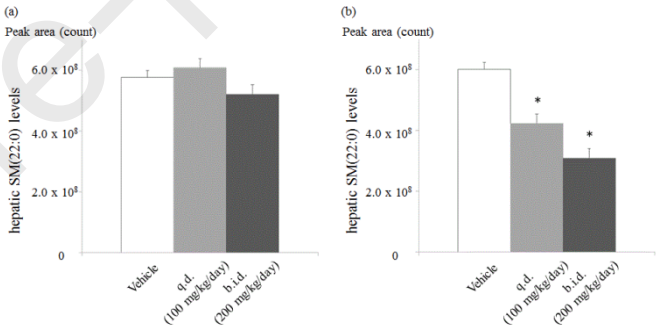


Figure 7. Reduction of hepatic SM (22:0) levels after seven days repeated treatment of **37** in KK-A^y/Ta mice (a) p.o. and (b) s.c. (*: $p < 0.05$ vs. vehicle).

4. Conclusions

In this study, 1,8-naphthyridin-2-one **37** was discovered as a potent and selective SMS2 inhibitor through the modification of the hit compound **10**. In the PK study of **37**, different PK profiles were observed following oral and subcutaneous administration. Subcutaneous administration of **37** significantly reduced the level of SM (22:0) as a target engaged biomarker for SMS inhibition, whereas oral administration of **37** did not reduce SM (22:0) level. In particular, the treatment of **37** (200 mg/kg/day, b.i.d., s.c.) achieved the strong reduction in the hepatic SM (22:0) level. These results suggest that the sufficient trough concentration of the drug would be crucial for in vivo efficacy in the repeated treatment.

Although several SMSs inhibitors have already been reported to date, there is still a demand for strong SMS2 inhibitors with high selectivity over SMS1 for the evaluation of SMS2-related biological functions. In this research, 1,8-naphthyridin-2-one **37** was identified to have excellent SMS2 activity and high selectivity against SMS1. Furthermore, subcutaneous treatment of **37** resulted in a significant reduction in the level of SM (22:0), which is an important in vivo biomarker for SMS inhibition.

would be an effective in vivo tool in addition to recently reported benzo[*d*]isoxazoles[30] and 2-benzyloxybenzamides,[31] to clarify the role of SMS2 enzyme and to develop a treatment for the SMS2-related diseases. Further optimization of **37** should generate more effective compounds for the treatment of SMS2-related diseases.

5. Experimental

General method. Melting points were determined in open capillary tubes on a Büchi melting pint apparatus B545 and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ADVANCE III (300 MHz) or Bruker Advance III plus (400 MHz) spectrometer. Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard in deuterated solvent and coupling constants (J) are in Hertz (Hz). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, ddd = doublet of doublet of doublet, dt = doublet of triplet, m = mutiplet, bs = broad signal), and coupling constants. All solvents and reagents were obtained from commercial suppliers and used without further purification. Thin-layer chromatography was performed using Purif-Pack (SI or NH, SHOKO SCIENTIFIC). LC-MS analysis was performed on a Shimadzu Liquid Chromatography-Mass Spectrometer System, operating in APCI (+ or -) or ESI (+ or -) ionization mode. Analytes were eluted using a linear gradient of 0.03% TFA containing water/acetonitrile or 5 mM ammonium acetate containing water/acetonitrile mobile phase, and detected at 220 nm. The purity of compounds submitted for biological evaluation was > 95% as determined by elemental analyses within ±0.4% of the calculated values. Yields have not yet been optimized.

5.1. Chemistry

*N-(3,5-Bis(trifluoromethyl)benzyl)-4-hydroxy-N,1-dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (**6**)*

To a mixture of **1** (1.0 g, 4.0 mmol) in toluene (20 ml) was added **2** (1.0 g, 4.0 mmol) at rt. The mixture was stirred at 120 °C for 12 h. The mixture was neutralized with aqueous 1 M HCl at 0 °C and extracted with AcOEt. The organic layer was separated, washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 30%–100% AcOEt in hexane). The residue was crystallized from AcOEt–IPE to give **6** (1.4 g, 76%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (3H, s), 3.68 (3H, s), 4.86 (2H, brs), 7.27–7.37 (2H, m), 7.58–7.73 (1H, m), 7.77–7.95 (3H, m), 8.05–8.19 (1H, m), 12.11 (1H, brs). LCMS (ESI$^+$) m/z 459.2.

N-(3,5-Dimethoxybenzyl)-4-hydroxy-N,1-dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (7)

Compound **7** was prepared in a manner similar to that described for **6** as a colorless solid. Yield: 85%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.80 (3H, brs), 3.60 (3H, s), 3.76 (6H, brs), 4.16–4.88 (2H, m), 6.21–8.13 (7H, m), 11.23 (1H, brs). LCMS (ESI$^+$) m/z 383.2.

*N-(3,5-Diethoxybenzyl)-4-hydroxy-N,1-dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (**8**)*

Compound **8** was prepared in a manner similar to that described for **6** as a white amorphous powder. Yield: quantative yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (6H, t, J = 7.0 Hz), 2.98 (3H, s), 3.66 (3H, s), 3.99 (4H, q, J = 7.0 Hz), 4.65 (2H, s), 5.99–6.61 (3H, m), 7.24 (1H, d, J = 0.8 Hz), 7.28–7.35 (1H, m), Hz), 12.23 (1H, brs). LCMS (ESI$^+$) m/z 411.2.

*N-(3,5-Bis(trifluoromethyl)benzyl)-4-chloro-N,1-dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (**9**)*

To a mixture of **6** (2.0 g, 4.4 mmol) in MeCN (10 ml) was added $POCl_3$ (10 ml, 110 mmol). The mixture was stirred at 90 °C for 3 h. The mixture was poured into iced water. The precipitation was filtered and washed with aqueous $NaHCO_3$ and water to afford **9** (1.8 g, 85%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.87–2.96 (3H, m), 3.63–3.72 (3H, m), 4.54–5.12 (2H, m), 7.39–7.50 (1H, m), 7.65–7.86 (2H, m), 7.98–8.18 (4H, m). LCMS (ESI$^+$) m/z 477.2.

*4-(3-(Benzyloxy)pyrrolidin-1-yl)-N-(3,5-bis(trifluoromethyl)benzyl)-N,1-dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (**10**)*

To a mixture of **9** (200 mg, 0.63 mmol) in DMSO (8.0 ml) was added 3-benzyloxypyrrolidine (150 mg, 0.84 mmol) and Et_3N (0.12 ml, 0.84 mmol). The mixture was stirred at 80 °C overnight. The mixture was quenched with water and extracted with AcOEt. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 5%–50% AcOEt in hexane) to afford **10** (225 mg, 87%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.94–2.22 (2H, m), 2.75–2.96 (3H, m), 2.99–3.28 (1H, m), 3.32–3.50 (2H, m), 3.53 (1H, d, J = 2.4 Hz), 3.59–3.61 (2H, m), 3.63–3.78 (1H, m), 4.17–4.31 (1H, m), 4.32–4.67 (3H, m), 4.77–5.27 (1H, m), 7.18–7.42 (6H, m), 7.45–7.54 (1H, m), 7.58–7.66 (1H, m), 7.93–8.02 (2H, m), 8.02–8.39 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.6, 31.6, 36.6, 49.2, 49.6, 58.4, 70.6, 77.6, 113.7, 115.4, 117.9, 121.2, 121.6, 123.9 (q, $J_{C–F}$ = 273 Hz), 127.0, 127.9, 128.0, 128.7, 128.9, 130.8 (q, $J_{C–F}$ = 33 Hz), 131.4, 138.9, 139.9, 141.4, 150.8, 160.2, 168.6. LCMS (ESI$^+$) m/z 618.3. Anal. Calcd for $C_{32}H_{29}F_6N_3O_3 \cdot 0.3H_2O$: C, 61.34; H, 4.79; N, 6.74. Found: C, 61.49; H, 4.96; N, 6.77.

