Scaffold hopping of fused piperidine-type NK3 receptor antagonists to reduce environmental impact

Koki Yamamoto, Shinsuke Inuki, Hiroaki Ohno, Shinya Oishi*

Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

*Corresponding Author:

Shinya Oishi, Ph.D.

Graduate School of Pharmaceutical Sciences

Kyoto University

Sakyo-ku, Kyoto, 606-8501, Japan

Tel.: +81-75-753-4561; Fax: +81-75-753-4570,

E-mail: soishi@pharm.kyoto-u.ac.jp

ABSTRACT

Neurokinin-3 receptor (NK3R) plays a pivotal role in the release of gonadotropin-releasing hormone in the hypothalamus–pituitary–gonadal (HPG) axis. To develop novel NK3R antagonists with less environmental toxicity, a series of heterocyclic scaffolds for the triazolopiperazine substructure in an NK3R antagonist fezolinetant were designed and synthesized. An isoxazolo[3,4-*c*]piperidine derivative exhibited moderate NK3R antagonistic activity and favorable properties that were decomposable under environmental conditions.

Keywords: environmental impact; fezolinetant; GnRH; NK3 receptor; scaffold hopping

This paper is dedicated to the memory of Prof. Kei-ichiro Maeda of The University of Tokyo, Japan.

Abbreviations: DMB, 2,4-dimethoxybenzoyl; 4-FB, 4-fluorobenzoyl; GnRH, gonadotropin-releasing hormone; NKB, neurokinin B; NK3R, neurokinin-3 receptor; PCOS, polycystic ovary syndrome.

1. Introduction

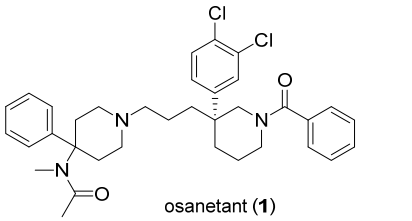
Pharmaceuticals include one or multiple bioactive ingredients that cure and/or alleviate symptoms in human or veterinary diseases. Many of these ingredients are excreted from the body in an unmetabolized form and/or as active metabolites via urine and/or feces. The fate of each ingredient following excretion depends on its chemical properties as well as the methods used to treat water it is excreted into.[1] Even after wastewater treatment, bioactive ingredients and their metabolites often survive to become ground and surface water contaminants in downstream areas.[2] High chemical stability of the ingredients is crucial for quality control of pharmaceuticals during long-term storage before administration. However, in terms of the impacts on the natural environment and ecosystems, stable bioactive substances with high bioactivity may have unfavorable effects on non-target species after excretion from patients or treated animals. Indeed, there have been cases of environmental contamination by bioactive ingredients derived from human and animal drugs that have led to the emergence of drug-resistant strains,[3] effects on the sex determination of aquatic fauna and flora,[4] and accumulation in organisms higher up in the food chain.[5] To overcome these disadvantages, drug design to turn off bioactivity after release into the environment is needed.

Neurokinin-3 receptor (NK3R) regulates pulsatile secretion of gonadotropin-releasing hormone (GnRH) in the hypothalamus–pituitary–gonadal (HPG) axis.[6] Activation of NK3R promotes the reproductive hormone cascade via activation of the GnRH neuron in the hypothalamus, leading to the pulsatile secretion of luteinizing hormone (LH) from the pituitary gland.[7–9] In contrast, NK3R antagonists, such as osanetant (**1**) and talnetant (**2a**), which were initially investigated for the treatment of schizophrenia, negatively regulate reproductive functions in mammals (Fig.1).[10,11] For example, talnetant decreased the secretion of testosterone in Guinia pigs.[12] Additionally, a recent clinical study revealed that oral administration of pavinetant (**2b**), which is expected to be applicable to treatment of polycystic ovarian syndrome (PCOS), effectively decreased the testosterone levels of healthy volunteers.[13,14] Alternatively, fezolinetant (**3**), which has a unique triazolopiperazine scaffold,[15] effectively decreased LH levels in rats and monkeys.[16,17] Clinical investigations of

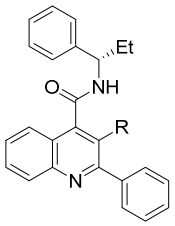
fezolinetant for the treatment of hot flashes are now ongoing. Thus, NK3R antagonists are expected to be therapeutic agents for various disorders of reproductive functions.

However, these NK3R antagonists and their bioactive metabolite(s) may affect the reproductive functions of non-target species via water pollution and/or soil contamination after excretion from treated humans and animals. To minimize the possibility of these adverse effects on non-target species, structures with potent NK3R antagonistic activity need to be converted into inactive form(s) by drug metabolizing systems in the body before excretion and/or by spontaneous degradation in the environment soon after excretion. In our structure-activity relationship studies of talnetant, we identified a talnetant analog, **2c**, that can be converted into inactive disulfide and isothiazolone forms by air oxidation.[18] Although the NK3R antagonism of thiol **2c** was favorably convertible under the conditions of the natural environment, its gradual inactivation in serum was also observed, which would potentially prevent prolonged in vivo biological activity. We also attempted to append the degradable properties onto fezolinetant by scaffold hopping, which is a process in which the core structure(s) are substituted and/or modified with other motifs.[19] Substitution of the triazolopiperazine scaffold in fezolinetant with nonplanar oxadiazolopiperazine synthesized by gold(I)-catalyzed domino cyclization unexpectedly led to loss of the NK3R antagonistic activity.[20] Based on these findings from our previous investigations, we expected that a series of aromatic heterocycles mimicking the planar 1,2,4-triazole moiety in fezolinetant could be appropriate scaffolds for novel NK3R antagonists with maintenance of NK3R antagonistic activity as well as favorable degradation properties. Herein we conducted a structure-activity relationship study of the triazolopiperazine part of fezolinetant by scaffold hopping for decomposable motif(s). The stability characteristics of a series of derivatives under simulated-sunlight irradiation and in serum were also investigated.

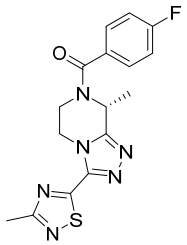
Fig.1. Structures of NK3R antagonists.



osanetant (**1**)



talnetant (**2a**, R = OH)
pavinetant (**2b**, R = $NHSO_2Me$)
our previous work (**2c**, R = SH)



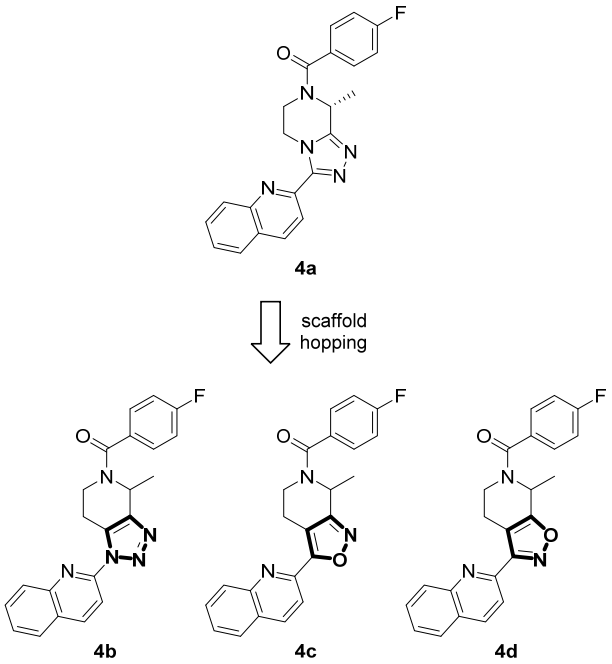
fezolinetant (**3**)

