Accepted Manuscript

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PII: S0968-0896(16)30389-3
DOI: http://dx.doi.org/10.1016/j.bmc.2016.05.054
Reference: BMC 13038

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 21 April 2016
Revised Date: 26 May 2016
Accepted Date: 27 May 2016

Please cite this article as: Yamamoto, K., Okazaki, S., Ohno, H., Matsuda, F., Ohkura, S., Maeda, K-I., Fujii, N., Oishi, S., Development of novel NK3 receptor antagonists with reduced environmental impact, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.05.054

Development of novel NK3 receptor antagonists with reduced environmental impact

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ABSTRACT

The neurokinin B (NKB)-neurokinin-3 receptor (NK3R) signaling positively regulates the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. The NK3R-selective antagonists may suppress the reproductive functions of mammals. For development of novel NK3R antagonists with reduced environmental toxicity, a structure–activity relationship study of an NK3R antagonist, talnetant, was carried out. Among several talnetant derivatives with labile functional groups in the natural environment, 3-mercaptoquinoline **2f** exhibited a comparable biological activity to that of the parent talnetant. Additionally, compound **2f** was converted into the disulfide **3f** or isothiazolone **8** by air-oxidation, both of which showed no binding affinity to NK3R.

Keywords: environmental toxicity, GnRH, neurokinin B, NK3 receptor, talnetant

Abbreviations: GnRH, gonadotropin-releasing hormone; NKB, neurokinin B; NK3R, neurokinin-3 receptor; PCOS, polycystic ovary syndrome.

1. Introduction

Pharmaceuticals are exploited to cure diseases and/or to alleviate symptoms in humans or veterinary therapy. For these purposes, the bioactive ingredient(s) are designed primarily for specific binding to the target molecule(s) of target species. The high chemical stability of the ingredients is crucial for the quality control of drugs during long-term storage before administration. However, in terms of the impacts on the natural environment and ecosystems, the stable bioactive substances excreted from the treated patients or animals may have unfavorable effects on non-target species [1]. Some representative examples of environmental contaminations are antibiotics and steroid hormones. Environmental exposure to antibiotics promotes the emergence of drug-resistant microbial strains, which threatens human and animal health [2-4]. Estradiol derivatives extensively used in veterinary medicine disrupt the endocrine systems of non-target species, thus leading to disabling reproductive functions and increasing developmental abnormalities [5,6]. For the appropriate control of drug delivery and distribution in time and space, a number of technologies have been developed to modify the bioactivities and properties of pharmaceutical agents [7].

Recent studies have revealed that neurokinin B (NKB)-neurokinin-3 receptor (NK3R) signaling plays an important role in controlling mammalian reproduction via regulation of the hypothalamo-pituitary-gonadal (HPG) axis [8,9]. NKB positively regulates the reproductive hormone cascade via activation of the gonadotropin-releasing hormone (GnRH) neuron in the hypothalamus, leading to the pulsatile secretion of luteinizing hormone (LH) from the pituitary gland [9]. Administration of senktide, an NK3R-selective agonist, stimulated LH secretion in diestrous rats [10]. GnRH generator activity was increased by intravenous administration of senktide into ovariectomized goats, which was monitored by recording multiple-unit activity in the mediobasal hypothalamus [11]. Through the structure-activity relationship studies of senktide, we identified novel NK3R selective agonists with high potency and resistance to proteolytic degradation [12,13]. In contrast, NK3R antagonists such as talnetant (**1a**) and AZD2624 (recently renamed AZD4901, **1b**), which were originally developed as antipsychotics, could be negative regulators for reproductive functions (Figure 1). For

example, oral administration of AZD4901 effectively decreased testosterone levels and is now expected to be applicable as a treatment for polycystic ovary syndrome (PCOS) [14]. ESN364 (**1c**) is also a potent NK3R antagonist with a unique triazolopiperazine scaffold [15,16]. Oral administration of ESN364 in monkeys led to reduction of gonadotropin and ovarian hormone levels throughout the menstrual cycle [17]. Clinical investigations also revealed that ESN364 was an effective inhibitor of the activity of the HPG axis by decreasing gonadotropin secretion [18]. Thus, both NK3R agonists and antagonists may be useful for the treatment of reproductive disorders. However, the NK3 modulators and the bioactive metabolite(s) excreted from treated humans or animals may cause environmental and health risks including reproductive disturbances of non-target species via water pollution and/or soil contamination. To minimize the possibility of these adverse effects on non-target species, the bioactive ingredient(s) need be converted into the inactive substance(s), by drug metabolizing systems in the body before excretion or by spontaneous degradation in the environment soon after excretion (Figure 2). Herein, we report the investigation of the structure–activity relationship of a quinoline-based antagonist, talnetant (**1a**), in an effect to develop novel NK3R antagonists with decreased environmental impact.

Figure 1. Structures of NK3 receptor antagonists.