*N-(3,5-Bis(trifluoromethyl)benzyl)-N,1-dimethyl-2-oxo-4-(pyrrolidin-1-yl)-1,2-dihydroquinoline-3-carboxamide (**11**)*

Compound **11** was prepared in a manner similar to that described for **10** as a colorless solid. Yield: 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.74–1.99 (4H, m), 2.75–3.02 (3H, m), 3.11–3.27 (2H, m), 3.42–3.66 (5H, m), 4.43–4.64 (1H, m), 4.77–5.23 (1H, m), 7.19–7.31 (1H, m), 7.43–7.56 (1H, m), 7.56–7.68 (1H, m), 7.89–7.98 (1H, m), 8.01 (1H, s), 8.11–8.32 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.1, 29.6, 36.7, 51.8, 113.6, 115.3, 118.2, 123.9 (q, $J_{C–F}$ = 273 Hz), 121.2, 121.6, 127.1, 129.0, 129.0, 130.8 (q, $J_{C–F}$ = 33 Hz), 131.3, 139.9, 141.4, 151.3, 160.3, 168.8. LCMS (ESI$^+$) m/z 512.2. Anal. Calcd for $C_{25}H_{23}F_6N_3O_2$: C, 58.71; H, 4.53; N, 8.22. Found: C, 58.77; H, 4.83; N, 8.27.

*N-(3,5-Bis(trifluoromethyl)benzyl)-N,1-dimethyl-2-oxo-4-(piperidin-1-yl)-1,2-dihydroquinoline-3-carboxamide (**12**)*

Compound **12** was prepared in a manner similar to that described for **10** as a colorless solid. Yield: 94%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.17–2.05 (6H, m), 2.75–2.99 (5H, m), 3.00–3.33 (2H, m), 3.63 (3H, s), 4.46–4.71 (1H, m), 4.79–5.20 (1H, m), 7.25–7.38 (1H, m), 7.45–7.60 (1H, m), 7.60–7.70 (1H, m), 7.90 (1H, dd, J = 8.1, 1.2 Hz), 7.98–8.06 (1H, m), 8.14–8.32 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.2, 26.3, 29.6, 32.4, 36.6, 49.7, 115.6, 118.4, 118.7, 121.2, 122.4, 123.9 (q, $J_{C–F}$ = 273 Hz), 126.0, 129.0, 130.8 (q, $J_{C–F}$ = 33 Hz), 131.6, 140.2, 141.3, 155.3, 160.2, 167.8. LCMS (ESI$^+$) m/z 526.2. Anal. Calcd for $C_{26}H_{25}F_6N_3O_2$: C, 59.43; H, 4.80; N, 8.00. Found: C, 59.48; H, 5.08; N, 8.02.

*dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (**13**)*

To a mixture of **6** (150 mg, 0.33 mmol) in DMF (6.0 ml) were added K_2CO_3 (140 mg, 0.98 mmol) and [(2-bromoethoxy)methyl]benzene (0.10 ml, 0.65 mmol) at rt. The mixture was stirred at 60 °C under N_2 overnight. The residue was purified by column chromatography (NH silica gel, eluted with 5%–100% AcOEt in hexane). The obtained residue was purified by column chromatography (silica gel, eluted with 10%–70% AcOEt in hexane) to give **13** (88 mg, 45%) as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.84–2.99 (3H, m), 3.53–3.68 (3H, m), 3.68–3.93 (2H, m), 4.09–4.36 (1H, m), 4.36–4.48 (1H, m), 4.48–4.62 (3H, m), 4.70–5.35 (1H, m), 7.23–7.42 (6H, m), 7.53–7.61 (1H, m), 7.65–7.75 (1H, m), 7.89–8.29 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.7, 36.4, 49.6, 69.0, 71.6, 72.6, 112.4, 115.3, 116.8, 121.2, 122.6, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.4, 128.0, 128.7, 128.8, 129.5, 130.9 (q, $J_{C–F}$ = 33 Hz), 132.5, 138.6, 139.6, 141.2, 158.2, 160.6, 166.6. LCMS (ESI$^+$) m/z 593.2. Anal. Calcd for $C_{30}H_{26}F_6N_2O_4$: C, 60.81; H, 4.42; N, 4.73. Found: C, 60.73; H, 4.45; N, 4.76.

*N-(3,5-Bis(trifluoromethyl)benzyl)-N,1-dimethyl-2-oxo-4-propoxy-1,2-dihydroquinoline-3-carboxamide (**14**)*

Compound **14** was prepared in a manner similar to that described for **13** as a colorless solid. Yield: 64%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86–1.10 (3H, m), 1.61–1.92 (2H, m), 2.75–3.09 (3H, m), 3.44–3.76 (3H, m), 3.89–4.07 (1H, m), 4.13–4.32 (1H, m), 4.46–5.35 (2H, m), 7.26–7.41 (1H, m), 7.48–7.64 (1H, m), 7.64–7.76 (1H, m), 7.88–8.30 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.7, 23.1, 29.7, 36.5, 49.6, 73.3, 111.7, 115.4, 116.8, 121.3, 122.6, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.2, 128.9, 130.9 (q, $J_{C–F}$ = 33 Hz), 132.4, 139.5, 141.2, 158.0, 160.6, 166.8. LCMS (ESI$^+$) m/z 501.1. Anal. Calcd for $C_{24}H_{22}F_6N_2O_3$: C, 57.60; H, 4.43; N, 5.60. Found: C, 57.64; H, 4.65; N, 5.66.

*N-(3,5-Bis(trifluoromethyl)benzyl)-N,1-dimethyl-4-(3-methylbutoxy)-2-oxo-1,2-dihydroquinoline-3-carboxamide (**15**)*

Compound **15** was prepared in a manner similar to that described for **13** as a colorless solid. Yield: 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.82–0.95 (6H, m), 1.54–1.88 (3H, m), 2.82–3.05 (3H, m), 3.50–3.73 (3H, m), 3.91–4.12 (1H, m), 4.20–4.38 (1H, m), 4.47–5.25 (2H, m), 7.34 (1H, t, J = 7.3 Hz), 7.49–7.63 (1H, m), 7.64–7.75 (1H, m), 7.87–8.28 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.6, 22.6, 24.9, 29.7, 36.5, 38.3, 49.6, 70.0, 111.5, 115.3, 116.8, 121.4, 122.6, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.2, 129.0, 129.6, 130.9 (q, $J_{C–F}$ = 33 Hz), 132.4, 139.5, 141.1, 157.9, 160.6, 166.8. LCMS (ESI$^+$) m/z 529.1. Anal. Calcd for $C_{26}H_{26}F_6N_2O_3$: C, 59.09; H, 4.96; N, 5.30. Found: C, 59.21; H, 4.86; N, 5.30.

