Published in final edited form as:

Bioorg Med Chem Lett. 2008 July 15; 18(14): 4064–4067. doi:10.1016/j.bmcl.2008.05.098.

Identifying Structural Features on 1,1-Diphenyl-hexahydro-oxazolo[3,4-*a*]pyrazin-3-ones Critical For Neuropeptide S Antagonist Activity

Yanan Zhang, **Brian P. Gilmour**, **Hernán A. Navarro**, and **Scott P. Runyon***

Organic and Medicinal Chemistry, Research Triangle Institute, P. O. Box 12194, Research Triangle Park, NC 27709

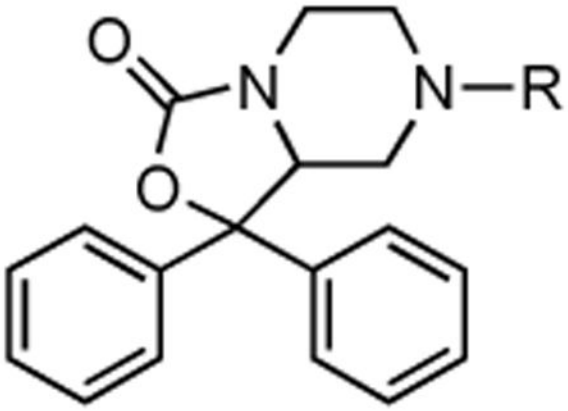
Abstract

A series of 7-substituted 1,1-diphenyl-hexahydro-oxazolo[3,4-*a*]pyrazin-3-ones were synthesized and tested for Neuropeptide S (NPS) antagonist activity. A concise synthetic route was developed, which features a DMAP catalyzed carbamate formation. 4-Fluorobenzyl urea (**1c**) and benzyl urea (**1d**) were identified as the most potent antagonists among the compounds examined. Structure-activity relationships (SARs) demonstrate that a 7-position urea functionality is required for potent antagonist activity and alkylation of the urea nitrogen (**1e**) or replacement with carbon or oxygen (**5a**) results in reduced potency. In addition, compounds with alpha-methyl substitution (**1b**) or elongated alkyl chains (**1h** and **1j**) had reduced potency, indicating a limited tolerance for 7-position substituents.

Graphical abstract

Description: Structural features important for Neuropeptide S antagonist activity have been identified though the synthesis and testing of a series of 7-substituted 1,1-diphenyl-hexahydro-oxazolo[3,4-a]pyrazin-3-ones.

*Corresponding author: Dr. Scott P. Runyon, Research Triangle Institute, Post Office Box 12194, Research Triangle Park, NC 27709-2194, Telephone: 919 316-3842, Fax: 919 541-8868, srunyon@rti.org.



The coupling of putative transmitters with orphan receptors has led to the identification of several interesting ligand-receptor pairings that are now beginning to show novel pharmacology.[1] In particular, the neuropeptide S receptor (NPSR) system deorphanized by Sato and co-workers[2] has recently been shown to modulate a variety of physiological states such as sleep,[3] feeding,[4,5] anxiety,[3,6,7] drug abuse,[8] and inflammation.[9]

The NPS receptor is activated by an endogenous 20 amino acid peptide (Figure 1) resulting in elevated intracellular calcium via its cognate proteins G_q and G_s.[3] Amino acids important for NPS agonist activity have been identified through Ala scanning mutagenesis. In particular, Phe 2, Arg 3, Asn 4, and Val 6 are critical for agonist activity whereas residues 5–13 are hypothesized to form an α-helical recognition sequence.[10] NMR and circular dichroism studies on NPS have indicated a significant degree of flexibility among the amino acids critical for receptor activation, thus making identification of the bioactive NPS conformer difficult using currently available data.[10,11]