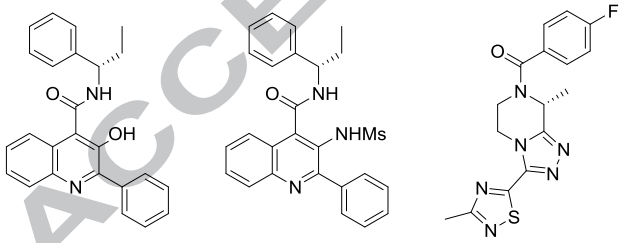
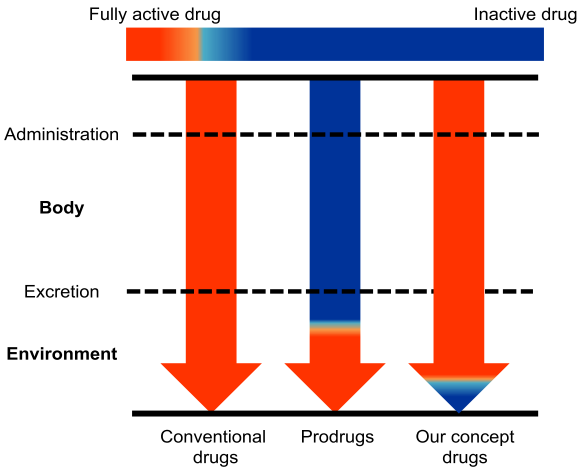


Figure 2. Drug activities over time and space for conventional drugs or our concept drugs.

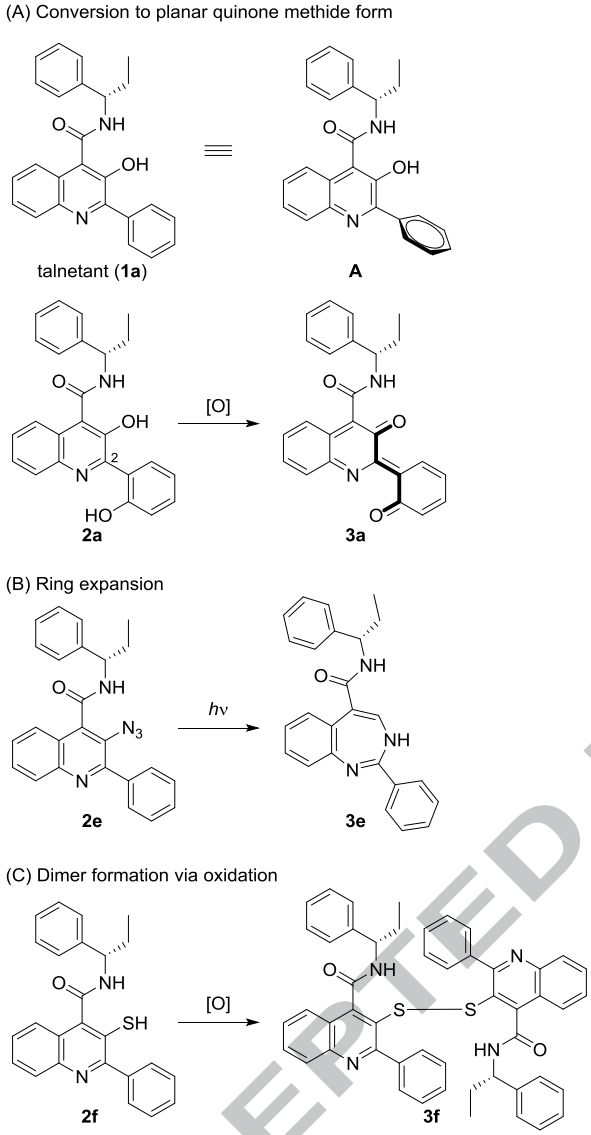


2. Results and Discussion

2.1. Design of NK3R antagonists with minimal environmental impact

We designed novel NK3R antagonists whose biological activities could be altered via three possible conversion processes under the conditions of the natural environment. Compound **2a** having an *o*-hydroxyphenyl group at the quinoline 2-position was designed to be converted under oxidative conditions to provide the quinone methide form **3a** (Figure 3A). It was expected that compound **3a** with a planar conformation would show decreased potency compared with the parent **2a** because talnetant (**1a**) binds to NK3R in the twisted biaryl conformation **A** [19]. Compounds **2c–d** could also be converted into the similar planar quinone methide-type products under oxidative conditions (Table 1). For the second class of NK3R antagonists, 3-azidoquinoline **2e** was designed so that it could be subjected to photoactivation to provide the ring-expanded benzodiazepine **3e** via nitrene formation. Because the quinoline ring is essential to the biological activity [20], we estimated that ring expansion leads to inactivation (Figure 3B). Compound **2f** with a 3-mercapto group was also designed to be dimerized to form a disulfide **3f** under oxidative conditions. We envisioned that the dimerization could lead to a loss or decrease in the biological activity (Figure 3C).

Figure 3. Design of NK3 receptor antagonists with reduced environmental toxicity.