*N-(3,5-Bis(trifluoromethyl)benzyl)-N,1-dimethyl-2-oxo-4-(4,4,4-trifluorobutoxy)-1,2-dihydroquinoline-3-carboxamide (**16**)*

Compound **16** was prepared in a manner similar to that described for **13** as a colorless solid. Yield: 65%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.90–2.07 (2H, m), 2.37–2.50 (2H, m), 2.85–3.02 (3H, m), 3.55–3.68 (3H, m), 4.03–4.16 (1H, m), 4.31–4.40 (1H, m), 4.51–5.26 (2H, m), 7.31–7.39 (1H, m), 7.55–7.62 (1H, m), 7.67–7.75 (1H, m), 7.91–8.28 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.7, 29.9, 125.1, 36.5, 49.6, 70.2, 111.9, 115.4, 116.6, 122.7, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.3, 125.2 (q, $J_{C–F}$ = 151 Hz), 128.9, 129.4, 129.8, 130.9 (q, $J_{C–F}$ = 33 Hz), 132.5, 139.5, 141.2, 157.7, 160.6, 166.6. LCMS (ESI$^+$) m/z 569.2. Anal. Calcd for $C_{25}H_{21}F_9N_2O_3$: C, 52.82; H, 3.72; N, 4.93. Found: C, 52.96; H, 3.91; N, 4.92.

*phenylpropoxy)-1,2-dihydroquinoline-3-carboxamide (**17**)*

Compound **17** was prepared in a manner similar to that described for **13** as a colorless amorphous powder. Yield: 81%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.94–2.18 (2H, m), 2.62–2.80 (2H, m), 2.81–2.95 (3H, m), 3.52–3.72 (3H, m), 3.86–4.09 (1H, m), 4.22–4.37 (1H, m), 4.40–5.23 (2H, m), 7.12–7.39 (6H, m), 7.50–7.61 (1H, m), 7.66–7.78 (1H, m), 7.86–8.26 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.7, 31.3, 31.8, 36.4, 49.5, 70.7, 111.1, 113.2, 115.3, 116.7, 121.3, 122.7, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.3, 126.4, 128.8, 128.9, 130.9 (q, $J_{C–F}$ = 33 Hz), 132.4, 139.5, 141.1, 141.4, 157.8, 160.6, 166.8. LCMS (ESI$^+$) m/z 577.2. Anal. Calcd for $C_{30}H_{26}F_6N_2O_3$: C, 62.50; H, 4.55; N, 4.86. Found: C, 62.60; H, 4.79; N, 4.90.

*N-(3,5-Bis(trifluoromethyl)benzyl)-4-(3-cyclohexylpropoxy)-N,1-dimethyl-2-oxo-1,2-dihydroquinoline-3-carboxamide (**18**)*

To a mixture of **6** (200 mg, 0.44 mmol) in toluene (8.0 ml) was added 3-cyclohexyl-1-propanol (0.20 ml, 1.3 mmol) and cyanomethylenetributylphosphorane (0.36 ml, 1.3 mmol) at rt. The mixture was stirred at 90 °C under Ar atmosphere overnight. The mixture was quenched with water and extracted with AcOEt. The organic layer was separated, washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 10%–50% AcOEt in hexane) to give **18** (96 mg, 38%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80–0.94 (2H, m), 1.08–1.30 (6H, m), 1.56–1.80 (7H, m), 2.84–3.00 (3H, m), 3.54–3.68 (3H, m), 3.87–4.02 (1H, m), 4.16–4.30 (1H, m), 4.54–5.19 (2H, m), 7.29–7.38 (1H, m), 7.53–7.61 (1H, m), 7.66–7.76 (1H, m), 7.88–8.28 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.3, 26.6, 27.1, 29.7, 33.2, 33.4, 36.5, 37.2, 49.6, 72.0, 111.5, 115.3, 116.8, 121.3, 122.6, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.2, 129.1, 131.1 (q, $J_{C–F}$ = 33 Hz), 132.4, 139.5, 141.1, 158.0, 160.6, 130.4. LCMS (ESI$^+$) m/z 583.2. Anal. Calcd for $C_{30}H_{32}F_6N_2O_3$: C, 61.85; H, 5.54; N, 4.81. Found: C, 61.88; H, 5.53; N, 4.87.

*tert-Butyl 4-(3-((3-((3,5-Bis(trifluoromethyl)benzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)propyl)piperidine-1-carboxylate (**19**)*

Compound **19** was prepared in a manner similar to that described for **13** as a colorless amorphous powder. Yield: 60%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91–1.05 (2H, m), 1.27–1.40 (12H, m), 1.58–1.79 (4H, m), 2.58–2.74 (2H, m), 2.96 (3H, s), 3.64 (3H, s), 3.87–4.02 (3H, m), 4.19–4.29 (1H, m), 4.52–5.21 (2H, m), 7.30–7.37 (1H, m), 7.53–7.61 (1H, m), 7.65–7.74 (1H, m), 7.88–8.28 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.9, 28.6, 29.7, 32.1, 32.6, 35.4, 36.5, 43.9, 49.6, 71.9, 78.8, 111.6, 115.3, 116.8, 121.3, 122.6, 123.9 (q, $J_{C–F}$ = 273 Hz), 124.2, 129.1, 130.9 (q, $J_{C–F}$ = 33 Hz), 132.4, 139.5, 141.2, 154.3, 158.0, 160.6, 166.7. LCMS (ESI$^+$) m/z 584.2.

*tert-Butyl 4-(3-((3-((3,5-Dimethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)propyl)piperidine-1-carboxylate (**20**)*

Compound **20** was prepared in a manner similar to that described for **13** as a colorless amorphous powder. Yield: 68%. 1H NMR (300 MHz, DMSO-d_6) δ 0.89–1.06 (2H, m), 1.26–1.45 (12H, m), 1.57–1.83 (4H, m), 2.55–2.77 (2H, m), 2.87 (3H, s), 3.56–3.67 (3H, m), 3.67–3.80 (6H, m), 3.85–4.09 (3H, m), 4.17–4.83 (3H, m), 6.36–6.69 (3H, m), 7.28–7.38 (1H, m), 7.51–7.61 (1H, m), 7.64–7.75 (1H, m), 7.90–7.98 (1H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.9, 28.6, 29.7, 32.1, 32.2, 32.5, 35.4, 36.1,

50.0 … 3.79 124.2, 132.3, 139.5, 139.6, 154.3, 157.7, 160.5, 161.1, 166.1. LCMS (ESI$^+$) m/z 608.3. Anal. Calcd for $C_{34}H_{45}N_3O_7 \cdot 0.4H_2O$: C, 66.41; H, 7.51; N, 6.83. Found: C, 66.54; H, 7.30; N, 6.84.