Several single nucleotide-polymorphisms (SNP) of the NPS receptor have been shown to exist. In particular, NPSR *Asn*107*Ile* and NPSR C-alt have been fully characterized for agonist sensitivity.[12] Radioligand binding of [^{125}I]Tyr^{10}-NPS is unaltered among receptor variants, however, a 5–10-fold enhancement in functional sensitivity using calcium flux is observed for the *Ile*^{107} variant.[12] Importantly, the SNP (*Asn*^{107} to *Ile*^{107}) in the NPS receptor has been implicated as a risk factor for asthma and has been shown to modulate the functional properties of NPS.[9]

The unique pharmacological properties of NPS were first described by Xu and colleagues who determined that NPS was involved in both arousal and anxiety.[3] Administration of NPS (i.c.v.) increased locomotor activity in both habituated and naïve mice. NPS-treated mice also demonstrated anxiolytic-like behaviors in the elevated plus maze, light dark box, and marble burying paradigm. In more recent studies, Rizzi and co-workers confirmed the arousal and anxiolytic promoting properties of NPS using stress-induced hypothermia, which is a behavioral model insensitive to alterations in locomotor activity.[6]

Recognizing that small molecule probes would greatly aid the pharmacological characterization of NPS receptors, our laboratory undertook a program to identify small

molecule antagonists. The development of small molecule antagonists based on a computationally generated structure of the endogenous peptide was not considered reliable due to the significant conformational flexibility inherent in the peptide.[11] Therefore, our laboratory chose to employ a previously patented scaffold as a lead structure.[13] Although no functional or receptor binding data was disclosed in the patent, structural features important for functional activity appeared to center around 7-position derivatives of 1,1-diphenyl-hexahydro-oxazolo[3,4-*a*]pyrazin-3-ones. Therefore, our laboratory synthesized and evaluated a diverse series of 7-substituted analogs (Tables 1 and 2). During the course of preparing this manuscript, two small molecules described in the Takeda patent were also synthesized and tested by Okamura et al.[14] These compounds (SHA-66, SHA-68) were shown to be potent and selective antagonists of both the Asn^{107} and Ile^{107} variants of the NPS receptor (Figure 1).

Target compounds (**1b**–**1s**), designed to identify structural features important for NPS antagonist activity, were prepared using the methods described in Schemes 1 and 2. The key intermediate 1,1-diphenyl-hexahydro-oxazolo[3,4-*a*]pyrazin-3-one (**1a**) was synthesized following a concise route as depicted in Scheme 1. Treatment of commercially available dibenzyl ester **2** with excess phenyllithium at -78 °C afforded (1,4-dib enzyl-piperazin-2-yl)-diphenyl-methanol in moderate yield. Subsequent debenzylation using palladium on barium sulfate under a hydrogen atmosphere with two equivalents of hydrochloric acid at 60 °C afforded **3** in nearly quantitative yield. Reprotection of the resulting amino groups as carbamates seemed ideal, since the desired cyclic carbamate **5** could be readily prepared upon subsequent heating of **4** with base. Based on this hypothesis, carboxybenzyl (Cbz), and 2-(trimethylsilyl)ethoxycarbonyl (Teoc) groups were investigated. Compounds **4a** and **4b** were obtained as planned and cyclization with sodium hydride in dimethyl formamide proceeded smoothly to provide mono-protected derivatives **5a** and **5b**. Unfortunately, removal of either protecting group proved problematic. Under normal deprotection conditions both cyclic carbamates **5a** and **5b** either collapsed to give **3** or decomposed. In an effort to circumvent these issues an alternate protecting group was explored. Knölker and colleagues reported that 1,2-aminoalcohols, when treated with di-*tert*-butyl dicarbonate (Boc_2O) and dimethylaminopyridine (DMAP), readily cyclize to provide oxazolidin-2-ones.[15] Using this rationale, diamine **3** was treated with Boc_2O, triethylamine, and DMAP in tetrahydrofuran to afford compound **5c** in excellent yield. Key intermediate **1a** was then obtained in quantitative yield by treatment of **5c** with trifluoroacetic acid (TFA) in dichloromethane. This approach now allows for the preparation of key intermediate **1a** from diamine **3** in two high yielding steps.