2. Results and discussion

2.1. Design of fezolinetant derivatives with decomposable scaffold

Initially, we designed potential NK3R antagonists, in which the scaffolds could be decomposed via hydrolysis or photolysis under environmental conditions (Fig. 2). We selected the quinoline derivative **4a**[21] as a lead compound for this structure-activity relationship study because of the synthetic feasibility of a series of derivatives. Based on substitution of the 1,2,4-triazole moiety in **4a** with five-membered aromatic heterocycles consisting of a combination of nitrogens and/or oxygens, three fused piperidine derivatives **4b**–**d** were possible. The arrangements of hydrogen bond acceptors derived from two imino nitrogens of 1,2,4-triazole in **4a** were reproduced in 1,2,3-triazole **4b** and isoxazoles **4c** and **4d**. The [1,2,3]triazolo[4,5-*c*]piperidine scaffold in **4b** was expected to degrade in response to sunlight exposure because 1,2,3-triazole is decomposed by UV irradiation.[22] Isoxazolo[3,4-*c*]piperidine **4c** and isoxazolo[5,4-*c*]piperidine **4d** were also designed on the basis of possible degradation of the isoxazole moiety via hydrolysis or photodegradation.[23,24]

Fig.2. Scaffold hopping from a fezolinetant derivative **4a** for design of novel NK3R antagonists with reduced environmental toxicity.

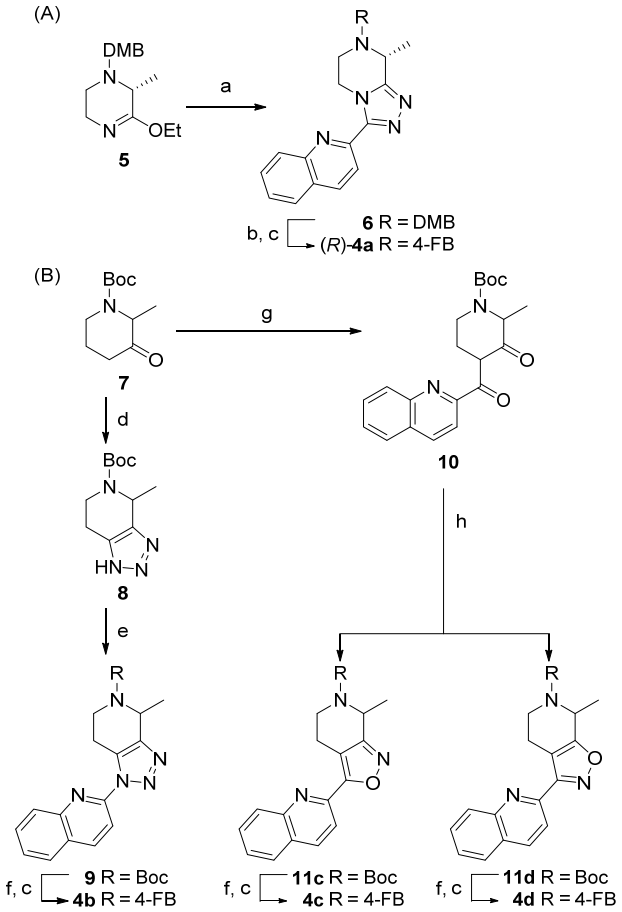


2.2. Synthesis of fezolinetant derivatives

We synthesized the fezolinetant derivatives **4a**–**d**. Triazolopiperazine **6** was obtained by treatment of piperazine **5** with quinoline-2-carbohydrazide. [1,2,4]Triazolo-[3,4-*a*]piperazine (*R*)-**4a** was prepared from **6** in two steps via deprotection of the 2,4-dimethoxybenzyl (DMB) group followed by acylation with 4-fluorobenzoyl chloride (Scheme 1A).[15] The fused piperidine derivatives **4b**–**d** were synthesized from a common substrate, 3-oxopiperidine derivative **7** (Scheme 1B). Reaction of ketone **7** with 1-azido-4-nitrobenzene provided 1,2,3-triazole **8**.[25] N^1-arylation on triazole **8** proceeded by treatment of triazole **8** with 2-chloroquinoline in the presence of *i*-Pr_2NEt to afford the desired 1-(quinolin-2-yl)triazole **9** after separation from a mixture of regioisomers. Boc deprotection of **9** followed by acylation with 4-fluorobenzoyl chloride gave the expected [1,2,3]triazolo[4,5-*c*]piperidine **4b**. The structure of **4b** was determined by X-ray crystal structure analysis. Synthesis of isoxazolopiperidines **4c** and **4d** began with treatment of ketone **7** with LDA and quinaldoyl chloride. The resulting diketone **10** was reacted with hydroxylamine under basic conditions to provide isoxazolo[3,4-*c*]piperidine **11c** and isoxazolo[5,4-*c*]piperidine **11d** after separation by column

chromatography. Isoxazoles **4c** and **4d** were obtained by the same manipulations of **11c** and **11d**, respectively, as for the synthesis of **4b**.

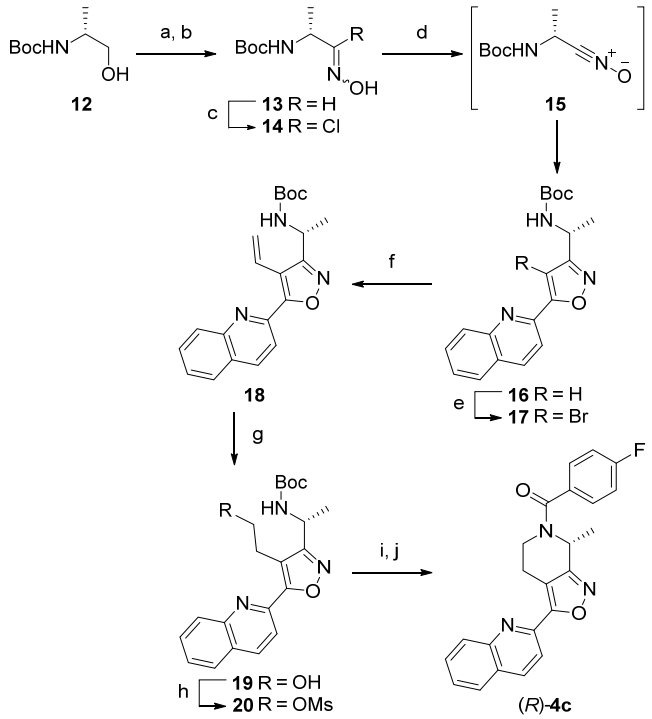
Scheme 1. Synthesis of fezolinetant derivatives **4a**–**d**. *Reagent and conditions:* (a) quinoline-2-carbohydrazide, EtOH, 70 °C; (b) TFA, CH_2Cl_2, 0 °C to rt; (c) 4-fluorobenzoyl chloride, Et_3N, CH_2Cl_2, rt; (d) 1-azido-4-nitrobenzene, NH_4OAc, DMF, 80 °C; (e) 2-chloroquinoline, *i*-Pr_2NEt, 120 °C; (f) HCl/dioxane, CH_2Cl_2, rt; (g) LDA, quinaldoyl chloride, THF, −78 °C to rt; (h) $NH_2OH{\cdot}HCl$, NaOH, *i*-PrOH, reflux. *Abbreviations*: DMB, 2,4-dimethoxybenzyl; 4-FB, 4-fluorobenzoyl.