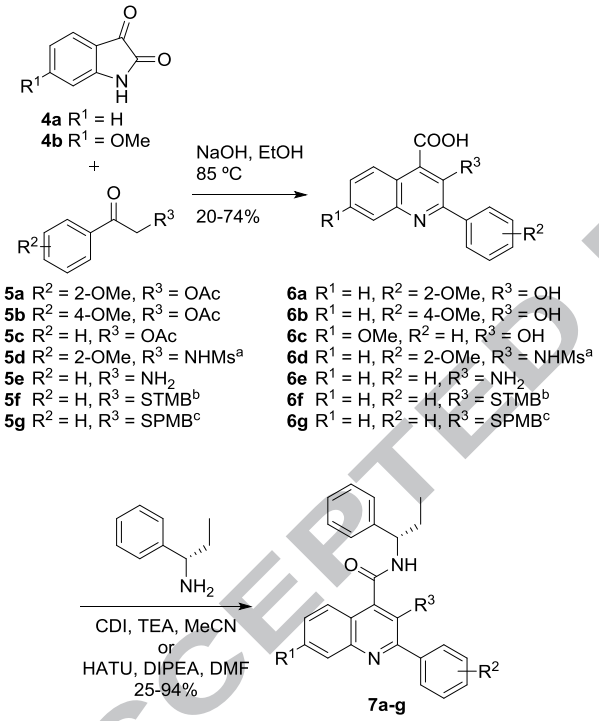


2.2. Synthesis of talnetant derivatives

We synthesized the derivatives with the potential to be inactivated via the above-mentioned three pathways. The Pfitzinger reaction of isatins **4a,b** with the appropriate aryl ketones **5a**–**g** provided quinoline-4-carboxylic acids **6a**–**g** in 20–74% yield. For the preparation of amides **7a**–**g**, carboxylic acids **6a**–**g** were coupled with (*S*)-1-phenylpropan-1-amine using carbonyldiimidazole (CDI) in acetonitrile or *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) in DMF (Scheme 1) [21]. The talnetant derivatives **2a**–**d** with a hydroxy group were obtained by treatment of the methoxy precursors **7a**–**d** with BBr_3 (Scheme 2A and 2B). For the

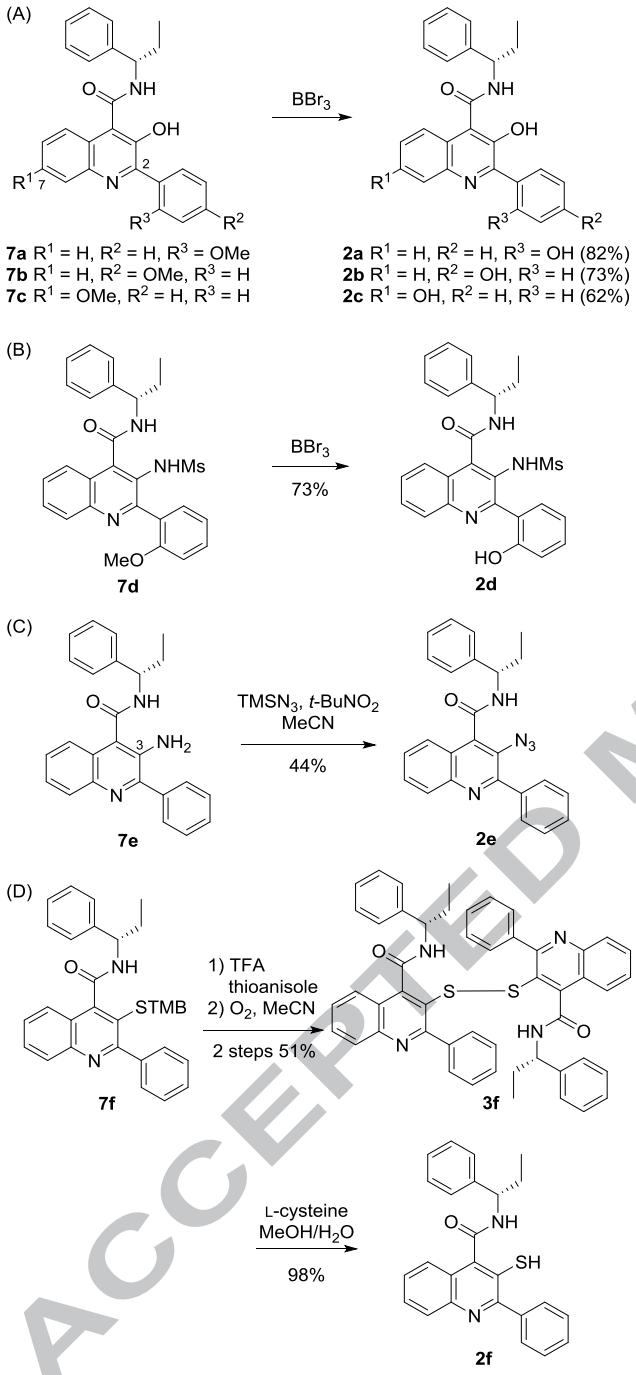
preparation of compound **2e** with an azide group at the quinoline 3-position, compound **7e** was treated with $TMSN_3$ and *t*-$BuNO_2$ under Sandmeyer conditions (Scheme 2C). The derivative **2f** with a 3-mercapto group was prepared from **7f** in three steps via deprotection of the trimethoxybenzyl group, formation of a temporary dimer by air-oxidation, and subsequent reduction with L-cysteine (Scheme 2D). Of note is that the deprotection of the *p*-methoxybenzyl group in **7g** by treatment with TFA, TfOH, and thioanisole for the synthesis of **2f** was unsuccessful.

Scheme 1. Synthesis of talnetant derivative precursors **7a**–**g**.



[a]Ms: methanesulfonyl. [b]TMB: 2,4,6-trimethoxybenzyl. [c]PMB: 4-methoxybenzyl.

Scheme 2. Synthesis of talnetant derivatives **2a**–**f**.