*tert-Butyl 4-(3-((3-((3,5-Diethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)propyl)piperidine-1-carboxylate (**21**)*

Compound **21** was prepared in a manner similar to that described for **13** as a colorless amorphous powder. Yield: 71%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89–1.03 (2H, m), 1.24–1.42 (18H, m), 1.55–1.82 (4H, m), 2.54–2.80 (2H, m), 2.82–2.90 (3H, m), 3.57–3.67 (3H, m), 3.86–4.07 (7H, m), 4.20–4.69 (3H, m), 6.32–6.62 (3H, m), 7.29–7.36 (1H, m), 7.52–7.60 (1H, m), 7.65–7.73 (1H, m), 7.88–7.98 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 15.2, 26.9, 28.6, 29.7, 32.1, 32.2, 32.5, 35.4, 36.1, 50.1, 63.5, 72.0, 78.9, 100.3, 106.7, 112.2, 115.3, 116.8, 122.6, 124.2, 132.3, 139.2, 139.5, 154.3, 157.7, 160.3, 160.5, 166.0. LCMS (ESI$^+$) m/z 636.4. Anal. Calcd for $C_{36}H_{49}N_3O_7 \cdot 0.2H_2O$: C, 67.62; H, 7.79; N, 6.57. Found: C, 67.44; H, 7.75; N, 6.51.

*Ethyl 1-Benzyl-4-(methylamino)-1,2,5,6-tetrahydropyridine-3-carboxylate (**23**)*

To a mixture of **22** (40 g, 130 mmol) in MeOH (150 ml) was added methylamine (69 ml, 670 mmol) at rt. The mixture was stirred at 50 °C under N_2 for 1 h. The mixture was concentrated in vacuo. The mixture was poured into water and extracted with AcOEt. The organic layer was separated, washed with brine, dried over $MgSO_4$, passed through short-pad column chromatography and concentrated in vacuo to give **23** (37 g, quantative yield) as an yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, *J* = 6.3 Hz), 2.37–2.43 (2H, m), 2.49–2.56 (2H, m), 2.84 (3H, d, *J* = 5.1 Hz), 3.26 (2H, s), 3.61 (2H, s), 4.10 (2H, q, *J* = 6.3Hz), 7.23–7.40 (5H, m), 8.68 (1H, brs).

*Ethyl 6-Benzyl-4-hydroxy-1-methyl-2-oxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carboxylate (**24**)*

A mixture of **23** (37 g, 140 mmol), ethyl malonylchloride (41 g, 270 mmol) and K_2CO_3 (56 g, 410 mmol) in DMF (200 ml) was stirred at 100 °C for 2 h. The mixture was neutralized with aqueous 1 M HCl. and extracted with AcOEt. 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 (silica gel, eluted with 0%–50% AcOEt in hexane). To a mixture of this residue in EtOH (200 ml) was added sodium ethoxide (35 g, 100 mmol) at rt. The mixture was stirred at 50 °C for 15 hr. The mixture was neutralized with aqueous 1 M HCl at 0 °C and extracted with AcOEt. 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 (silica gel, eluted with 0%–10% MeOH in AcOEt) to give 24 (8.4 g, 48%) as a light brown amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (3H, t, *J* = 6.3 Hz), 2.73 (4H, s), 3.41 (3H, s), 3.46 (2H, s), 3.71 (2H, s), 4.43 (2H, q, *J* = 6.3 Hz), 7.23–7.40 (5H, m), 13.58 (1H, s). LCMS (ESI$^+$) m/z 343.2.

*6-Benzyl-N-(3,5-dimethoxybenzyl)-4-hydroxy-N,1-dimethyl-2-oxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridine-3-carboxamide (**25**)*

To a mixture of **24** (6.5 g, 19 mmol) in toluene (100 ml) was added **3** (4.0 g, 22 mmol) at rt. The mixture was stirred at 110 °C for 15 h. The mixture was concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0%–30% MeOH in AcOEt) to give **25** (9.0 g, 99%) as a light brown amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (2H, s), 3.62 (2H, s), 6.34–6.37 (2H, m), 6.43 (1H, brs), 6.48–6.50 (1H, m), 7.27–7.40 (5H, m). LCMS (ESI$^+$) m/z 478.3.

*tert-Butyl 4-(3-((6-Benzyl-3-((3,5-dimethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**26**)*

To a mixture of **25** (3.0 g, 6.3 mmol) and K_2CO_3 (1.7 g, 13 mmol) in DMF (30 ml) was added *tert*-butyl 4-(3-((methylsulfonyl)oxy)propyl)piperidine-1-carboxylate (3.0 g, 9.4 mmol). The mixture was stirred at 100 °C for 2 h. After being cooled to rt, the mixture was poured into water and extracted with AcOEt and IPA. The organic layer was separated, washed with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 50%–100% AcOEt in hexane) to afford **26** (3.5 g, 78%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.72–0.96 (2H, m), 0.98–1.15 (2H, m), 1.18–1.67 (15H, m), 2.55–2.70 (3H, m), 2.72–2.84 (6H, m), 3.05–3.29 (2H, m), 3.34–3.46 (3H, m), 3.54–3.81 (9H, m), 3.83–3.96 (2H, m), 4.11–4.70 (2H, m), 6.30–6.64 (3H, m), 7.18–7.40 (5H, m). LCMS (ESI$^+$) m/z 703.5.

*tert-Butyl 4-(3-((3-((3,5-Dimethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2,5,6,7,8-hexahydro-1,6-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**27**)*

To a mixture of **26** (3.2 g, 4.6 mmol) in EtOH (20 ml) and THF (10 ml) was added 10% Pd on activated carbon (0.24 g, 0.23 mmol). The mixture was stirred at 60 °C under H_2 atmosphere (0.1 MPa) for 3 h. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 30%–100% AcOEt in hexane) to **27** (2.4 g, 86%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.81–1.03 (2H, m), 1.18–1.44 (13H, m), 1.50–1.69 (4H, m), 2.34 (1H, brs), 2.53–2.74 (4H, m), 2.75–2.84 (3H, m), 2.86–2.98 (2H, m), 3.34–3.40 (3H, m), 3.51 (2H, s), 3.63–3.79 (7H, m), 3.83–3.96 (2H, m), 4.14–4.70 (2H, m), 6.34–6.41 (1H, m), 6.47–6.65 (2H, m). LCMS (ESI$^+$) m/z 613.5.

*tert-Butyl 4-(3-((3-((3,5-Dimethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,6-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**28**)*

To a mixture of **27** (400 mg, 0.65 mmol) in *n*-BuOH (5.0 ml) was added 10% Pd on activated carbon (35 mg, 0.030 mmol). The mixture was stirred at 160 °C under microwave irradiation for 1.5 h. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (NH silica gel, eluted with 50%–100% AcOEt in hexane) to afford **28** (250 mg, 62%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.91–1.03 (2H, m), 1.26–1.42 (12H, m), 1.59–1.82 (4H, m), 2.55–2.77 (2H, m), 2.85–2.91 (3H, m), 3.54–3.59 (3H, m), 3.68–3.77 (6H, m), 3.88–4.08 (3H, m), 4.27–4.75 (3H, m), 6.37–6.64 (3H, m), 7.47–7.53 (1H, m), 8.61–8.68 (1H, m), 8.99–9.07 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.8, 28.6, 29.5, 32.1, 32.1, 32.5, 35.4, 36.1, 50.1, 55.6, 72.0, 78.9, 99.4, 106.3, 109.4, 112.4, 113.0, 139.5, 144.3, 146.6, 151.0, 154.3, 157.4, 160.9, 161.1, 165.5. LCMS (ESI$^+$) m/z 609.3. Anal. Calcd for $C_{33}H_{44}N_4O_7 \cdot 1.0H_2O$: C, 63.24; H, 7.40; N, 8.94. Found: C, 63.33; H, 7.32; N, 8.85.