To identify the structures of compounds **4c** and **4d**, which cannot be distinguished by X-ray analysis, the isoxazolo[3,4-*c*]piperidine **4c** was synthesized via an alternative route from *N*-Boc-D-alaninol **12** (Scheme 2). Swern oxidation of alcohol **12** followed by treatment with hydroxylamine gave the oxime **13**. After chlorination of **13** using *N*-chlorosuccinimide (NCS), the nitrile oxide intermediate **15** was generated under basic conditions, then subjected to 1,3-dipolar cycloaddition with 2-ethynylquinoline to provide the expected isoxazole **16**. Treatment of isoxazole **16** with *N*-bromosuccinimide (NBS) in

the presence of HBr afforded a 4-bromoisoxazole **17**. Subsequently, Suzuki-Miyaura coupling of **17** with vinyl boronic acid pinacol ester provided the 4-vinylisoxazole **18**. 9-BBN-mediated hydroboration of **18** followed by oxidative treatment gave the 4-hydroxyethyl isoxazole **19**. After the hydroxy group in **19** was activated with a mesyl group, Boc group deprotection and acylation was conducted using 4-fluorobenzoyl chloride to afford the isoxazolo[3,4-*c*]piperidine (*R*)-**4c**. The spectral data of (*R*)-**4c** coincided well with those of isoxazolo[3,4-*c*]piperidine **4c**.

Scheme 2. Synthesis of (*R*)-**4c**. *Reagent and conditions:* (a) $(COCl)_2$, DMSO, *i*-Pr_2NEt, CH_2Cl_2, −78 °C to 0 °C; (b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, 80 °C; (c) NCS, HCl/dioxane, DMF, 40 °C; (d) 2-ethynylquinoline, $NaHCO_3$, EtOAc, 40 °C; (e) NBS, HBr, DMF, 60 °C; (f) vinylboronic acid pinacol ester, $Pd(Ph_3P)_4$, Cs_2CO_3, DMF, 100 °C; (g) 9-BBN, THF, 0 °C to rt, then H_2O_2, NaOH, MeOH, THF, rt; (h) MsCl, Et_3N, CH_2Cl_2, rt; (i) HCl/dioxane, CH_2Cl_2, rt, then NaOH aq.; (j) 4-fluorobenzoyl chloride, Et_3N, CH_2Cl_2, rt.

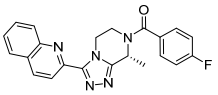
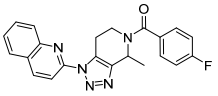
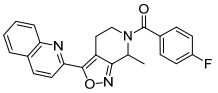
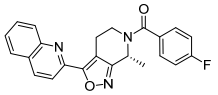
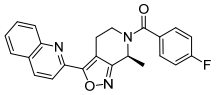
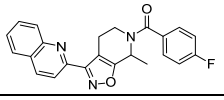


2.3. Biological evaluation of fezolinetant derivatives

We investigated the biological activity of fused piperidine derivatives **4b**–**d** by evaluating the antagonism of NKB-induced activation for human NK3R (Table 1).[20] 1,2,3-Triazole **4b** exhibited 15-fold less potent NK3R inhibition than 1,2,4-triazole (*R*)-**4a** [IC_{50} ((*R*)-**4a**): 0.26 μM, IC_{50} (**4b**): 4.1

μM]. Similarly, isoxazoles **4c** and **4d** showed moderate NK3R inhibition [IC_{50} (**4c**): 10.3 μM, IC_{50} (**4d**): 8.2 μM]. These findings suggested that the presence of two nitrogens of piperazine in **4a** was favorable to NK3R inhibition.

Table 1. NK3R antagonism of fused piperidine derivatives.

compound	structure	IC_{50} (μM)[a]
(*R*)-**4a**		0.26 ± 0.02
4b		4.1 ± 0.5
4c		10.3 ± 1.5
(*R*)-**4c**		11.8 ± 1.8
(*S*)-**4c**		13.7 ± 1.8
4d		8.2 ± 1.7

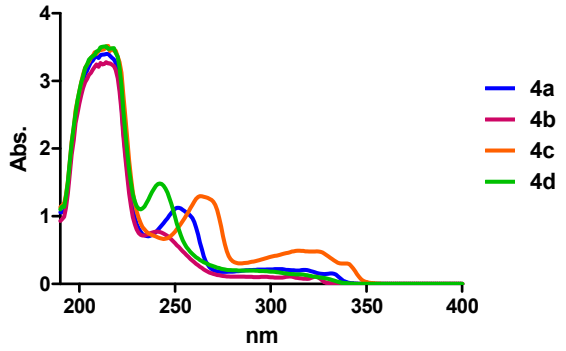
[a]IC_{50} values are the concentrations required for 50% inhibition of the NKB-mediated activation of NK3R.

To identify the stereoisomer of isoxazolo[3,4-*c*]piperidine **4c** that predominantly contributed to the bioactivity, two isoxazolo[3,4-*c*]piperidines (*R*)-**4c** and (*S*)-**4c** were evaluated for NK3R antagonistic activity after separation by chiral chromatography using a COSMOSIL CHiRAL 5A column. In contrast to the (*R*)-isomer of fezolinetant having more potent activity toward NK3R than the (*S*)-isomer in the SAR study,[15a] the derivatives (*R*)-**4c** and (*S*)-**4c** exhibited essentially identical NK3R antagonism [IC_{50} ((*R*)-**4c**): 11.8 ± 1.8 μM, IC_{50} ((*S*)-**4c**): 13.7 ± 1.8 μM]. These findings suggest that the chirality of the 7-methyl group of the isoxazolo[3,4-*c*]piperidine in **4c** had little effect on the biological activity.