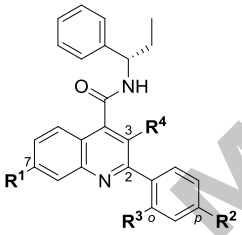


2.3. Biological activity of talnetant derivatives

We investigated the biological activities of talnetant derivatives **2a–f** along with the synthetic intermediates **7a–c** and **7g** (Table 1). The biological activities were evaluated by a competitive binding assay using ([^{125}I]-His^{3}, MePhe^{7})-NKB for human NK3R. The *o*-hydroxy modification at the

2-phenyl group on the quinoline (**2a** and **2d**) led to slightly more potent NK3R inhibition [IC_{50} (**2a**): 6.0 nM, IC_{50} (**2d**): 3.9 nM] compared with talnetant (IC_{50}: 8.4 nM). The 3-mercapto modification (**2f**) also inhibited the NKB binding to NK3R with high potency [IC_{50}(**2f**): 5.6 nM]. In contrast, the 7-methoxy, 7-hydroxyl, 3-azido and 3-(*p*-methoxybenzyl)thio modifications of the quinoline ring (**7c**, **2c**, **2e**, and **7g**) decreased NK3R inhibition [IC_{50} (**7c**): 44 nM, IC_{50} (**2c**): 245 nM, IC_{50} (**2e**): 24 nM, IC_{50} (**7g**): 154 nM]. The *o*-methoxy (**7a**), *p*-methoxy (**7b**), or *p*-hydroxy (**2b**) modifications at the 2-phenyl group were also not favorable.

Table 1. Biological evaluation of talnetant derivatives.



compound	R^1	R^2	R^3	R^4	IC_{50} (nM)
Talnetant	H	H	H	OH	8.4 ± 2.0
AZD2624	H	H	H	NH-Ms[b]	1.6 ± 0.7
7a	H	H	OMe	OH	1527 ± 234
7b	H	OMe	H	OH	965 ± 133
7c	OMe	H	H	OH	44 ± 8.0
2a	H	H	OH	OH	6.0 ± 2.0
2b	H	OH	H	OH	390 ± 68
2c	OH	H	H	OH	245 ± 45
2d	H	H	OH	NH-Ms[b]	3.9 ± 0.8
2e	H	H	H	N_3	24 ± 12
2f	H	H	H	SH	5.6 ± 1.8
7g	H	H	H	S-PMB[c]	154 ± 65

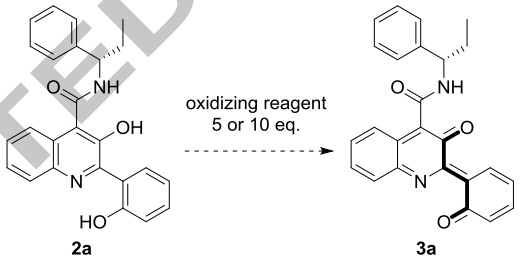
[a]IC_{50} values are the concentrations required for 50% inhibition of the ([^{125}I]His^3, MePhe^7)-NKB binding to the NK3 receptor (n = 6). [b]Ms: methanesulfonyl. [c]PMB: 4-methoxybenzyl.

2.4. Investigation of the stability of potent NK3 derivatives under oxidative conditions or in serum

We examined the structural conversion or degradation from the highly potent diol **2a** and thiol **2f**

under oxidative conditions (Table 2 and Figure 4). When compound **2a** was treated with mild oxidizing agents including hydrogen peroxide (H_2O_2), no reaction occurred to give the possible quinone methide form **3a** with the expected planar conformation. Treatment of **2a** with highly reactive oxidizing agents such as pyridinium chlorochromate (PCC) led to degradation without formation of **3a**. These results that a severe oxidizing condition is needed for conversion from **2a** to the inactive form. In contrast, quinolinethiol **2f** was easily converted to the disulfide form **3f** in MeCN under air for 24 h at 37 °C (Figure 4A). In a solution of 20 mM phosphate buffer (pH 7.4)-MeCN, thiol **2f** was converted to isothiazolone **8** by the same treatment for 12 h at 37 °C (Figure 4B). Both oxidation products (**3f** and **8**) did not inhibit NK3R binding (IC_{50} >10 μM), suggesting that the thiol **2f** has an appropriate chemical property to offset the pharmacological effects under the conditions of the natural environment.

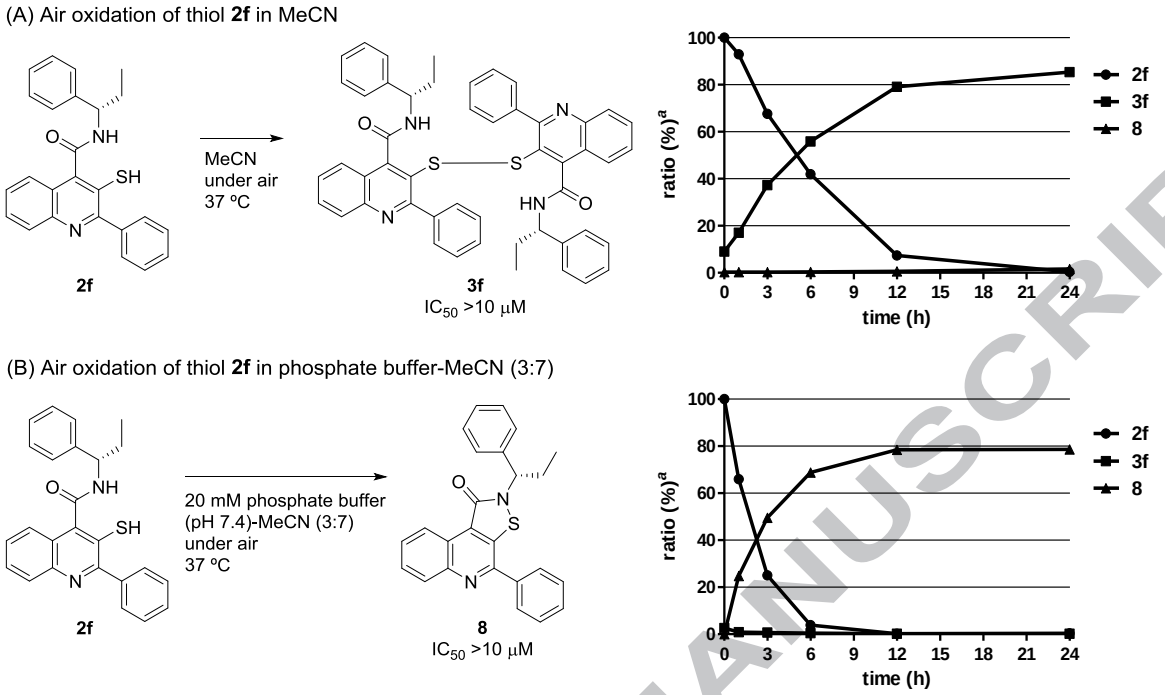
Table 2. Conversion of diol **2a** under oxidative conditions.