*Ethyl 2-(Methylamino)nicotinate (**30**)*

To a mixture of **29** (1.0 g, 5.4 mmol) in THF (10 ml) was added methylamine (0.66 ml, 6.5 mmol) at rt. The mixture was stirred at 40 °C for 3 h. The mixture was concentrated in vacuo.

The eluted with 0%–50% AcOEt in hexane) to give **30** (1.0 g, quantative yield) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.45 (3H, m), 3.06 (3H, d, *J* = 4.8 Hz), 4.32 (2H, q, *J* = 7.2Hz), 6.51 (1H, dd, *J* = 7.7, 4.8 Hz), 7.92 (1H, brs), 8.11 (1H, dd, *J* = 7.7, 2.0 Hz), 8.31 (1H, dd, *J* = 4.8, 1.9 Hz). LCMS (ESI^+) m/z 181.1.

*Ethyl 4-Hydroxy-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate (**31**)*

To a mixture of **30** (42 g, 230 mmol) and DMAP (2.9 g, 23 mmol) in THF (800 ml) was added ethyl 3-chloro-3-oxopropanoate (44 ml, 350 mmol) at 0 °C. After being stirred at rt for 20 min., DIPEA (61 ml, 350 mmol) was added to the reaction mixture. The mixture was stirred at rt under a dry atmosphere overnight. The precipitate was removed by filtration, and the filtrate was concentrated in vacuo. The obtained residue was dissolved in THF (800 ml) and *t*-BuOK was added to the reaction mixture at 0 °C. The mixture was stirred at rt for 3 h. The mixture was quenched with aqueous 6 M HCl to pH 4–5 and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluted with 0%–85% AcOEt in hexane) to give **27** (39 g, 67%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, t, *J* = 7.1 Hz), 3.78 (3H, s), 4.51 (2H, q, *J* = 7.2 Hz), 7.21 (1H, dd, *J* = 7.9, 4.7 Hz), 8.44 (1H, dd, *J* = 7.9, 1.9 Hz), 8.71 (1H, dd, *J* = 4.7, 1.9 Hz), 14.18 (1H, s). LCMS (ESI^+) m/z 249.1.

*tert-Butyl 4-(3-((3-((3,5-Dimethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**32**)*

To a mixture of **31** (10 g, 40 mmol) in toluene (200 ml) was added **3** (7.3 g, 40 mmol) at rt. The mixture was stirred at 100 °C overnight. The mixture was concentrated in vacuo. The solid was collected by filtration, washed with IPE, and dried in vacuo. This residue was dissolved in DMF (200 ml) and *tert*-butyl 4-(3-((methylsulfonyl)oxy)propyl)piperidine-1-carboxylate (12 g, 37 mmol) and K_2CO_3 (5.4 g, 39 mmol) were added to the mixture. The mixture was stirred at 90 °C overnight. The mixture was poured into water and extracted with AcOEt. 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 (silica gel, eluted with 0%–95% AcOEt in hexane) to give **32** (13 g, 82%) as a colorless amorphous powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.92–1.02 (2H, m), 1.26–1.40 (12H, m), 1.58–1.79 (4H, m), 2.57–2.76 (2H, m), 2.85–2.93 (3H, m), 3.65–3.78 (9H, m), 3.89–4.06 (3H, m), 4.25–4.76 (3H, m), 6.37–6.66 (3H, m), 7.33–7.40 (1H, m), 8.25–8.33 (1H, m), 8.65–8.73 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 26.3, 28.0, 31.5, 31.6, 31.9, 32.0, 34.9, 35.5, 49.5, 55.1, 71.4, 78.3, 98.8, 105.8, 111.7, 112.0, 118.3, 132.8, 138.9, 148.7, 150.9, 153.8, 156.1, 160.6, 160.8, 165.1. LCMS (ESI^+) m/z 609.3. Anal. Calcd for $C_{33}H_{44}N_4O_7 \cdot 0.3H_2O$: C, 64.54; H, 7.32; N, 9.12. Found: C, 64.68; H, 7.49; N, 8.92.

*tert-Butyl 4-(3-((3-((3,5-Bis(trifluoromethyl)benzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**33**)*

Compound **33** was prepared in a manner similar to that described for **32** as a colorless amorphous powder. Yield: 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01–1.89 (17H, m), 2.60–2.75 (3H, m), 3.01–3.09 (3H, m), 3.64 (1H, d, *J* = 3.5 Hz), 3.75–3.83 (3H, m), 3.86–3.96 (1H, m), 4.37–5.04 (4H, m), 7.21 (1H, dd, *J* = 7.9, 4.7 Hz), 7.65–7.86 (2H, m), 7.98 (1H, s), 8.23 (1H, dd, *J* = m/z 585.4.

*tert-Butyl 4-(3-((3-((3-Methoxy-5-(trifluoromethyl)benzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**34**)*

Compound **34** was prepared in a manner similar to that described for **32** as a colorless amorphous powder. Yield: 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01–1.92 (18H, m), 2.60–2.77 (2H, m), 2.98 (3H, s), 3.59–4.90 (12H, m), 7.01–7.24 (3H, m), 7.33–7.41 (1H, m), 8.18–8.29 (1H, m), 8.58–8.69 (1H, m). LCMS (ESI^+) m/z 647.5.

*1-Methylcyclopropyl 4-(3-((3-((3,5-Dimethoxybenzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**35**)*

To a mixture of **32** (300 mg, 0.49 mmol) in AcOEt (10 ml) was added 4 M HCl in AcOEt (1.0 ml). After being stirred at rt overnight, the mixture was concentrated in vacuo. This product was subjected to the next reaction without further purification. To a mixture of this residue and Et_3N (8.8 ml, 63 mmol) in THF (200 ml) was added 1-methylcyclopropyl(4-nitrophenyl)carbonate (6.0 g, 25 mmol). The mixture was stirred at rt overnight. The mixture was quenched with aqueous NH_4Cl and extracted with AcOEt. 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 (NH silica gel, eluted with 0%–70% AcOEt in hexane) to give **30** (11 g, 82%) as a colorless amorphous powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.52–0.65 (2H, m), 0.69–0.82 (2H, m), 0.89–1.03 (2H, m), 1.24–1.40 (3H, m), 1.46 (3H, s), 1.57–1.81 (4H, m), 2.60–2.76 (2H, m), 2.83–2.93 (3H, m), 3.64–3.78 (9H, m), 3.80–4.08 (3H, m), 4.24–4.75 (3H, m), 6.37–6.66 (3H, m), 7.33–7.41 (1H, m), 8.25–8.32 (1H, m), 8.65–8.73 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.1, 21.9, 26.8, 28.5, 32.0, 32.1, 32.3, 32.5, 35.3, 36.1, 50.1, 55.6, 56.2, 72.0, 99.4, 106.3, 112.2, 112.5, 118.8, 133.4, 139.5, 149.2, 151.4, 154.6, 156.6, 161.1, 161.3, 165.6. LCMS (ESI^+) m/z 607.4. Anal. Calcd for $C_{33}H_{42}N_4O_7 \cdot 0.2H_2O$: C, 64.94; H, 7.00; N, 9.18. Found: C, 64.82; H, 7.25; N, 9.03.