2.4. Investigation of photodegradation of fezolinetant derivatives

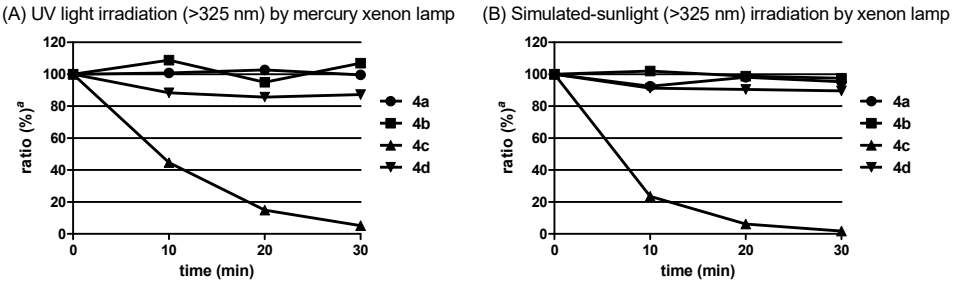
We investigated the spectral features and photodegradation profiles of **4a**–**d**. It has been reported that the absorption spectra of photodegradable compounds overlap with the actinic spectrum.[26] Initially, the absorption spectra of all derivatives were evaluated to identify those with the potential for photodegradation by sunlight exposure. The UV-vis spectrum of isoxazolo[3,4-*c*]piperidine **4c** showed strong absorption in the wavelength range of 300–350 nm (Fig. 3). In contrast, [1,2,4]triazolo[3,4-*a*]piperazine **4a**, [1,2,3]triazolo[4,5-*c*]piperidine **4b** and isoxazolo[5,4-*c*]piperidine **4d** exhibited low UV-visible absorption at above 300 nm.

Fig. 3. Absorption spectra of fused piperidine-type NK3R antagonists in 50 mM phosphine buffer (pH 7.4)–EtOH (7:3).



The photochemical stability was assessed by irradiation via a mercury xenon lamp through a UV cut-off filter (below 325 nm) in which the optical spectrum corresponds to that of UV-A (Fig. 4A).[27] Among triazolopiperazine **4a** and fused piperidines **4b**–**d**, isoxazolo[3,4-*c*]piperidine **4c** was promptly decomposed, while other scaffolds (**4a**, **4b** and **4d**) were stable under irradiation with a mercury xenon lamp. The simulated sunlight irradiation by the xenon lamp (>325 nm) also led to decomposition of **4c** to provide multiple products (Fig. 4B and Supplementary Fig. S1). After UV-irradiation of **4c**, the sample did not show NK3R inhibition (IC_{50} >100 μM). These findings indicated that decomposition would depend on the excitation characteristics of the scaffold by photoirradiation, and that isoxazole **4c** could be inactivated by sunlight exposure.

Fig.4. Photodegradation of fused piperidine-type NK3R antagonists.



[a]The ratios of compounds were calculated by HPLC analysis using calibration curves.

2.5. Investigation of the stability of fezolinetant derivatives under aqueous conditions

We also assessed the stability of fused piperidine derivatives in aqueous buffer by monitoring the intact compounds by HPLC (Supplementary Fig. S2A–C). No hydrolysate was observed from any derivatives at any pH (pH 4.0, 7.4 and 10.0), suggesting that fused piperidine scaffolds were unlikely to be decomposed via hydrolysis under environmental conditions. Additionally, we investigated the stability of fused piperidine derivatives in rat serum (Supplementary Fig. S3) and found no degradation of any of the compounds. Thus, derivative **4c** could be a novel NK3R antagonist that is stable in serum and inactivated in the natural environment via solar light irradiation after excretion.

3. Conclusion

We designed and synthesized a series of fezolinetant derivatives with fused piperidine scaffolds, in which the 1,2,4-triazole moiety in fezolinetant was substituted with five-membered aromatic heterocycles. The resulting derivatives showed moderate NK3R antagonistic activity. Among them, isoxazolo[3,4-*c*]piperidine **4c** was stable in aqueous buffer and rat serum and had a favorable photodegradable property, being decomposed by UV irradiation. These results indicated isoxazolo[3,4-*c*]piperidine **4c** may be a candidate for the treatment of sex-hormone disorders with less environmental impact.

4. Experimental Section

4.1. Synthesis

4.1.1. General synthesis

^{1}H NMR spectra were recorded using a JEOL AL-400 or a JEOL ECA-500 spectrometer. Chemical shifts are reported in δ (ppm) relative to Me_4Si as an internal standard. ^{13}C NMR spectra were recorded using a JEOL AL-400 or a JEOL ECA-500 and referenced to the residual solvent signal. Exact mass (HRMS) spectra were recorded on Shimadzu LC-ESI-IT-TOF-MS equipment. IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. Melting points were measured by a hot stage melting points apparatus (uncorrected). Optical rotations were measured with a JASCO P-1020 polarimeter. For chromatography, Wakogel C-300E (Wako) was employed.

(*R*)-2-[7-(2,4-Dimethoxylbenzyl)-8-methyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrazin-3-yl]quinoline (6). To a solution of (*R*)-1-(2,4-dimethoxylbenzyl)-5-ethoxy-6-methylpiperazine **5**[15] (158 mg, 1.00 mmol) in EtOH (2.00 mL) was added quinoline-2-carbohydrazide[28] (187 mg, 1.00 mmol). After being stirred for 17 h at 70 °C, the reaction mixture was concentrated. The residue was dissolved with EtOAc, and the whole was washed with 1 M NaOH and brine, and dried over Na_2SO_4. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 1/3) to give the title compound **6** (107 mg, 26%): pale yellow amorphous solid; $[\alpha]^{24}_{D}$ +13.3 (*c* 1.06, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.77 (d, *J* = 6.9 Hz, 3H), 2.67-2.72 (m, 1H), 3.20-3.24 (m, 1H), 3.56 (d, *J* = 13.7 Hz, 1H), 3.78 (s, 6H), 3.94 (d, *J* = 13.7 Hz, 1H), 4.03-4.08 (m, 1H), 4.45-4.50 (m, 1H), 4.75-4.80 (m, 1H), 6.46-6.49 (m, 2H), 7.27-7.28 (m, 1H), 7.46-7.48 (m, 1H), 7.62-7.65 (m, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.36-8.37 (m, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.3, 45.2, 45.6, 49.8, 53.6, 54.9, 55.0, 98.0, 103.7, 117.6, 119.6, 126.6, 127.1, 127.3, 128.9, 129.3, 130.6, 136.1, 146.7, 147.5, 150.5, 155.7, 158.4, 159.8; HRMS (ESI) calcd for $C_{24}H_{26}N_5O_2$ $[M + H]^+$: 416.2081, found: 416.2086.