entry	oxidizing agent	solvent	time	result
1	Air (K_2CO_3)	acetone	24 h	no reaction
2	Air (DBU)	MeOH	24 h	no reaction
3	H_2O_2/$NaHCO_3$	acetone	24 h	no reaction
4	$FeCl_3$	MeOH	24 h	no reaction
5	DDQ	MeOH	24 h	no reaction
6	MnO_2	acetone	24 h	no reaction
7	$KMnO_4$	acetone	24 h	no reaction
8	PCC	MeOH	5 min	decomp.
9	CAN	MeCN	5 min	decomp.
10	O_3	MeOH	30 min	mixture
11	NBS	MeOH	3 h	mixture
12	Ag_2O	acetone	3 h	mixture

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DDQ: 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; CAN: ammonium cerium (IV) nitrate; PCC: pyridinium chlorochromate, NBS: *N*-bromosuccinimide.

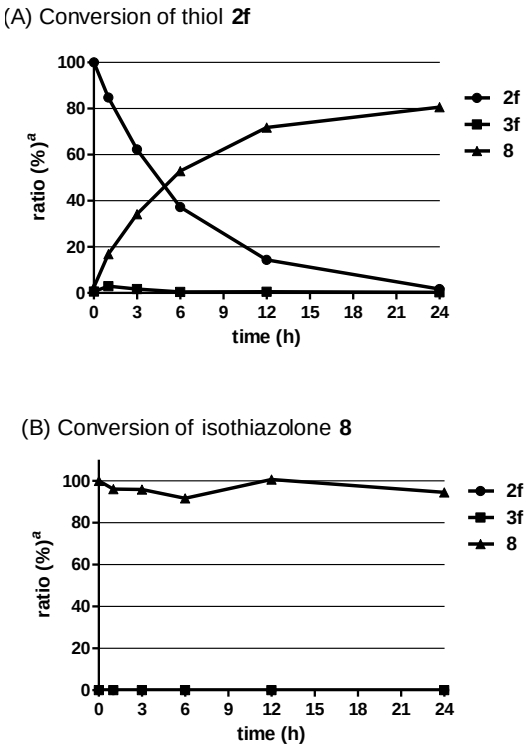
Figure 4. Investigation of the oxidation process of thiol **2f**.



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

We also investigated the stability of thiol **2f** in pig serum by HPLC analysis. Compound **2f** was slowly converted to isothiazolone **8** via air-oxidation in pig serum (Figure 5A), while no change from **8** was observed under the identical conditions over 24 h (Figure 5B). Thus, compound **2f** could be a novel NK3R antagonist, which is irreversibly converted into the inactive form **8** in the body after administration as well as under aerobic conditions after excretion from the body [22]. The inactive compound **8** would be the predominant form present if compound **2f**-derived substances were uptaken from the environment to other organisms.

Figure 5. Stability of thiol **2f** and isothiazolone **8** in pig serum under air at 37 °C.



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

3. Conclusions

In this study, we revealed that talnetant derivatives **2a**,**d** and **2f** with hydroxyl- or mercapto-group modifications exhibited comparable NK3 receptor binding inhibition to that of the parent compound talnetant. Among these potent derivatives, thiol **2f** was spontaneously converted to the inactive disulfide (**3f**) or isothiazolone (**8**) forms under aerobic conditions. Accordingly, the thiol **2f** may be a novel NK3 receptor antagonist for the treatment of sex-hormone disorders with decreased environmental impact.

4. Experimental Section

4.1. Synthesis

4.1.1. General synthesis

^{1}H NMR spectra were recorded using 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 referenced to the residual solvent signal. Exact mass spectra (HRMS) were recorded on a JMS-HX/HX 110A mass spectrometer or Shimadzu LC-ESI-IT-TOF-MS equipment. IR spectra were determined on a JASCO FT/IR-4100 spectrometer. Optical rotations were measured with a JASCO P-1020 polarimeter. For flash chromatography, Wakogel C-300E (Wako) was employed. The purity of the compounds was determined as no less than 95% by combustion analysis or HPLC analysis.