*1-Methylcyclopropyl 4-(3-((3-((3,5-Bis(trifluoromethyl)benzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**36**)*

Compound **36** was prepared in a manner similar to that described for **35** as a colorless amorphous powder. Yield: 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.54–0.64 (2H, m), 0.70–0.80 (2H, m), 0.90–1.04 (2H, m), 1.24–1.42 (3H, m), 1.46 (3H, s), 1.56–1.80 (4H, m), 2.59–2.79 (2H, m), 2.90–3.03 (3H, m), 3.60–3.72 (3H, m), 3.75–4.04 (3H, m), 4.20–4.31 (1H, m), 4.59–5.16 (2H, m), 7.34–7.42 (1H, m), 7.99–8.33 (4H, m), 8.66–8.75 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ 13.1, 21.9, 26.8, 28.6, 32.0, 32.4, 32.8, 35.3, 36.5, 49.7, 56.2, 71.9, 112.5, 118.9, 122.5, 123.8 (q, J_{C-F} = 272 Hz), 125.2, 129.1, 130.9 (q, J_{C-F} = 33 Hz), 133.4, 141.1, 149.3, 151.6, 154.6, 156.9, 161.4, 166.3. LCMS (ESI^+) m/z 683.2. Anal. Calcd for $C_{33}H_{36}F_6N_4O_5$: C, 58.06; H, 5.32; N, 8.21. Found: C, 58.18; H, 5.39; N, 8.12.

*1-Methylcyclopropyl 4-(3-((3-((3-Methoxy-5-(trifluoromethyl)benzyl)(methyl)carbamoyl)-1-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-4-yl)oxy)propyl)piperidine-1-carboxylate (**37**)*

described for **35** as a colorless amorphous powder. Yield: 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.53–0.67 (2H, m), 0.69–0.83 (2H, m), 0.89–1.05 (2H, m), 1.24–1.41 (3H, m), 1.46 (3H, s), 1.56–1.80 (4H, m), 2.61–2.76 (2H, m), 2.86–2.97 (3H, m), 3.60–3.71 (3H, m), 3.80–4.05 (6H, m), 4.24–4.97 (3H, m), 7.12–7.43 (4H, m), 8.24–8.36 (1H, m), 8.64–8.75 (1H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.1, 21.9, 26.8, 28.6, 32.0, 32.0, 32.4, 32.7, 35.3, 36.3, 56.2, 56.2, 71.9, 109.8, 111.9, 112.5, 116.9, 117.9, 118.8, 124.5 (q, $J_{C–F}$ = 274 Hz), 130.9 (q, $J_{C–F}$ = 31 Hz), 133.4, 140.6, 149.2, 151.5, 154.6, 156.8, 160.4, 161.4, 166.0. LCMS (ESI$^+$) m/z 645.3. Anal. Calcd for $C_{33}H_{39}F_3N_4O_6 \cdot 0.3H_2O$: C, 60.97; H, 6.14; N, 8.62. Found: C, 60.72; H, 6.30; N, 8.50. Purity: 98.97%.

X-ray structure analysis

Crystal data for **36**: $C_{33}H_{36}F_6N_4O_5$, MW= 682.66; crystal size, 0.16 x 0.09 x 0.04 mm; colorless, platelet; monoclinic, space group $P2_1/c$, a = 20.2006(4) Å, b = 13.7155(3) Å, c = 11.5867(2) Å, $\alpha = \gamma = 90°$, β = 93.189(7)°, V = 3205.26(11) Å^3, Z = 4, Dx = 1.415 g/cm^3, T = 100 K, μ = 1.024 mm^{-1}, λ = 1.54187 Å, R_1 = 0.064, wR_2 = 0.122.

All measurements were made on a Rigaku R-AXIS RAPID-191R diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SIR2008 and was refined using full-matrix least-squares on F^2 with SHELXL-97. All non-H atoms were refined with anisotropic displacement parameters.

5.2. Biology

5.2.1. In vitro assay

Protein preparation

The full-length coding sequence of human SMS2 is identical to NCBI accession number NM_152621. The resulting PCR product for C-terminal-FLAG-tagged human SMS2 was subcloned into pcDNA3.3 vector (Life Technologies) to generate an internal plasmid ID of TMCC-3206. To prepare overexpressed SMS2 membrane in FreeStyle293 cells (Life Technologies), the expression vector was transiently transfected into FreeStyle293 cells using NeoFection (ASTEC, Fukuoka, Japan) in accordance with the instruction manual. After culture for 2 days, the cells were centrifuged (1,000 × g, 10 min, 4 °C). Cells were homogenized in ice-cold 20 mM Tris-HCl buffer (pH 7.5 at 25 °C) containing 2 mM EDTA and 1 × Complete protease inhibitor cocktail (Roche). Cell homogenates were centrifuged (890 × g, 10 min, 4 °C), and the supernatant was recovered. Total membrane fractions were isolated by ultracentrifugation (140,000 × g, 60 min, 4 °C). The pellets were re-suspended in the same buffer, and stored at -80 °C. The protein concentration in the homogenate was determined using the BCA Protein Assay Kit (Pierce Biotechnology, Inc., IL, USA) according to the instruction manual.

The full-length coding sequences of human SMS1 and mouse SMS2 are identical to NCBI accession numbers NM_147156 and NM_028943, respectively. The sequences were inserted into pcDNA3.3 expression vectors with a C-terminal-FLAG tag to generate mammalian expression plasmids with the internal plasmid ID of TMCC-3208 and TMCC-3824, respectively. The membrane proteins for these genes were prepared by the same method as human SMS2.

RapidFire/MS assay

After pre-incubation of the SMS enzyme with the test compounds for 60 min, the reaction was performed in 20 µL of mM KCl, 0.1% BSA, 0.03% NP-40 substitute (WAKO), 1 mM DTT, and 10% DMSO, supplemented with 30 µg/mL of SMS enzyme, 50 µM C14-Phosphatidylcholine-D72 (TAIYO NIPPON SANSO), and 1 µM C17-ceramide (Avanti Polar Lipids) as substrates using a 384 well assay plate (REMP). After incubation at room temperature for 30 min, 60 µL of 2-propanol containing 0.45% formic acid and 33 nM C17-SM (Avanti Polar Lipids) was added to the reaction mixture, mixed, and centrifuged to precipitate the proteins. Subsequently, high-throughput online solid phase extraction was performed using a RapidFire 300™ (Agilent Technologies). The samples were loaded on to the SPE C8 cartridge (Agilent Technologies) in 0.1% trifluoroacetic acid in acetonitrile/deionized water (60/40, v/v) at a flow rate of 1.5 mL/min and eluted using 0.1% trifluoroacetic acid in acetonitrile/deionized water (80/20, v/v) at a flow rate of 1.25 mL/min. The injection needle was washed with deionized water followed by acetonitrile. The aspiration time (injection volume 10 µL), load/wash time, elution time, and re-equilibration time were adjusted to 350, 3000, 4000, and 500 ms, respectively, for a total cycle time of approximately 10.0 sec.