(*R*)-(4-Fluorophenyl)[8-methyl-3-(quinolin-2-yl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]methanone (4a). To a solution of compound **6** (83.0 mg, 0.200 mmol) in CH_2Cl_2 (1.00 mL) was added TFA (115 μL, 1.50 mmol) at 0 °C. After being stirred for 4.5 h at room temperature, the reaction mixture was diluted with EtOAc and H_2O. The aqueous layer was basified by 2 M NaOH and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. After the filtrate was concentrated, the residue was dissolved in CH_2Cl_2 (2.00 mL). To the solution were added Et_3N (55.8 μL, 0.0642 mmol) and 4-fluorobenzoyl chloride (3.88 μL, 0.0321 mmol). After being stirred for 15 min, the reaction mixture was diluted with CH_2Cl_2, washed with 1 M HCl and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 1/3) to give the title compound **4a** (20.3 mg, 26%): colorless solid; mp 232-234 °C; $[\alpha]^{24}_D$ +57.3 (*c* 0.60, MeOH); IR (neat) 1641 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ 1.67 (d, *J* = 6.9 Hz, 3H), 3.66-3.71 (m, 1H), 4.19-4.22 (m, 1H), 4.42-4.46 (m, 1H), 5.08-5.11 (m, 1H), 5.67 (br s, 1H), 7.28-7.31 (m, 2H), 7.59-7.67 (m, 3H), 7.80-7.83 (m, 1H), 8.01-8.10 (m, 2H), 8.29-8.30 (m, 1H), 8.49 (d, *J* = 8.0 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ 18.6, 38.4, 45.3, 46.2, 115.0 (d, $J_{C\text{-}F}$ = 21.6 Hz, 2C), 119.2, 126.9, 127.0, 128.6, 128.9 (d, $J_{C\text{-}F}$ = 9.6 Hz, 2C), 129.7 (2C), 131.5 (d, $J_{C\text{-}F}$ = 3.6 Hz), 136.8, 146.3, 147.1, 150.1, 153.2, 163.4, 168.4; HRMS (ESI) calcd for $C_{22}H_{19}FN_5O$ $[M + H]^+$: 388.1568, found: 388.1566.

***tert*-Butyl 4-methyl-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate (8).** To a solution of *tert*-butyl 2-methyl-3-oxopiperidine-1-carboxylate **7** (213 mg, 1.00 mmol) in DMF (2.00 mL) were added 1-azido-4-nitrobenzene[29] (213 mg, 1.30 mmol) and NH_4OAc (385 mg, 5.00 mmol). After being stirred for 11 h at 80 °C, the reaction mixture was extracted with EtOAc, washed with 1 M HCl and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 3/1) to give the title compound **8** (148 mg, 62%): colorless amorphous solid; IR (neat) 1693 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.47 (d, *J* = 6.3 Hz, 3H), 1.50 (s, 9H), 2.78-2.83 (m, 2H), 3.03-3.09 (m, 1H), 4.44 (br s, 1H), 5.40

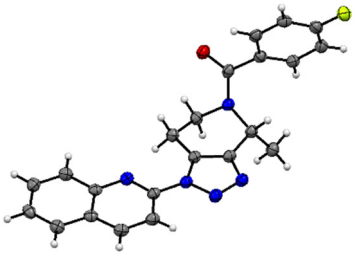
(br s, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 19.4, 22.4, 28.5 (3C), 37.4, 47.0, 80.4, 140.7, 145.3, 154.7; HRMS (ESI) calcd for $C_{11}H_{18}N_4O_2$ $[M + H]^+$: 239.1503, found: 239.1501.

***tert*-Butyl 4-methyl-1-(quinolin-2-yl)-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridine-5-carboxylate (9).** A solution of triazole **8** (71.4 mg, 0.300 mmol) and 2-chloroquinoline (172 mg, 0.900 mmol) in *i*-Pr_2NEt (209 μL, 1.20 mmol) was stirred for 58 h at 120 °C. The reaction mixture was extracted with EtOAc, washed brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 6/1) to give the title compound **9** (23.0 mg, 21%): colorless amorphous solid; IR (neat) 1695 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.56 (d, *J* = 6.9 Hz, 3H), 3.08-3.13 (m, 1H), 3.27-3.34 (m, 1H), 3.55-3.59 (m, 1H), 4.51 (br s, 1H), 5.45 (br s, 1H), 7.57-7.60 (m, 1H), 7.74-7.77 (m, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 8.01 (d, *J* = 8.6 Hz, 1H), 8.29-8.35 (m, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 19.0, 25.0, 28.5 (3C), 36.5, 47.0, 80.2, 113.9, 127.0, 127.4, 127.7, 128.9, 130.5, 131.4, 139.3, 146.3, 146.9, 149.6, 154.5; HRMS (ESI) calcd for $C_{20}H_{24}N_5O_2$ $[M + H]^+$: 366.1925, found: 366.1921.

(4-Fluorophenyl)[4-methyl-1-(quinolin-2-yl)-1,4,6,7-tetrahydro-5*H*-[1,2,3]triazolo[4,5-*c*]pyridin-5-yl]methanone (4b). To a solution of quinoline **9** (7.80 mg, 0.0214 mmol) in CH_2Cl_2 (500 μL) was added HCl (4 M in dioxane; 250 μL, 1.00 mmol). After being stirred for 1 h, the reaction mixture was basified by 2 M NaOH solution at 0 °C, and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was dissolved in CH_2Cl_2 (500 μL). To the solution were added Et_3N (8.95 μL, 0.0642 mmol) and 4-fluorobenzoyl chloride (3.88 μL, 0.0321 mmol). After being stirred for 15 min, the reaction mixture was diluted with CH_2Cl_2, washed with 1 M HCl and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 3/1) to give the title compound **4b** (7.30 mg, 88%): colorless solid; mp 199-201 °C; IR (neat) 1637 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.58 (d, *J* = 6.9 Hz, 3H), 3.30-3.54 (m, 3H), 4.17 (br s, 1H), 5.50 (br s, 1H), 7.26-7.29 (m, 2H), 7.53-7.55 (m, 2H), 7.65-7.67 (m, 1H), 7.83-7.84 (m, 1H), 8.01-8.07 (m,

2H), 8.22 (d, J = 8.6 Hz, 1H), 8.62 (d, J = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 18.5, 23.8, 38.0, 46.1, 113.2, 115.0 (d, $J_{C\text{-}F}$ = 21.6 Hz, 2C), 126.7 (2C), 127.5, 127.9, 128.6 (d, $J_{C\text{-}F}$ = 4.8 Hz, 2C), 130.3, 130.7, 132.4 (d, $J_{C\text{-}F}$ = 3.6 Hz), 139.6, 145.2, 145.3, 148.6, 162.3 (d, $J_{C\text{-}F}$ = 247.1 Hz), 168.7; HRMS (ESI) calcd for $C_{22}H_{19}FN_5O$ $[M + H]^+$: 388.1568, found: 388.1566.

Crystallography of 4b. The data of the compound **4b** ($C_{22}H_{18}FN_5O$) was collected with a Rigaku XtaLAB P200 diffractometer using multi-layer mirror monochromated Cu-Kα radiation at 93 K. The substance was crystallized from $CHCl_3$–*n*-hexane as clear colorless block crystals and solved in primitive orthorhombic space group $P2_1/c$ with Z = 4. The unit cell dimensions are a = 7.5117(3), b = 35.6861(8), c = 7.4465(4), V = 1788.70(15) Å^3, Dcalc = 1.439 g/cm^3, Mw: 387.41. R = 0.0746, GOF = 1.036. The CCDC deposition number: CCDC 1893155.