4.1.2. 3-Hydroxy-2-(2-methoxyphenyl)quinoline-4-carboxylic acid (6a)

2-Acetoxy-2'-methoxyacetophenone (**5a**) (920 mg, 4.12 mmol) in EtOH (5.2 mL) was added to a solution of isatin (**4a**) (500 mg, 3.40 mmol) in 10N NaOH (3.0 mL) and EtOH (800 μL) at 85 °C. After the mixture was stirred at 85 °C for 1 h, H_2O (9.0 mL) was added to the reaction mixture at 0 °C. The mixture was acidified to pH 1 with 1N HCl. The precipitate was collected by filtration and washed with H_2O and EtOH to give the title compound **6a** as pale yellow solids (583 mg, 58%); mp 227-229 °C (recrystallized from EtOH); IR (neat) cm^{-1}: 1654 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.77 (s, 3H, CH_3), 7.13 (dd, J = 7.4, 7.4 Hz, 1H, Ar), 7.21 (d, J = 8.0 Hz, 1H, Ar), 7.48 (d, J = 6.9 Hz, 1H, Ar), 7.52-7.56 (m, 1H, Ar), 7.61-7.64 (m, 1H, Ar), 7.68-7.71 (m, 1H, Ar), 8.01 (d, J = 8.0 Hz, 1H, Ar), 8.91 (d, J = 6.3 Hz, 1H, Ar); ^{13}C NMR (125 MHz, DMSO-d_6): δ 55.7, 111.5 (2C), 120.3 (2C), 124.8, 126.0, 126.5 (2C), 128.6, 130.8 (2C), 131.1, 151.6, 157.3 (2C), 170.4; HRMS (ESI): *m/z* calcd for $C_{17}H_{14}NO_4$ $[M+H]^+$ 296.0917, found: 296.0916.

4.1.3. (*S*)-3-Hydroxy-2-(2-methoxyphenyl)-*N*-(1-phenylpropyl)quinoline-4-carboxamide (7a)

CDI (243 mg, 1.50 mmol) was added to a solution of compound **6a** (400 mg, 1.36 mmol) and TEA (247 μL, 1.77 mmol) in MeCN (1.60 mL). After the mixture was stirred at 50 °C for 5 h, (*S*)-1-phenylpropylamine (218 μL, 1.50 mmol) was added to the reaction mixture at 50 °C. After being stirred at 50 °C for 5 h, the reaction mixture was stirred at room temperature overnight. After the reaction mixture was concentrated, the residue was dissolved in EtOAc. The extract was washed with 10% $NaHCO_3$, 1N HCl, H_2O, and brine, and dried over $MgSO_4$. The filtrate was concentrated, and the residue was recrystallized from *n*-hexane–*n*-PrOAc to give the title compound **7a** as colorless crystals (137 mg, 25%); $[\alpha]^{28}_{D}$ –22.4 (*c* 0.93, $CHCl_3$); mp 156-158 °C; IR (neat) cm^{-1}: 3349-3112 (OH), 1638 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.94 (t, J = 7.4 Hz, 3H, CH_3), 1.75-1.81 (m, 2H, CH_2), 3.73 (s, 3H, CH_3), 5.03 (dt, J = 7.6, 7.6 Hz, 1H, CH), 7.07 (ddd, J = 7.4, 7.4, 1.1 Hz, 1H, Ar), 7.13 (d, J = 8.0 Hz, 1H, Ar), 7.25-7.27 (m, 2H, Ar), 7.30 (dd, J = 7.4, 1.7 Hz, 1H, Ar), 7.35 (dd, J = 7.7, 7.7 Hz, 1H, Ar), 7.43-7.49 (m, 4H, Ar), 7.53-7.55 (m, 2H, Ar), 7.92 (d, J = 8.6 Hz, 1H, Ar), 9.08 (d, J = 8.0 Hz, 1H, NH), 9.18 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.2, 29.3, 54.7, 55.4, 111.4, 120.2, 123.5, 125.7, 125.9, 126.3, 126.7 (3C), 126.8, 127.0, 128.2 (2C), 128.8, 130.1, 130.8, 141.9, 143.6, 145.1, 152.0, 157.3, 164.7; *Anal.* calcd for $C_{26}H_{24}N_2O_3$: C, 75.71; H, 5.86; N, 6.79. found: C, 75.68; H, 5.91; N, 6.82.

4.1.4. (*S*)-2-Phenyl-*N*-(1-phenylpropyl)-3-[(2,4,6-trimethoxybenzyl)thio]quinoline-4-carboxamide (7f)

HATU (6.34 g, 16.7 mmol), (*S*)-1-phenylpropylamine (3.20 mL, 22.2 mmol) and DIPEA (7.70 mL, 44.5 mmol) were added to a solution of 2-phenyl-3-[(2,4,6-trimethoxybenzyl)thio]quinoline-4-carboxylic acid (**6f**) (5.10 g, 11.1 mmol) in DMF (16.7 mL). After being stirred at room temperature for 4 h, the mixture was concentrated. After the residue was dissolved in EtOAc, the whole was washed with 1N HCl, H_2O, and brine, and dried over $MgSO_4$. The filtrate was concentrated, and the resulting residue was purified by flash chromatography over silica gel with *n*-hexane–EtOAc (2:1)

to give the title compound **7f** as brown amorphous solids (5.80 g, 91%); $[\alpha]^{28}_{D}$ +55.2 (*c* 0.53, $CHCl_3$); IR (neat) cm^{-1}: 1647 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 0.99 (t, *J* = 7.2 Hz, 3H, CH_3), 1.80-1.83 (m, 2H, CH_2), 3.35 (s, 6H, $CH_3 \times 2$), 3.71-3.72 (m, 5H, CH_2, CH_3), 5.09 (dt, *J* = 7.6, 7.5 Hz, 1H, CH), 6.00 (s, 2H, Ar), 7.27 (dd, *J* = 7.4, 7.4 Hz, 1H, Ar), 7.35 (dd, *J* = 7.4, 7.4 Hz, 2H, Ar), 7.39-7.41 (m, 3H, Ar), 7.46 (d, *J* = 6.9 Hz, 2H, Ar), 7.55-7.56 (m, 4H, Ar), 7.76-7.80 (m, 1H, Ar), 8.03 (d, *J* = 8.6 Hz, 1H, Ar), 8.96 (d, *J* = 8.6 Hz, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.2, 29.3, 29.7, 54.8, 55.2, 55.3 (2C), 90.5 (2C), 105.3, 124.1, 124.8, 124.9, 126.7 (2C), 126.8, 127.2 (3C), 127.9, 128.1 (2C), 129.0, 129.6 (2C), 130.3, 140.3, 143.2, 146.3, 150.3, 158.4 (2C), 160.3, 161.9, 165.3; HRMS (ESI): *m/z* calcd for $C_{35}H_{35}N_2O_4S$ $[M + H]^+$ 579.2312, found: 579.2309.