The four-step procedure described in Scheme 1 provides key intermediate **1a** in good yield. Although the yield of step a in Scheme 1 occurs in moderate yield (≈ 50%), steps b,e and f are accomplished in almost quantitative yield. Compared to the procedure adopted by both Takeda Pharmaceuticals[13] and Okamura et al.,[14] orthogonal protection of the two amino groups was not needed in our approach. In addition, the DMAP mediated cyclization of **3** to **5c** makes this route an attractive alternative.

Target ureas **1b–l** and thioureas **1r** and 1**s** were prepared by reacting **1a** with various commercially available isocyanates or isothiocyanates (Scheme 2). For **1l**, where the

isocyanate was not available, reaction of **1a** with triphosgene and *N*-(2-aminoethyl)piperidine proceeded well to give **1l** in moderate yield. Alternatively, **1a** was coupled to a number of acids with benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) in tetrahydrofuran to provide amides **1m–o**. The *N*-alkyl amines **1p** and **1q** were prepared by reductive amination of **1a** with 4-(4-fluorophenyl)-butanal, which was obtained through oxidation of 4-(4-fluorophenyl)butanol[16] with pyridinium chlorochromate in dichloromethane, or formaldehyde using sodium triacetoxyborohydride in dichloroethane.[17] Target compounds were fully characterized by NMR and mass spectroscopy.

Considering the potential pharmacological significance of small molecule NPS receptor antagonists, we have defined key structural features of 7-substituted 1,1-diphenyl-hexahydro-oxazolo[3,4-*a*]pyrazin-3-ones required for antagonist activity. In keeping with earlier reports,[12,14] NPS was more potent at the Ile^{107} variant (EC_{50} of 2.5 nM) than at the Asn^{107} variant (EC_{50} 16.7 nM) using a calcium flux functional assay.[18] The number of spare receptors in an expression system can affect potency, however, taken together, these data support there being a functional difference between the two variants. Compound **1c** and a closely related 1,2,3,6-tetrahydro-pyridine analog of **1l** were resolved into their individual enantiomers in the Takeda Patent although no indication of biological activity was provided (Table 1).[13] Okamura and colleagues recently reported K_b's of 27.9 nM (Ile^{107}) and 16.9 nM (Asn^{107}) for racemic **1c** and 32.6 nM (Ile^{107}) and 21.7 nM (Asn^{107}) for the desfluoro analog **1d**, respectively.[14] In our hands, racemic **1c** (K_e = 13.7 and 51 nM) and **1d** (K_e = 9.9 and 47 nM) were the most potent NPS antagonists among the compounds described herein at both the Ile^{107} and Asn^{107} variants, respectively. In contrast to Okamura, we found these compounds to be slightly more selective for the Ile^{107} variant. Methylation of urea **1c** (**1b**, K_e = 214 and 588 nM) reduced potency by 16-fold at Ile^{107} and 12-fold at Asn^{107}. Similarly, replacement of the urea nitrogen with carbon (**1m**, K_e = 339 and 460 nM) or oxygen (**5a**, K_e = 354 and 353 nM) significantly reduced potency at both receptor variants. This suggests a hydrogen on the 7-position urea is critical for antagonist activity. In order to determine if a hydrogen bond donor placed in close proximity to the urea hydrogen could serve as a suitable surrogate, phenylalanine derivative **1o** was prepared. A 100-fold reduction in potency was observed for **1o** (K_e = 911 nM and 4640 nM). Because the urea functionality appeared critical for NPS antagonist activity, we began to evaluate alternate substitution of the 7-position urea. Addition of an alpha methyl group (**1e**, K_e = 317 and 229 nM) reduced potency by 32- and 48-fold compared to **1d**. Lengthening the alkyl spacer between the terminal phenyl ring to two carbons (**1f**, K_e = 321 nM) reduced potency by 25-fold compared to **1c**. Further elongation of the alkyl spacer to three (**1h**, K_e = 309 nM) and four carbons (**1i**, K_e = 537 nM) significantly reduced potency compared to the parent **1d**. These results suggest that the hypothetical receptor pocket has specific size requirements. In order to assess if hydrophilic or hydrophobic character of the urea substituent was essential for antagonist potency, carboxylic acid **1k** and piperidine **1l** were prepared. Intermediate ester **1j** (K_e = 726 nM) and target acid **1k** (K_e > 4 μM) were both inactive as antagonists. Interestingly, 2-piperidinoethyl analog **1l** had intermediate potency at the Ile^{107} receptor variant (K_e = 109 nM) indicating bulky amine substituents are tolerated, albeit at slightly reduced potency. Converting urea **1l** to thiourea **1s** moderately reduced potency (K_e = 280