***tert*-Butyl 2-methyl-3-oxo-4-(quinoline-2-carbonyl)piperidine-1-carboxylate (10).** To a solution of *i*-Pr_2NH (425 μL, 3.00 mmol) in THF (2.00 mL) was added *n*-BuLi (1.39 M in hexane; 2.45 mL, 3.40 mmol) at −78 °C under argon and the mixture was stirred for 20 min at 0 °C. A solution of ketone **7** (426 mg, 2.00 mmol) in THF (2.00 mL) was added to the mixture at −78 °C and the mixture was stirred for 30 min at this temperature. A solution of quinaldoyl chloride (497 mg, 2.60 mmol) in THF (4.00 mL) was added to the mixture. After being stirred for 6 h at room temperature, the reaction mixture was quenched with 1 M HCl and the whole was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 10/1) to give the title compound **10** (271 mg, 37%): yellow solid; mp 123-125 °C; IR (neat) 1687 (C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.49-1.50 (m, 12H), 2.78-2.81 (m, 1H), 2.97-2.99 (m, 2H), 4.10-4.12 (m, 1H),

4.75 (br s, 1H), 7.62-7.66 (m, 1H), 7.76-7.80 (m, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 1H), 8.31 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 17.5, 25.9, 28.5 (3C), 37.6, 52.8, 80.0, 106.8, 120.6, 127.7, 128.4, 128.6, 129.4, 130.4, 137.3, 137.5, 145.6, 153.9, 154.1, 186.6; HRMS (ESI) calcd for $C_{21}H_{25}N_2O_4$ $[M + H]^+$: 369.1809, found: 369.1811.

***tert*-Butyl 7-methyl-3-(quinolin-2-yl)-4,5-dihydroisoxazolo[3,4-*c*]pyridine-6-(7*H*)-carboxylate (11c) and *tert*-butyl 7-methyl-3-(quinolin-2-yl)-4,7-dihydroisoxazolo[5,4-*c*]pyridine-6-(5*H*)-carboxylate (11d).** To a solution of diketone **10** (159 mg, 0.432 mmol) in *i*-PrOH (2.00 mL) were added NH_2OH·HCl (60.0 mg, 0.864 mmol) and NaOH (52.0 mg, 1.30 mmol). After being stirred for 2 h under reflux conditions, the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 20/1) to give the title compounds **11c** (35.2 mg, 22%) and **11d** (43.5 mg, 28%).

Compound **11c**: colorless solid; mp 137-139 °C; IR (neat) 1698 (C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 1.56 (d, *J* = 7.0 Hz, 3H), 2.93-3.08 (m, 2H), 3.39-3.44 (m, 1H), 4.43 (br s, 1H), 5.55 (br s, 1H), 7.55-7.59 (m, 1H), 7.72-7.76 (m, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 8.24 (d, *J* = 8.7 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 19.7, 22.1, 28.4 (3C), 36.3, 46.4, 80.3, 113.1, 118.5, 127.3, 127.5, 127.6, 129.7, 130.0, 136.9, 147.6, 147.9, 154.4, 161.9, 163.9; HRMS (ESI) calcd for $C_{21}H_{24}N_3O_3$ $[M + H]^+$: 366.1812, found: 366.1812.

Compound **11d**: colorless solid; mp 127-129 °C; IR (neat) 1697 (C=O) cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.51-1.53 (m, 12H), 2.93-3.05 (m, 2H), 3.24-3.28 (m, 1H), 4.35-4.48 (br m, 1H), 5.29-5.45 (br m, 1H), 7.56-7.60 (m, 1H), 7.71-7.76 (m, 1H), 7.83-7.85 (m, 1H), 8.10-8.14 (m, 2H), 8.23 (d, *J* = 8.2 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 17.9, 22.5, 28.4 (3C), 37.0, 47.6, 80.4, 111.7, 119.6, 127.2, 127.6, 127.9, 129.7, 129.8, 136.7, 147.9, 149.7, 154.3, 159.6, 169.3; HRMS (ESI) calcd for $C_{21}H_{24}N_3O_3$ $[M + H]^+$: 366.1812, found: 366.1822.

(4-Fluorophenyl)[7-methyl-3-(quinolin-2-yl)-4,5-dihydroisoxazolo[3,4-*c*]pyridin-6(7*H*)-

yl]methanone (4c). By use of a procedure similar to that described for the preparation of the compound **4b** from **9**, the compound **11c** (43.5 mg, 0.0214 mmol) was converted to the title compound **4c** (36.8 mg, 80%): colorless solid; mp 172-174 °C; IR (neat) 1640 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.60 (d, J = 6.9 Hz, 3H), 2.99-3.06 (m, 1H), 3.30-3.42 (m, 2H), 4.04-4.06 (m, 1H), 5.63-5.65 (m, 1H), 7.26-7.30 (m, 2H), 7.54-7.56 (m, 2H), 7.63-7.67 (m, 1H), 7.80-7.83 (m, 1H), 7.97-8.02 (m, 2H), 8.06 (d, J = 8.6 Hz, 1H), 8.51 (d, J = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 18.9, 21.3, 38.4, 45.4, 112.0, 115.0 (d, $J_{C\text{-}F}$ = 21.6 Hz, 2C), 117.9, 127.0 (d, $J_{C\text{-}F}$ = 13.2 Hz, 2C), 127.5, 128.6, 128.7, 130.0, 132.1 (d, $J_{C\text{-}F}$ = 3.6 Hz), 137.1, 146.5, 147.0, 161.2, 161.3, 162.3 (d, $J_{C\text{-}F}$ = 248.3 Hz), 163.3, 168.7; HRMS (ESI) calcd for $C_{23}H_{19}FN_3O_2$ $[M + H]^+$: 388.1456, found: 388.1458.

(4-Fluorophenyl)[7-methyl-3-(quinolin-2-yl)-4,7-dihydroisoxazolo[5,4-*c*]pyridin-6(5*H*)-yl]methanone (4d). By use of a procedure similar to that described for the preparation of the compound **4b** from **9**, the compound **11d** (35.2 mg, 0.0961 mmol) was converted to the title compound **4d** (25.2 mg, 68%): colorless solid; mp 161-163 °C; IR (neat) 1603 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.60 (d, J = 6.9 Hz, 3H), 2.99-3.06 (m, 1H), 3.30-3.42 (m, 2H), 4.04-4.06 (m, 1H), 5.63-5.65 (m, 1H), 7.26-7.30 (m, 2H), 7.54-7.56 (m, 2H), 7.63-7.67 (m, 1H), 7.80-7.83 (m, 1H), 7.97-8.02 (m, 2H), 8.06 (d, J = 8.6 Hz, 1H), 8.51 (d, J = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 17.1, 21.8, 37.2, 46.3, 110.6, 115.0 (d, $J_{C\text{-}F}$ = 19.2 Hz, 2C), 118.7, 127.0, 127.4 (d, $J_{C\text{-}F}$ = 18.0 Hz, 2C), 128.7 (2C), 129.6, 132.1 (d, $J_{C\text{-}F}$ = 3.6 Hz) , 136.8, 146.9, 148.6, 158.8, 161.3, 162.3 (d, $J_{C\text{-}F}$ = 247.1 Hz), 168.4, 168.6; HRMS (ESI) calcd for $C_{23}H_{19}FN_3O_2$ $[M + H]^+$: 388.1456, found: 388.1464.