Mass spectrometric analysis of analyte formation was performed using an API-4000TM triple quadrupole mass spectrometer (AB SCIEX) equipped with an electro spray ion source (TurboIon Spray®) operated in positive selected reaction monitoring (SRM) mode. The SRM conditions for the analytes are shown below. The parameters were optimized as follows: capillary temperature, 650 °C; ion spray voltage, 5.5 kV. Collision gas, curtain gas, ion source gas 1 and 2 pressures were set at 10, 20, 60, and 60 psi, respectively. Analytical data were acquired and analyzed using Analyst software version 1.5.0 (AB SCIEX).

Analyte	Precursor → Product (m/z)	DP (V)	CE (V)	CXP (V)
C17-SM-d13	730.4 → 197.1	81	37	16
C17-SM (I.S.)	717.4 → 184.2	71	33	14
C17-CER	552.4 → 264.2	66	35	8
C14-PC-d72	750.0 → 198.2	81	10	16

The inhibitory activity was calculated as follows: % inhibition = (A - B) / (A - C) × 100, where, A, B, and C are peak area ratios with vehicle, with test sample, and without reaction, respectively.

5.2.2. Solubility

A small volume of each compound in DMSO was added to aqueous buffer. After incubation, the precipitates were separated by filtration through a filter plate. The filtrate was analyzed for compounds in solution by LC/MS/MS analysis.

5.2.3. In vivo assay

Ten week old male KK-A^y/Ta mice (CLEA Japan) were used. Test compounds were administered orally or subcutaneously to the animals once or twice per day for 7 days. Control animals were treated with the vehicle. The next morning after last dosing, the animals were sacrificed under anesthesia and the livers were dissected. The pieces were weighed and stored in a freezer.

The sample was homogenized in isopropanol (100 mg/mL) by a ball-mill (MM301, Retsch GmbH, Haan, Germany), and centrifuged at 15,000 rpm for 5 min. The supernatant was analysed by liquid chromatography mass spectrometry (LC/MS). Chromatographic separation was performed using a Ultimate3000 liquid chromatography system (Thermo Fisher Scientific Inc., San Jose, CA, USA) equipped with a reverse phase column, a XBridge C18 (2.1×50 mm, 2.5 µm, Waters co. ltd., Milford, USA), by gradient elution of mobile phase A, 0.01% acetic acid, 1 mM ammonia and 10 µM EDTA-2Na in

Mill... mM ammonia in ethanol/isopropanol (3:2), with flow rate at 0.5 mL/min. The eluate of the liquid chromatography directly introduced to an Orbitrap XL mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA), and obtained full mass spectrum (MS) ranging from m/z 150 to m/z 2000 with a mass resolving power of 60000 FWHM (Full width at half maximum) at m/z 400. The raw data of the LC/MS were processed by Expressionist Refiner MS software (ver.8.2, Genedata AG, Basel, Switzerland), and the peak intensities of each molecules were exported as a tab-separated file in addition to the monoisotopic m/z value and the retention time information. Sphingolipids were identified based on the accurate m/z value, retention time and standard reagent information with reference to a lipid database downloaded from lipidMAPS (http://www.lipidmaps.org/) and Human Metabolome Database (http://www.hmdb.ca/).

Acknowledgements

We would like to acknowledge Dr. Shota Ikeda, Dr. Tsuyoshi Maekawa, Dr. Takashi Ichikawa, Dr. Takahiko Taniguchi, and Dr. Tohru Yamashita for helpful discussion during the preparation of the manuscript. We also thank Mika Inoue for the high throughput synthesis and Megumi Hirayama for the analysis of sphingolipids. We also thank Mitsuyoshi Nishitani for structural analysis of the synthesized compound.