nM) whereas replacement of the piperidine with a more water soluble morpholino group (**1r**, K_e = 3760 nM) dramatically reduced potency. Finally, evaluation of 4-(4-fluorophenyl)butyl analog **1p** (K_e = 1100) and methyl analog **1q** (K_e > **4** μM) were inactive as antagonists. In general, it appears that tolerance to 7-substitution is limited.

This study demonstrates that antagonists were generally more potent at the *Ile*^{107} variant (1–5 fold) with limited exceptions (Tables 1 and 2). This suggests that future development of antagonists selective for one receptor variant may be possible. In addition, none of the compounds presented in this study possessed measurable intrinsic activity at 10 μM in the NPS *Ile*^{107} cell line.

In conclusion, we have provided a novel synthetic route to 7-substituted 1,1-diphenyl-hexahydro-oxazolo[3,4-*a*]pyrazin-3-ones and begun to identify the key structural features required for NPS antagonist activity. In particular, we have demonstrated the importance of the urea functionality possessing a free hydrogen and that ethylpiperidine (**1l**) can serve as a substitute for the benzyl group of **1d**. The combination of these results provides a basis for the design and testing of novel scaffolds with enhanced potency and drug-like properties for the NPS receptor.

Acknowledgments

This work was supported by a United States National Institute of Mental Health research grant (MH081247-01). We also wish to thank Mr. Keith Warner, Ms. Tiffany Langston and Ms. Kathleen Kitsopoulos for their valuable technical assistance.

References

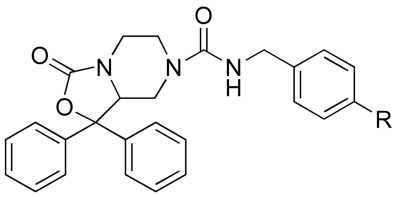
1. Civelli O. GPCR deorphanizations: the novel, the known and the unexpected transmitters. Trends Pharmacol Sci. 2005; 26:15. [PubMed: 15629200]
2. Sato, S., S, Y., Miyajima, N., Yoshimura, K. Novel G-protein coupled receptor protein and DNA thereof. World Patent Application WO 02/31145 A1. 2002.
3. Xu YL, Reinscheid RK, Huitron-Resendiz S, Clark SD, Wang Z, Lin SH, Brucher FA, Zeng J, Ly NK, Henriksen SJ, de Lecea L, Civelli O. Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. Neuron. 2004; 43:487. [PubMed: 15312648]
4. Smith KL, Patterson M, Dhillo WS, Patel SR, Semjonous NM, Gardiner JV, Ghatei MA, Bloom SR. Neuropeptide S stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake. Endocrinology. 2006; 147:3510. [PubMed: 16574794]
5. Beck B, Fernette B, Stricker-Krongrad A. Peptide S is a novel potent inhibitor of voluntary and fast-induced food intake in rats. Biochem Biophys Res Commun. 2005; 332:859. [PubMed: 15919054]
6. Rizzi A, Vergura R, Marzola G, Ruzza C, Guerrini R, Salvadori S, Regoli D, Calo G. Neuropeptide S is a stimulatory anxiolytic agent: a behavioural study in mice. Br J Pharmacol. 2008
7. Leonard SK, Dwyer JM, Sukoff Rizzo SJ, Platt B, Logue SF, Neal SJ, Malberg JE, Beyer CE, Schechter LE, Rosenzweig-Lipson S, Ring RH. Pharmacology of neuropeptide S in mice: therapeutic relevance to anxiety disorders. Psychopharmacology (Berl). 2008; 197:601. [PubMed: 18311561]
8. Ciocciopo, R., Economidou, D., Cannella, N., Braconi, S., Stopponi, S. In Society for Neuroscience. Vol. 2007. San Diego; 2007. Neuropeptide s system activation facilitates conditioned reinstatement of cocaine-seeking in the rat; p. 271.18/Z1
9. Laitinen T, Polvi A, Rydman P, Vendelin J, Pulkkinen V, Salmikangas P, Makela S, Rehn M, Pirskanen A, Rautanen A, Zucchelli M, Gullsten H, Leino M, Alenius H, Petays T, Haahtela T,