4.1.5. (*S*)-3-Hydroxy-2-(2-hydroxyphenyl)-*N*-(1-phenylpropyl)quinoline-4-carboxamide (2a)

BBr_3 (1.21 mL, 2.17 mmol) was added to compound **7a** (50.0 mg, 0.121 mmol) at 0 °C. After being stirred at room temperature for 5 h, the mixture was quenched with H_2O and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. After concentration, the residue was purified by flash chromatography over silica gel with *n*-hexane–EtOAc (2:1) to give the title compound **2a** as colorless crystals (39.6 mg, 82%); $[\alpha]^{28}_{D}$ –21.7 (*c* 1.10, DMSO) mp 185–187 °C (recrystallized from *n*-hexane–*n*-PrOAc); IR (neat) cm^{-1}: 1636 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 0.96 (t, *J* = 7.2 Hz, 3H, CH_3), 1.78-1.80 (m, 2H, CH_2), 5.05 (dt, *J* = 7.6, 7.6 Hz, 1H, CH), 6.96-7.01 (m, 2H, Ar), 7.27 (dd, *J* = 7.4, 7.4 Hz, 1H, Ar) , 7.33-7.38 (m, 3H, Ar), 7.44 (d, *J* = 7.4 Hz, 2H, Ar), 7.49-7.52 (m, 1H, Ar), 7.55-7.61 (m, 2H, Ar), 7.83-7.85 (m, 1H, Ar), 7.97 (d, *J* = 8.0 Hz, 1H, Ar), 9.15 (d, *J* = 7.4 Hz, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.2, 29.4, 54.8, 116.5, 119.0, 123.1, 123.6, 125.4, 126.7 (3C), 126.8, 127.0, 127.1, 128.2 (3C), 128.3, 130.7, 131.4, 143.5 (2C), 151.6, 156.4, 164.5; *Anal.* calcd for $C_{25}H_{22}N_2O_3$: C, 75.36; H, 5.57; N, 7.03. found: C, 75.27; H, 5.70; N, 7.00.

4.1.6. (*S*)-3-Azido-2-phenyl-*N*-(1-phenylpropyl)quinoline-4-carboxamide (2e)

Trimethylsilyl azide (39.0 μL, 0.296 mmol) and *tert*-butyl nitrite (35.4 μL, 0.296 mmol) were added to a solution of (*S*)-3-amino-2-phenyl-*N*-(1-phenylpropyl)quinoline-4-carboxamide (**7e**) [23] (50.0 mg, 0.197 mmol) at 0 °C. After being stirred at room temperature for 5h, the reaction mixture was concentrated. Crude product was purified by flash chromatography over silica gel with EtOAc–*n*-hexane (2:1) to give the title compound **2e** as a pale yellow solid (35.0 mg, 44%.); $[\alpha]^{28}_{D}$ –20.3 (*c* 0.67, $CHCl_3$); mp 135–137 °C (recrystallized from *n*-hexane–EtOAc); IR (neat) cm^{-1}: 1632 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.96 (t, *J* = 7.2 Hz, 3H, CH_3), 1.78-1.83 (m, 2H, CH_2), 5.05 (dt, *J* = 7.6, 7.6 Hz, 1H, CH), 7.29 (dd, *J* = 7.4, 7.4 Hz, 1H, Ar), 7.38 (dd, *J* = 7.7, 7.7 Hz, 2H, Ar), 7.43 (d, *J* = 6.9 Hz, 2H, Ar), 7.53-7.65 (m, 5H, Ar), 7.77-7.82 (m, 3H, Ar), 8.08 (d, *J* = 8.6 Hz, 1H, Ar) , 9.44 (d, *J* = 8.6 Hz, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.0, 29.3, 55.0, 124.1, 124.3, 126.6 (2C), 126.9, 127.0, 128.1, 128.3 (2C), 128.5 (2C), 129.2 (3C), 129.3, 129.8, 135.8, 137.3, 143.0, 144.9, 153.6, 163.2; *Anal.* calcd for $C_{25}H_{21}N_5O$: C,73.69; H, 5.19; N, 17.19. found: C, 73.53; H, 5.23; N, 17.25.