***tert*-Butyl (*R*)-{1-[5-(quinolin-2-yl)isoxazol-3-yl]ethyl}carbamate (16).** DMSO (710 μL, 10.0 mmol) was added dropwise to a solution of oxalyl chloride (396 μL, 7.50 mmol) in CH_2Cl_2 (10.0 mL) under argon at −78 °C. After the reaction mixture was stirred for 15 min at same temperature, a solution of *N*-(*tert*-butoxycarbonyl)-D-alaninol **12** (876 mg, 5.00 mmol) in CH_2Cl_2 (5.00 mL) was added to the mixture dropwise. After the reaction mixture was stirred for 30 min at same temperature, *i*-Pr_2NEt (4.35 mL, 25.0 mmol) was added to the mixture dropwise. After being stirred for 30 min at

0 °C, the reaction mixture was washed with 1 M HCl and brine, dried over $MgSO_4$. After the filtrate was concentrated, the residue was dissolved in EtOH (5.00 mL). To the mixture were added $NH_2OH \cdot HCl$ (417 mg, 6.00 mmol) and NaOAc (492 mg, 6.00 mmol) and the reaction was continued for 30 min at 80 °C. The reaction mixture was diluted with EtOAc and washed with H_2O and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was dissolved in DMF (10.0 mL). To the mixture were added NCS (1.00 g, 7.50 mmol) and HCl (4 M in dioxane; 125 µL, 0.500 mmol and the reaction was continued for 1 h at 40 °C. The reaction mixture was diluted with EtOAc, washed with H_2O and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was dissolved in EtOAc (10.0 mL). To the mixture were added 2-ethynylquinoline[30] (459 mmol, 3.00 mmol) and $NaHCO_3$ (1.01 g, 12.0 mmol). After being stirred for 15 h at 40 °C, the mixture was diluted with EtOAc and washed with H_2O and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 20/1 to 5/1) to give the title compound **16** (575 mg, 57%): colorless needles; $[\alpha]^{24}_D$ +51.0 (*c* 1.35, $CHCl_3$); mp 135-137 °C; IR (neat) 1684 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.48 (s, 9H), 1.60 (d, *J* = 6.9 Hz, 3H), 5.07 (br s, 1H), 5.20 (br s, 1H), 7.06 (s, 1H), 7.57-7.60 (m, 1H), 7.74-7.78 (m, 1H), 7.83-7.85 (m, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 8.12-8.14 (m, 1H), 8.26 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 20.8, 28.3 (3C), 44.0, 79.8, 101.4, 118.2, 127.5, 127.7, 128.0, 129.7, 130.3, 137.2, 146.2, 148.0, 155.0, 166.8, 169.7; HRMS (ESI) calcd for $C_{19}H_{21}N_3O_3Na$ $[M + Na]^+$: 362.1475, found: 362.1476.

***tert*-Butyl (*R*)-{1-[4-bromo-5-(quinolin-2-yl)isoxazol-3-yl]ethyl}carbamate (17).** To a solution of isoxazole **16** (400 mg, 1.18 mmol) and NBS (630 mg, 3.54 mmol) in DMF (6.00 mL) was added HBr (47% solution in H_2O; 13.5 µL, 0.118 mmol). After being stirred for 12 h at 60 °C, the reaction mixture was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 20/1 to 6/1) to give the title compound **17** (172 mg, 35%): colorless solid; $[\alpha]^{27}_D$ –7.81 (*c* 0.97,

$CHCl_3$); mp 117-119 °C; IR (neat) 1712 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.60 (d, *J* = 7.4 Hz, 3H), 5.12-5.15 (m, 1H), 5.29 (d, *J* = 8.0 Hz, 1H), 7.61-7.64 (m, 1H), 7.77-7.81 (m, 1H), 7.86-7.88 (m, 1H), 8.05-8.07 (m, 1H), 8.23 (d, *J* = 8.6 Hz, 1H), 8.30 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 20.5, 28.4 (3C), 43.7, 79.9, 91.7, 119.2, 127.6, 127.9, 128.0, 130.1, 130.4, 137.1, 145.9, 147.9, 154.8, 164.1, 165.2; HRMS (ESI) calcd for $C_{19}H_{21}BrN_3O_3$ $[M + H]^+$: 418.0761, found: 418.0751.

***tert*-Butyl (*R*)-{1-[5-(quinolin-2-yl)-4-vinylisoxazol-3-yl]ethyl}carbamate (18).** To a solution of bromide **17** (313 mg, 0.750 mmol), vinylboronic acid pinacol ester (257 μL, 1.50 mmol), and $Pd(Ph_3P)_4$ (86.7 mg, 0.0750 mmol) in DMF (3.75 mL) was added 2 M Cs_2CO_3 solution (3.75 mL). After being stirred for 4 h at 100 °C, the reaction mixture was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 20/1 to 10/1) to give the title compound **18** (195 mg, 71%): colorless solid; $[\alpha]^{25}_D$ –54.5 (*c* 0.98, $CHCl_3$); mp 122-124 °C; IR (neat) 1710 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.61 (d, *J* = 6.3 Hz, 3H), 5.27-5.28 (m, 1H), 5.35-5.37 (m, 1H), 5.58 (d, *J* = 12.0 Hz, 1H), 5.80-5.84 (m, 1H), 7.55-7.62 (m, 2H), 7.75-7.78 (m, 1H), 7.85-7.86 (m, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 8.17 (d, *J* = 8.6 Hz, 1H), 8.27 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 20.9, 28.4 (3C), 43.6, 79.8, 114.8, 119.2, 119.5, 125.3, 127.6 (3C), 129.9, 130.2, 136.9, 147.6, 147.8, 155.0, 163.6, 164.5; HRMS (ESI) calcd for $C_{21}H_{24}N_3O_3$ $[M + H]^+$: 366.1812, found: 366.1819.

***tert*-Butyl (*R*)-{1-[4-(2-hydroxyethyl)-5-(quinolin-2-yl)isoxazol-3-yl]ethyl}carbamate (19).** To a solution of compound **18** (146 mg, 0.40 mmol) in THF (2.40 mL) was added 9-BBN (0.5 M in THF; 2.40 mL, 1.20 mmol) at 0 °C and the mixture was stirred for 11 h at room temperature. To the reaction mixture were added MeOH (400 μL), 2 M NaOH (400 μL), and H_2O_2 (30% solution in H_2O; 81.7 μL, 0.800 mmol). After being stirred for 5 h at room temperature, the reaction mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$. After the filtrate was concentrated, the residue was

purified by column chromatography on silica gel (*n*-hexane/EtOAc = 20/1 to 10/1) to give the title compound **19** (53.3 mg, 35%): orange solid; $[\alpha]^{25}_{D}$ –9.23 (*c* 0.81, $CHCl_3$); mp 66-68 °C; IR (neat) 1678 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.64 (d, *J* = 6.9 Hz, 3H), 3.12-3.27 (m, 2H), 4.04-4.06 (m, 2H), 5.08-5.09 (m, 1H), 5.24 (d, *J* = 8.6 Hz, 1H), 6.07 (br s, 1H), 7.60-7.63 (m, 1H), 7.76-7.79 (m, 1H), 7.86-7.88 (m, 1H), 8.00 (d, *J* = 8.6 Hz, 1H), 8.14 (d, *J* = 8.6 Hz, 1H), 8.33 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 20.5, 25.4, 28.3 (3C), 42.4, 62.9, 80.0, 114.9, 119.3, 127.7, 127.8, 127.9, 128.8, 130.7, 137.9, 146.7 (2C), 154.9, 165.0, 165.9; HRMS (ESI) calcd for $C_{21}H_{25}N_3O_4Na$ $[M + Na]^+$: 406.1737, found: 406.1736.

(*R*)-(4-Fluorophenyl)[7-methyl-3-(quinolin-2-yl)-4,5-dihydroisoxazolo[3,4-c]pyridin-6(7*H*)-yl]methanone ((*R*)-4c). To a solution of compound **19** (38.3 mg, 0.100 mmol) in CH_2Cl_2 (500 μL) were added Et_3N (41.8 μL, 0.300 mmol) and MsCl (11.6 μL, 0.150 mmol). After being stirred for 2.5 h at room temperature, the reaction mixture was washed with brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was dissolved in CH_2Cl_2 (1.00 mL) and HCl (4 M in dioxane; 1.00 mL, 4.00 mmol). After being stirred for 2 h, the reaction mixture was basified by 2 M NaOH solution, stirred for 1 h at room temperature, and the whole was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was dissolved in CH_2Cl_2 (1.00 mL). To the solution were added Et_3N (40.9 μL, 0.300 mmol) and 4-fluorobenzoyl chloride (18.1 μL, 0.150 mmol). After being stirred for 30 min, the reaction mixture was diluted with CH_2Cl_2, washed with 1 M HCl and brine, and dried over $MgSO_4$. After the filtrate was concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 3/1) to give the title compound (*R*)-**4c** (15.3 mg, 40%): pale yellow solid; $[\alpha]^{25}_{D}$ +132 (*c* 0.46, $CHCl_3$); mp 181-183 °C; IR (neat) 1639 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-*d*$_6$) δ 1.60 (d, *J* = 6.9 Hz, 3H), 3.02-3.07 (m, 1H), 3.31-3.41 (m, 2H), 4.03 (br s, 1H), 5.63 (br s, 1H), 7.26-7.29 (m, 2H), 7.53-7.56 (m, 2H), 7.64-7.67 (m, 1H), 7.81-7.84 (m, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 8.01-8.03 (m, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 8.53 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-*d*$_6$) δ 18.9, 21.3, 38.3, 45.4, 112.0, 115.0 (d,

$J_{C\text{-}F}$ = 20.4 Hz, 2C), 117.9, 127.0 (d, $J_{C\text{-}F}$ = 13.2 Hz, 2C), 127.5, 128.6, 128.7 (2C), 129.9, 132.1 (d, $J_{C\text{-}F}$ = 3.6 Hz), 137.1, 146.5, 147.1, 161.2, 162.3 (d, $J_{C\text{-}F}$ = 247.1 Hz), 162.7, 168.7; HRMS (ESI) calcd for $C_{23}H_{19}FN_3O_2$ $[M + H]^+$: 388.1456, found: 388.1460.

(*S*)-(4-Fluorophenyl)[7-methyl-3-(quinolin-2-yl)-4,5-dihydroisoxazolo[3,4-c]pyridin-6(7*H*)-yl]methanone ((*S*)-4c). The compound **4c** (8.50 mg, 0.0220 mmol) was separated by COSMOSIL CHiRAL 5A column (4.6 × 250 mm, Nacalai Tesque, Inc., 35% EtOH in *n*-hexane). After the fractions were concentrated, the residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc = 3/1) to give the title compound (*S*)-**4c** (4.06 mg, 48%): pale yellow solid; $[\alpha]^{24}_D$ –98.0 (*c* 0.25, $CHCl_3$); mp 174-176 °C; IR (neat) 1640 (C=O) cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 1.60 (d, *J* = 6.9 Hz, 3H), 3.02-3.06 (m, 1H), 3.30-3.41 (m, 2H), 4.03 (br s, 1H), 5.63 (br s, 1H), 7.26-7.29 (m, 2H), 7.53-7.56 (m, 2H), 7.64-7.67 (m, 1H), 7.80-7.84 (m, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 8.02 (d, *J* = 8.6 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 8.52 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ 18.9, 21.3, 38.4, 45.4, 112.0, 115.0 (d, $J_{C\text{-}F}$ = 20.4 Hz, 2C), 117.9, 127.0 (d, $J_{C\text{-}F}$ = 14.4 Hz, 2C), 127.5, 128.6, 128.7 (2C), 129.9, 132.1, 137.1, 146.5, 147.1, 161.2, 162.4 (d, $J_{C\text{-}F}$ = 260.3 Hz), 162.7, 168.7; HRMS (ESI) calcd for $C_{23}H_{19}FN_3O_2$ $[M + H]^+$: 388.1456, found: 388.1453.

4.2. Inhibitory activity of the fezolinetant derivatives against NK3R

NK3R antagonistic activity of the fezolinetant derivatives was evaluated by $[Ca^{2+}]_i$ flux assay. NK3R expressing CHO cells (4.0×10^4 cells/50 μL/well) were inoculated in 10% FBS/Ham's F-12 onto a 96-well black clear-bottom plate (Greiner), followed by incubation at 37 °C for 24 h in 5% CO_2. After the medium was removed, 50 μL of the pigment mixture (Calcium 4 assay kit, Molecular Devices) and 50 μL of the compound solution at different concentrations in assay buffer (HANKS/HEPES containing 2.5 mM probenecid, 0.2 % BSA, and 0.1% CHAPS) was dispensed into each well of the plate, followed by incubation at 37 °C for 1 h. Separately, an NK3R agonist solution (0.1 nM senktide) in assay buffer was prepared on a 96-well sample plate (V-bottom plate, Coster). The cell and agonist solution plates were set in FDSS/μcell (Hamamatsu) and 25 μL of agonist solution was automatically

transferred to the cell plate.

4.3. UV-vis spectra

UV-vis spectra were recorded on Shimadzu UV-2450 UV-vis spectrophotometer. Compounds (30 μM) were dissolved in a mixture of 50 mM phosphate buffer (pH 7.4) and EtOH [70:30 (v/v)] (containing 0.1% DMSO).

4.4. Investigation of photodegradation of fezolinetant derivatives

Compounds (30 μM) were dissolved in a mixture of 50 mM phosphate buffer (pH 7.4) and EtOH [70:30 (v/v)] (containing 0.1% DMSO) and the reaction mixture was exposed to UV-light irradiation using an MUV-202U (Moritex Co., Japan) equipped a mercury xenon lamp (1500 W/m^2) or a MAX-303 (Asahi-bunko, Japan) equipped a xenon arc lamp (900 W/m^2) and special glass filters restricting the transmission of wavelength below 325 nm. A 50 μL aliquot was sampled at the indicated intervals, and distilled with MeCN (50 μL). An aliquot of the sample was analyzed by HPLC and the peak area was recorded by UV detection at 254 nm. The ratios of the resulting compounds were calculated from the calibration curves.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at ##############.

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