Laitinen A, Laprise C, Hudson TJ, Laitinen LA, Kere J. Characterization of a common susceptibility locus for asthma-related traits. Science. 2004; 304:300. [PubMed: 15073379]

10. Bernier V, Stocco R, Bogusky MJ, Joyce JG, Parachoniak C, Grenier K, Arget M, Mathieu MC, O'Neill GP, Slipetz D, Crackower MA, Tan CM, Therien AG. Structure-function relationships in the neuropeptide S receptor: molecular consequences of the asthma-associated mutation N107I. J Biol Chem. 2006; 281:24704. [PubMed: 16790440]
11. Tancredi T, Guerrini R, Marzola E, Trapella C, Calo G, Regoli D, Reinscheid RK, Camarda V, Salvadori S, Temussi PA. Conformation-activity relationship of neuropeptide S and some structural mutants: helicity affects their interaction with the receptor. J Med Chem. 2007; 50:4501. [PubMed: 17696420]
12. Reinscheid RK, Xu YL, Okamura N, Zeng J, Chung S, Pai R, Wang Z, Civelli O. Pharmacological characterization of human and murine neuropeptide s receptor variants. J Pharmacol Exp Ther. 2005; 315:1338. [PubMed: 16144971]
13. Fukatsu K, Nakayama Y, Tarui N, Mori M, Matsumoto H, Kurasawa O, Banno H. Bicyclic Piperazine Compound And Use Thereof. Jun 7.2006 Patent Application No.: PCT/JP2004/012683 (EPO 4772639.3).
14. Okamura N, Habay SA, Zeng J, Chamberlin AR, Reinscheid RK. Synthesis and pharmacological in vitro and in vivo profile of SHA 68 (3-Oxo-1,1-diphenyl-tetrahydro-oxazolo[3,4-a]pyrazine-7-carboxylic acid 4-fluoro-benzylamide), a selective antagonist of the Neuropeptide S receptor. J Pharmacol Exp Ther. 2008; doi: 10.1124/jpet.107.135103
15. Knölker HJ, Braxmeier T. Isocyanates. Part 5. Synthesis of chiral oxazolidin-2-ones and imidazolidin-2-ones via DMAP-catalyzed isocyanation of amines with di-tert-butyl dicarbonate. Tetrahedron Lett. 1998; 39:9407.
16. Wan AS, Ngiam TL, Leung SL, Go ML, Heng PW, Natarajan PN, Shafiee A, Vossoghi M, Savabi F, Francisco CG, et al. Long-acting contraceptive agents: norethisterone esters of arylcarboxylic acids. Steroids. 1983; 41:309. [PubMed: 6658878]
17. Matthews JM, Dyatkin AB, Evangelisto M, Gauthier DA, Hecker LR, Hoekstra WJ, Liu F, Poulter BL, Sorgi KL, Maryanoff BE. Synthesis, resolution, and absolute configuration of novel tricyclic benzodiazepines. Tetrahedron. 2004; 15:1259.
18. Functional Determinations: Identification of functional agonists as well as antagonists at the NPS receptor utilized RD-HGA16 cells (Molecular Devices), a CHO cell line stably over-expressing the promiscuous Gq-protein Gα16. Two individual cell lines were created that stably express each NPS receptor variant (NPS Ile^{107} and Asn^{107}). Cells are loaded with the calcium sensitive dye calcium3 (Molecular Devices) for 1 h and compounds are assayed in separate experiments for intrinsic activity and for the ability to inhibit NPS activity as measured by calcium mobilization in the FlexStation assay. Test compound Ke values were determined by running an 8-point half-log NPS concentration response curve in the presence and absence of a single concentration of test compound. EC_{50} values were calculated for NPS (A) and NPS + test compound (A′), and these used to calculate the test compound K_e. A three-parameter logistic equation was fit to the concentration response data with Prism (v5 for Windows, GraphPad Software; San Diego, CA) to calculate the EC_{50} values. At least two different concentrations of test compound were used for these experiments, and these were chosen such that they at least caused a four-fold rightward shift in the NPS EC_{50}. The K_e was calculated from the formula: $K_e = [L]/(DR-1)$, where [L] equals the concentration of test compound in the assay and DR equals the dose ratio (A′/A). The data represent the mean ± SE from at least three independent experiments.

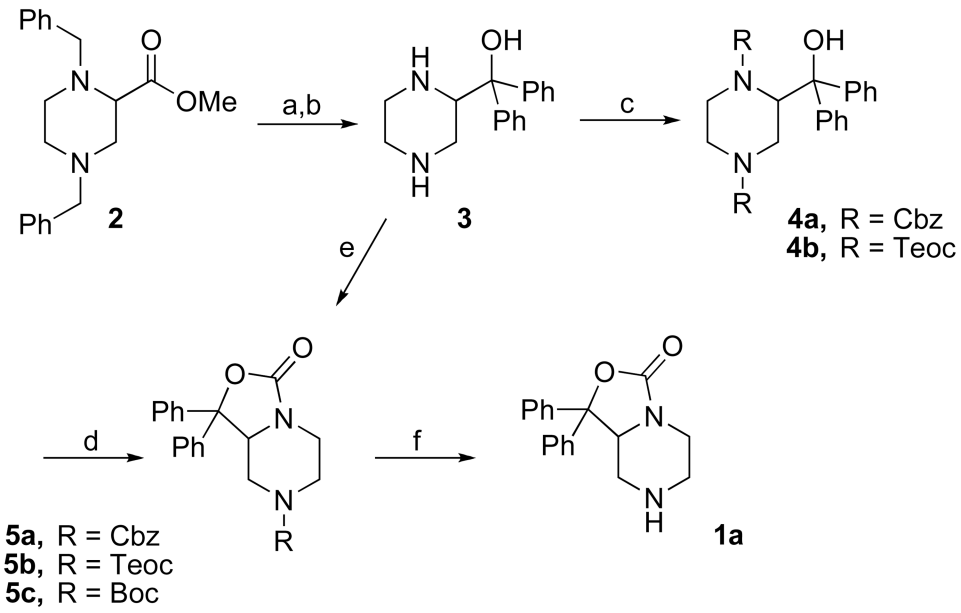
SFRNGVGTGMKKTSFQRAKS

NPS SEQUENCE



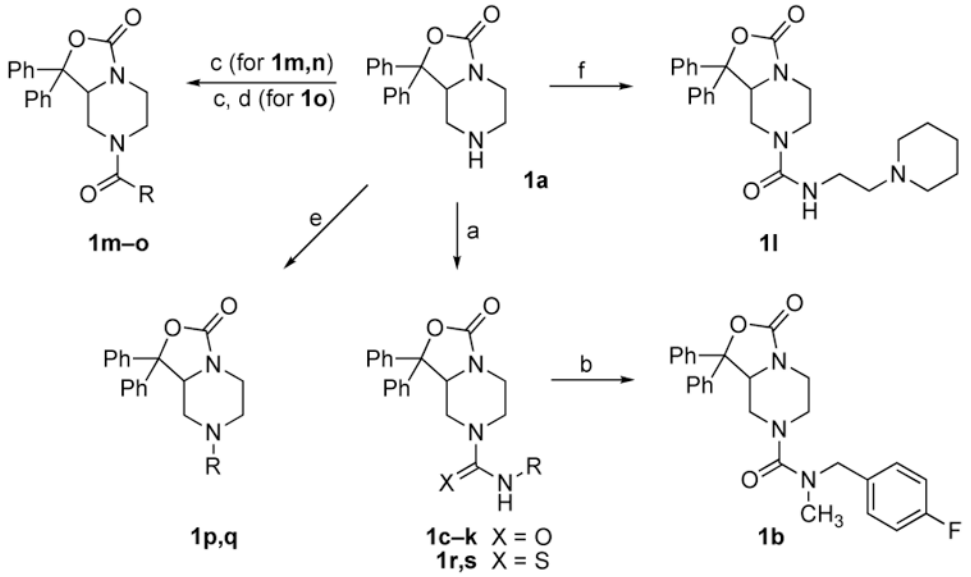
R = H, SHA-66
R = F, SHA-68

Figure 1. Neuropeptide S and previously described NPS antagonists



Scheme 1. Synthesis of key intermediate 1a

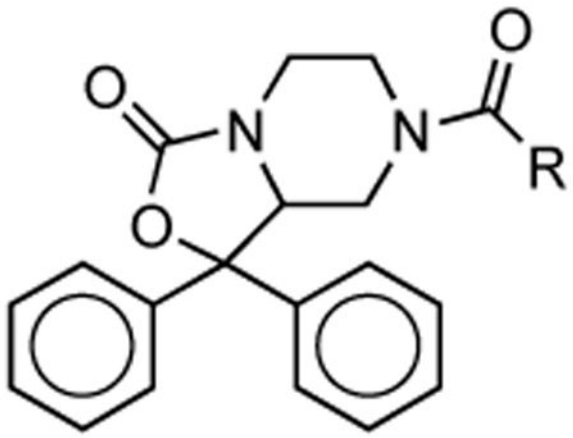
Reagents and Conditions. Synthesis of amine **1a**: (a) PhLi, THF, -78 °C; (b) H_2, 40 psi, Pd/$BaSO_4$, EtOH, 2eq, HCl, 60 °C ; (c) **4a**, CbzCl, K_2CO_3, H_2O/THF, **4b**, Teoc-Succinimide, Et_3N, CH_3CN; (d) NaH, DMF; (e) Boc_2O, DMAP, Et_3N, THF; (f) TFA,CH_2Cl_2.

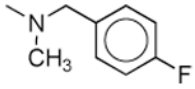
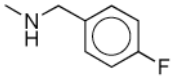
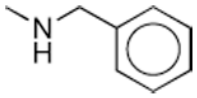
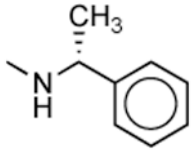
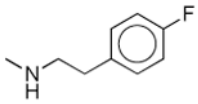
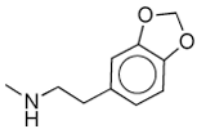
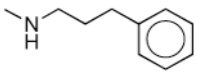


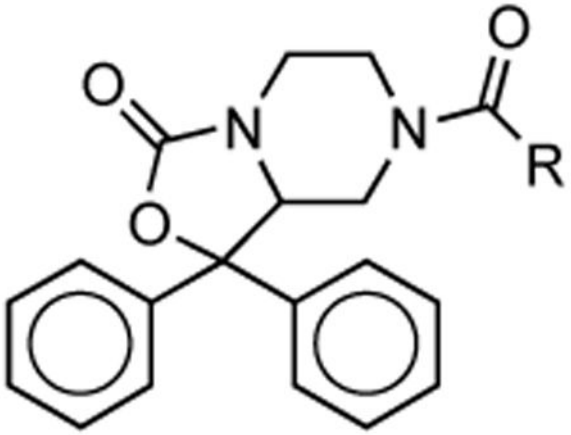
Scheme 2. Synthesis of target compounds 1b–1s

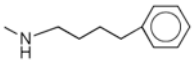
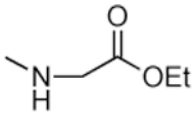
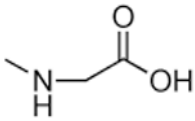
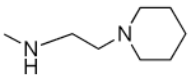
Reagents and Conditions. Synthesis of target compounds **1b–s**: (a) Appropriate isocyanate or isothiocyanate, CH_2Cl_2; (b) MeI, NaHMDS, THF; (c) Appropriate acid, BOP, Et_3H, THF; (d) TFA, CH_2Cl_2; (e) Appropriate aldehyde, $NaB(OAc)_3H$, dichloroethane; (f) *N*-(2-aminoethyl)piperidine, triphosgene, Et_3N, CH_2Cl_2.

Table 1
NPS Antagonist Activity for Urea Derivatives



Compound[a]	R	NPS *Ile*^{107} K_e	NPS *Asn*^{107} K_e	*Asn/Ile*
1b		214 ± 42	588 ± 150	2.8
1c[b]		13.7 ± 2.8	52 ± 2	3.8
1d[c]		9.9 ± 1.6	47 ± 15	4.8
1e		317 ± 5	229 ± 75	0.7
1f		321 ± 120	326 ± 140	1.0
1g		567 ± 190	690 ± 390	1.2
1h		309 ± 52	417 ± 71	1.3



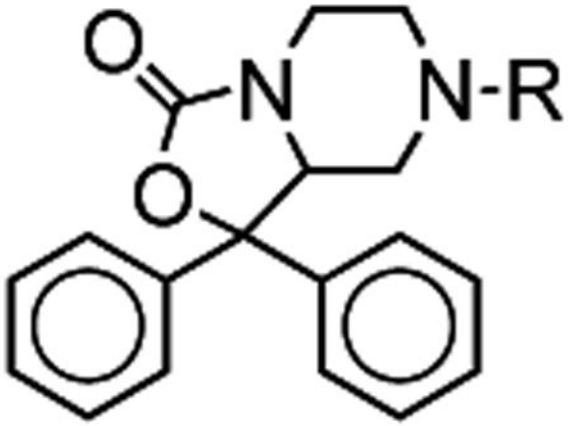
Compound[a]	R	NPS *Ile*^{107} K_e	NPS *Asn*^{107} K_e	*Asn/Ile*
1i		537 ± 190	437 ± 150	0.8
1j		726 ± 107	1260 ± 250	1.7
1k		> 4 mM	> 4 mM	--
1l		109 ± 23	490 ± 173	4.5

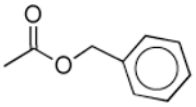
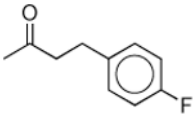
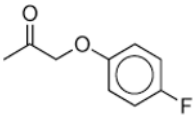
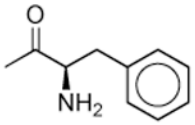
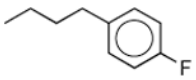
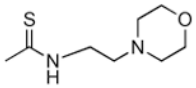
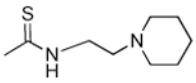
[a]All target compounds were fully characterized using NMR and Mass Spectrometry.

[b]SHA-68.

[c]SHA-66.

Table 2
NPS Antagonist Activity for Alternate 7-Substitution



Compound	R	NPS *Ile*^{107} K_e	NPS *Asn*^{107} K_e	*Asn/Ile*
5a		354 ± 51	353 ± 22	1.0
1m		339 ± 76	460 ± 130	1.4
1n		244 ± 42	267 ± 41	1.1
1o		911 ± 130	4640 ± 1400	5.1
1p		1110 ± 30	1100 ± 300	1.0
1q	$-CH_3$	> 4 mM	> 4 mM	--
1r		3760 ± 470	2560 ± 70	0.7
1s		280 ± 41	354 ± 96	1.3

[a]All target compounds were fully characterized using NMR and Mass Spectrometry.