4.1.7. 3,3'-Dithiobis{2-phenyl-*N*-[(*S*)-1-phenylpropyl]quinoline-4-carboxamide} (3f)

Compound **7f** (5.70 g, 9.90 mmol) was added to a solution of thioanisole (11.6 mL, 99.0 mmol) in TFA (99.0 mL). After being stirred at room temperature for 2 h, the mixture was concentrated. MeCN (49.5 mL) was added to the residue. After being stirred at room temperature under O_2 for 4 days, the mixture was concentrated. Crude product was purified by flash chromatography over silica gel with EtOAc–*n*-hexane (2:1) to give the title compound **3f** as a pale yellow solid (2.0 g, 51%.); $[\alpha]^{28}_{D}$ –98.7 (*c* 1.12, MeOH); mp 222–224 °C (recrystallized from *n*-hexane–EtOAc); IR (neat) cm^{-1}: 1630 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.72 (t, *J* = 7.4 Hz, 6H, CH_3 × 2), 1.46-1.60 (br m, 4H, CH_2 × 2), 4.67 (dt, *J* = 7.3, 7.3 Hz, 2H, CH × 2), 7.12-7.16 (m, 8H, Ar), 7.19-7.27 (m, 8H, Ar), 7.35 (d, *J* = 6.9 Hz, 4H, Ar), 7.51-7.56 (m, 4H, Ar), 7.78-7.82 (m, 2H, Ar), 8.02 (d, *J* = 8.0 Hz, 2H,

Ar), 8.54 (d, J = 7.4 Hz, 2H, NH × 2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 10.4 (2C), 28.4 (2C), 55.1 (2C), 124.0 (2C), 124.4 (2C), 125.3 (2C), 126.6 (2C), 126.7 (2C), 126.8 (2C), 127.1 (4C), 127.3 (2C), 127.7 (2C), 128.0 (2C), 128.1 (2C) 129.2 (2C), 129.4 (2C), 129.5 (2C), 130.9 (2C), 140.0 (2C), 142.1 (2C), 147.1 (2C), 149.5 (2C), 161.4 (2C), 164.5 (2C); *Anal.* calcd for $C_{50}H_{42}N_4O_2S_2 \cdot 1.5H_2O$: C, 73.05; H, 5.52; N, 6.82. found: C, 72.80; H, 5.31; N, 6.70.

4.1.8. (*S*)-3-Mercapto-2-phenyl-*N*-(1-phenylpropyl)quinoline-4-carboxamide (2f)

L-Cysteine hydrochloride (426 mg, 3.52 mmol) was added to a solution of compound **3f** (1.0 g, 1.26 mmol) in MeOH (50.0 mL) and H_2O (15.0 mL) at 0 °C. After being stirred at room temperature for 30 min, the mixture was filtrated. The filtrate was concentrated and the precipitate was collected by filtration to give the title compound **2f** as a pink solid (746 mg, 74%); $[\alpha]^{28}_D$ –61.8 (*c* 0.75, MeOH); mp 87-89 °C (recrystallized from H_2O–MeOH); IR (neat) cm^{-1}: 3330-3111 (SH), 1629 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.98 (t, J = 7.2 Hz, 3H, CH_3), 1.75-1.87 (m, 2H, CH_2), 5.06 (dt, J = 7.6, 7.6 Hz, 1H, CH), 7.28-7.30 (m, 2H, Ar), 7.39 (dd, J = 7.2, 7.2 Hz, 2H, Ar), 7.44-7.45 (m, 2H, Ar), 7.52-7.58 (m, 4H, Ar), 7.62-7.63 (m, 2H, Ar), 7.74 (dd, J = 7.4, 7.4 Hz, 1H, Ar), 8.02 (d, J = 8.6 Hz, 1H, Ar), 9.47 (d, J = 8.0 Hz, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 11.3, 29.2, 55.0, 123.9, 124.0, 126.7 (3C), 127.0, 127.8, 128.3 (4C), 128.9 (3C), 129.1, 129.4, 139.2, 142.3, 143.1, 144.5, 157.9, 165.4; *Anal.* calcd for $C_{25}H_{22}N_2OS$: C, 75.35; H, 5.56; N, 7.03. found: C, 75.13; H, 5.72; N, 6.78.

4.2. Inhibitory activity of talnetant derivatives against NKB binding to NK3R (Table 1)

Binding inhibition assays were performed according to the procedure in our previous research [12]. Membranes from NK3R-expressing CHO cells were incubated with 50 μL solution of talnetant derivatives, 25 μL of radioactive ligand solution [([^{125}I]His^3, MePhe^7)-NKB, 0.4 nM, PerkinElmer

Life Sciences], and 25 μL of NK3R membrane suspension (10 μg) in assay buffer [50 mM HEPES (pH 7.4), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% BSA]. Reaction mixtures were filtered through GF/B filters, pretreated with 0.3% polyethyleneimine. Filters were washed with wash buffer [50 mM HEPES (pH 7.4), 500 mM NaCl, 0.1% BSA] and dried at 55 °C. Bound radioactibity was measured by TopCount (PerkinElmer Life Sciences) in the presence of MicroScint-O (30 μL) (PerkinElmer Life Sciences).

4.3. Quantitative analysis of air oxidation process from thiol 2f

Compound **2f** (1 mM) was incubated in MeCN or a mixture of 20 mM phosphate buffer (pH 7.4) and MeCN [30:70 (v/v)] under air at 37 °C. An aliquot of the sample was analyzed by HPLC at the indicated intervals and the peak area was recorded by UV detection at 254 nm. The ratios of the resulting compounds were calculated from the calibration curves.

4.4. Quantitative analysis of the conversion of thiol 2f to isothiazolone 8 in pig serum

Compound **2f** (0.1 mM) was incubated in pig serum (containing 0.5% DMSO) at 37 °C. A 20 μL aliquot was sampled at the indicated intervals, and extracted with MeCN (80 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.

Acknowledgements

This work was supported by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry from MAFF, Japan; Grants-in-Aid for Scientific Research from JSPS, Japan; Platform for Drug Discovery, Informatics, and Structural Life Science from MEXT, Japan. We are grateful to Dr. Akira Hirasawa (Kyoto University) for his generous support of this research.

Supplementary data

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

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Graphical abstract