References and notes

1. Holthuis, J. C.; Pomorski, T.; Raggers, R. J.; Sprong, H.; Van Meer, G. *Physiol. Rev.*, **2001**, *81*, 1689.
2. Hannun, Y. A.; Obeid, L. M. *Nat. Rev. Mol. Cell Biol.*, **2008**, *9*, 139.
3. Breslow, D. K. and Weissman, J. S. *Mol. Cell*, **2010**, *40*, 267.
4. Simons, K. and Ikonen, E. *Nature*, **1997**, *387*, 569.
5. Brown, D. A. and Rose J. K. *Cell*, **1992**, *68*, 533.
6. Parton, R. G. and Simons K. *Science*, **1995**, *269*, 1398.
7. Harder, T.; Scheiffele, P.; Verkade, P.; Simons K. *J. Cell Biol.*, **1998**, *141*, 929.
8. Li, Z.; Hailemariam, T. K.; Zhou, H.; Li, Y.; Duckworth, D. C.; Peake, D. A.; Zhang, Y.; Kuo, M.-S.; Cao, G.; Jiang, X.-C. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids*, **2007**, *1771*, 1186.
9. Tafesse F. G.; Ternes, P.; Holthuis, J. C. M. *J. Biol. Chem.*, **2006**, *281*, 29421.
10. Yamaoka, S.; Miyaji, M.; Kitano, T.; Umehara, H.; Okazaki, T. *J. Biol. Chem.*, **2004**, *279*, 18688.
11. Huitema, K.; van den Dikkenberg, J.; Brouwers, J. F.; Holthuis, J. C. *Embo. J.*, **2004**, *23*, 33.
12. Yeang, C.; Ding, T.; Chirico, W. J.; Jiang, X.-C. *Nutr. Metab.*, **2011**, *8*, 89.
13. Vacaru, A. M.; Tafesse, F. G.; Ternes, P.; Kondylis, V.; Hermansson, M.; Brouwers, J. F.; Somerharju, P.; Rabouille, C.; Holthuis, J. C. *J. Cell Biol.*, **2009**, *185*, 1013.
14. Hailemariam, T. K.; Huan, C.; Liu, J.; Li, Z.; Roman, C.; Kalbfeisch, M.; Bui, H. H.; Peake, D. A.; Kuo, M.-S.; Cao, G.; Wadgaonkar, R.; Jiang, X.-C. *Arterioscler. Thromb. Vasc. Biol.*, **2008**, *28*, 1519.
15. Liu, J.; Huan, C.; Chakraborty, M.; Zhang, H.; Lu, D.; Kuo, M.-S.; Cao, G.; Jiang, X.-C. *Circ. Res.*, **2009**, *105*, 295.
16. Jiang, X.-C.; Yeang, C.; Li, Z.; Chakraborty, M.; Liu, J.; Zhang, H.; Fan, Y. *Clin. Lipidol.*, **2009**, *4*, 595.
17. Li, Z.; Zhang, H.; Liu, J.; Liang, C.-P.; Li, Y.; Li, Y.; Teitelman, G.; Beyer, T.; Bui, H. H.; Peake, D. A.; Zhang, Y.; Sanders, P. E.; Kuo, M.-S.; Park, T.-S.; Cao, G.; Jiang, X.-C. *Mol. Cell. Biol.*, **2011**, *31*, 4205.
18. Mitsutake, S.; Zama, K.; Yokota, H.; Yoshida, T.; Tanaka, M.; Mitsui, M.; Ikawa, M.; Okabe, M.; Tanaka, Y.; Yamashita, T.; Takemoto, H.; Okazaki, T.; Watanabe, K.; Igarashi, Y. *J. Biol. Chem.*, **2011**, *286*, 28544.
19. Yano, M.; Yamamoto, T.; Nishimura, N.; Gotoh, T.; Watanabe, K.; Ikeda, K.; Garan, Y.; Taguchi, R.; Node, K.; Okazaki, T.; Oike, Y. *PLoS ONE*, **2013**, *8*, e61380.
20. ... T.; Lu, M.; Kadomatsu, T.; Tsukano, H.; Ikawa, M.; Okabe, M.; Yamaoka, S.; Okazaki, T.; Umehara, H.; Gotoh, T.; Song, W.-J.; Node, K.; Taguchi, R.; Yamagata, K.; Oike, Y. *J. Biol. Chem.*, **2011**, *286*, 3992.
21. Lu, M.-H.; Takemoto, M.; Watanabe, K.; Luo, H.; Nishimura, M.; Yano, M.; Tomimoto, H.; Okazaki, T.; Oike, Y.; Song, W.-J. *J. Physiol.*, **2012**, *590*, 4029.
22. Dong, L.; Watanabe, K.; Itoh, M.; Huan, C.-R.; Tong, X.-P.; Nakamura, T.; Miki, M.; Iwao, H.; Nakajima, A.; Sakai, T.; Kawanami, T.; Sawaki, T.; Masaki, Y.; Fukushima, T.; Fujita, Y.; Tanaka, M.; Yano, M.; Okazaki, T.; Umehara, H. *Int. Immunol.*, **2012**, *24*, 327.
23. Zama, K.; Mitsutake, S.; Watanabe, K.; Okazaki, T.; Igarashi, Y. *Chem. Phys. Lipids*, **2012**, *165*, 760.
24. Meng, A.; Luberto, C.; Meier, P.; Bai, A.; Yang, X.; Hannun, Y. A.; Zhou, D. *Exp. Cell Res.*, **2004**, *292*, 385.
25. Bai, A.; Meier, G. P.; Wang, Y.; Luberto, C.; Hannun, Y. A.; Zhou, D. *J. Pharmacol. Exp. Ther.*, **2004**, *309*, 1051.
26. Gusain, A.; Hatcher, J. F.; Adibhatla, R. M.; Wesley, U. V.; Dempsey, R. J. *Mol. Neurobiol.*, **2012**, *45*, 455.
27. Adibhatla, R. M.; Hatcher, J. F.; Gusain, A. *Neurochem. Res.*, **2012**, *37*, 671.
28. Sauer, G.; Amtmann, E.; Melber, K.; Knapp, A.; Müller, K.; Hummel, K.; Scherm, A. *Proc. Natl. Acad. Sci. USA*, **1984**, *81*, 3263.
29. Adachi, R.; Ogawa, K.; Matsumoto, S.; Satou, T.; Tanaka, Y.; Sakamoto, J.; Nakataha, T.; Okamoto, R.; Kamaura, M.; Kawamoto, T. *Eur. J. Med. Chem.,* **2017**, *136*, 283.
30. Mo, M., Yang, J., Jiang, X.-C., Cao, Y., Fei, J., Chen, Y., Qi, X., Chu, Y., Zhou, L., Ye, D. *J. Med. Chem.*, **2018**, *62*, 8241.
31. Li, Y.; Huang, T.; Lou, B.; Ye, D.; Qi, X.; Li, X.; Hu, S.; Ding, T.; Chen, Y.; Cao, Y.; Mo, M.; Dong, J.; Wei, M.; Chu, Y.; Li, H., Jiang, X.-C.; Cheng, N.; Zhou, L.. *Eur. J. Med. Chem.* **2019**, *163*, 864.
32. Deng, X.; Lin, F.; Zhang, Y.; Li, Y.; Zhou, L.; Lou, B.; Li, Y.; Dong, J.; Ding, T.; Jiang, X.; Wang, R.; Ye, D. *Eur. J. Med. Chem.*, **2014**, *73*, 1.
33. Yeang, C.; Varshney, S.; Wang, R.: Zhang, Y.; Ye, D.; Jiang, X.-C. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids*, **2008**, *1781*, 610.
34. Zhang, Y.; Lin, F.; Deng, X.; Wang, R.; Ye, D. *Chin. J. Chem.*, **2011**, *29*, 1567.
35. Qi, X.-Y.; Cao, Y., Li, Y.-L., Mo, M.-G., Zhou, L., Ye, D.-Y. *Bioorg. Med. Chem.Lett.*, **2017**, *27*, 3511.
36. Tsunoda, T.; Ozaki, F.; Ito, S. *Tetrahedron Lett.*, **1994**, *35*, 5081.
37. Avdeef, A.; Strafford, M.; Block, E.; Balogh, M. P.; Chambliss, W.; Khan, I. *Eur. J. Pharm. Sci.* **2001**, *14*, 271.
38. Bochevarov, A. D.; Harder, E.; Hughes, T. F.; Greenwood, J. R.; Braden, D. A.; Philipp, D. M.; Rinaldo, D.; Halls, M. D.; Zhang, J.; Friesner, R. A. *Int . J. Quantum Chem.* **2007**, *107*, 1390.
39. Snider, E. J., Wright, S. W. *Tetrahedron Lett*., **2011**, 52, 3171.

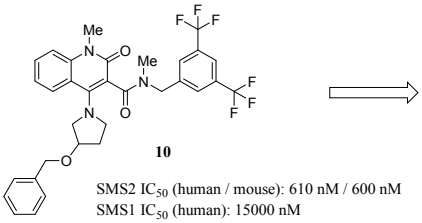
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Discovery of 1,8-Naphthyridin-2-one Derivative as a Potent and Selective Sphingomyelin Synthase 2 Inhibitor

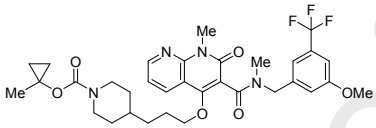
Leave this area blank for abstract info.

Takafumi Yukawa*, Takashi Nakahata, Rei Okamoto, Yuji Ishichi, Yasufumi Miyamoto, Satoshi Nishimura, Tatsuo Oikawa, Kazuki Kubo, Ryutaro Adachi, Yoshinori Satomi, Masanori Nakakariya, Nobuyuki Amano, Masahiro Kamaura, and Nobuyuki Matsunaga
Pharmaceutical Research Division, Takeda Pharmaceutical Company, Ltd., 26-1, Muraoka-higashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan



10

SMS2 IC_{50} (human / mouse): 610 nM / 600 nM
SMS1 IC_{50} (human): 15000 nM



37

SMS2 IC_{50} (human / mouse): 45 nM / 2.2 nM
SMS1 IC_{50} (human): 2400 nM
Significant hepatic SM (22:0) reduction (200 mg/kg/day, b.i.d., s.c.)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: