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The Discovery of Indole Full Agonists of the Neurotensin Receptor 1 (NTSR1)

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Abstract

Neurotensin (NT) is an endogenous tridecapeptide found in the central nervous system (CNS) and in peripheral tissues. Neurotensin exerts a wide range of physiological effects and it has been found to play a critical role in a number of human diseases, such as schizophrenia, Parkinson's disease and drug addiction. The discovery of small-molecule non-peptide neurotensin receptor (NTSR) modulators would represent an important breakthrough as such compounds could be used as pharmacological tools, to further decipher the cellular functions of neurotensin, and potentially as therapeutic agents to treat human disease. Herein, we report the identification of non-peptide low-micromolar neurotensin receptor 1 (NTSR1) full agonists, discovered through structural optimization of the known NTSR1 partial agonist **1**. *In vitro* cellular screenings, based on an intracellular Ca^{2+} mobilization assay, revealed our best hit molecule **8** (SR-12062) to have an EC_{50} of 2 μM at NTSR1 with full agonist behaviour ($E_{\text{max}} = 100\%$), showing a higher efficacy and ~ 90-fold potency improvement compared to parent compound **1** ($\text{EC}_{50} = 178 \mu\text{M}$; $E_{\text{max}} = 17\%$).

Keywords

SR-12062; Neurotensin; Neurotensin receptors; Neurotensin agonists; Indole agonists

Neurotensin (NT), a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) isolated in 1973 from bovine hypothalami,¹ is widely distributed throughout the central and peripheral nervous systems where it acts as a neurotransmitter and neuromodulator, and in the gastrointestinal tract where it behaves as a hormone. Neurotensin has been found to modulate numerous cellular signalling pathways, playing a critical role in a variety of physiological and pathological processes.² Indeed, neurotensin has important roles in the

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://>. These data include assay protocols, representative synthetic procedures and structural characterization of the most relevant compounds described in this article.

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modulation of dopamine neurotransmission, hypothermia, hypotension, opioid-independent analgesia and food intake.^{3,4} In addition, neurotensin has been shown to be implicated in numerous brain disorders such as schizophrenia, Parkinson's disease and drug addiction.^{5,6} Both central and peripheral actions of neurotensin depend on recognition of the peptide by specific receptors. To date, four different neurotensin receptors (NTSRs) have been cloned from human: NTSR1 and 2 are seven-transmembrane spanning domain G-protein-coupled receptors (GPCRs), whereas NTSR3 and NTSR4 are members of the sortilin protein family with a single-transmembrane spanning domain.^{7–10}

Among the neurotensin receptors, NTSR1 is the most widely studied, mediates most of the known neurotensin effects and holds the potential as an interesting therapeutic target.¹¹ Since its discovery roughly 40 years ago, efforts to design and synthesize both peptidomimetic and small molecule modulators of NTSR1 have led to a better understanding of the receptors function *in vivo*.^{13–19} Most of this work has focused on the identification of abbreviated peptide fragments and improving their oral and CNS exposure.^{18–21} Despite the challenge of developing peptide therapeutics, some of these efforts have advanced as far as early clinical trials. In contrast, the generation of small molecule agonists of NTSR1 remains a challenge, however, reports characterizing novel agonist scaffolds are beginning to appear in the primary literature. In addition, the crystal structure of *Rattus Norvegicus* NTSR1, bound to a peptide agonist, has been recently reported.¹² This finding, combined with suitable molecular modeling techniques, may assist medicinal chemistry efforts towards the discovery of small molecule NTSR1 ligands, which could be used as valuable tools to help further define the biological roles of neurotensin, and potentially as candidate agents to treat NT-dependent pathologies.

The majority of NTSR1 modulators reported in the literature are represented by peptides, which often show poor *in vivo* pharmacokinetic profiles (e.g., low oral bioavailability and CNS penetration). Nonetheless, a number of orally bioavailable and brain penetrant peptidic neurotensin mimetics have been described in the literature and currently employed as useful pharmacological probes. Conversely, only a small number of non-peptide positive and negative modulators of the NTSR1 have been discovered to date (Figure 1). These include the partial agonist **1** reported by researchers at Wyeth,²² the pyrazole-derived agonist **2**,²³ the β -arrestin biased brain penetrant agonist ML314 (**3**)²⁴ and the recently discovered positive modulator ML301 (**4**).²⁵ Among NTSR1 antagonists, stand out two trisubstituted pyrazole derivatives structurally related to agonists **2** and **4**: SR 48692 (**5**)²⁶ and SR 142948A (**6**),²⁷ which show binding affinities in the low nanomolar range.

However, the need for more potent, selective and brain permeable NTSR1 modulators still remains a major challenge. Herein, we report the discovery of single-digit micromolar NTSR1 full agonists based on an indole scaffold. Molecular design, synthetic strategies, SAR investigation and *in vitro* cellular assays are presented and discussed.

Previously, researchers at Wyeth reported the identification, through a ligand-based virtual screening campaign, of compound **1** as a partial agonist of the NTSR1 (Figure 1).²² Compound **1** represents a promising starting point for hit-to-lead development but has only poor potency ($EC_{50} = 178 \mu M$) and poor efficacy ($E_{max} = 17\%$). In an effort to discover

potent small-molecule NTSR1 full agonists we decided to embark on the chemical synthesis and biological evaluation of analogues of compound **1**, variously decorated at the 1-, 5- and 6-positions of the indole core structure. Successfully, many of the newly synthesized compounds showed NTSR1 full agonist activity with EC₅₀ values in the low micromolar range, validating this chemical series as a robust chemotype for effective NTSR1 modulation. We found it crucial to begin our investigation exploring the stereochemistry of compound **1**. Encouragingly, inversion of configuration at the α -carbon of the leucine residue, from D-leucine (cmpd **1**) to standard L-leucine (cmpd **7**), significantly improved both potency and efficacy. Furthermore, compound **8** (SR-12062), our best hit, was found to have an EC₅₀ of 2 μ M and a full efficacy profile ($E_{\max}$ = 100%), showing ~ 90-fold potency improvement to parent compound **1** (Figure 2).

Compound **8** was efficiently prepared in 5 steps and 42% overall yield from commercially available 5-bromoindole (**9**) (Scheme 1). The synthesis commenced with a palladium-catalyzed Suzuki *C-C* cross coupling reaction between **9** and quinolin-3-ylboronic acid. This allowed the preparation of the arylindole framework in good yield (68%). *N*-Alkylation of the indole nitrogen was accomplished with NaH and ethyl 2-bromoacetate in DMF. The resulting ethyl ester was hydrolyzed with LiOH to furnish compound **11** in high yield (71% over 2 steps). HATU-mediated amide coupling of **11** with L-leucine methyl ester and subsequent hydrolysis of the ester group afforded compound **8** in excellent yield (87% over 2 steps). Analogous compounds of hit **8** were synthesized according to the synthetic route outlined in Scheme 1.

NTSR1 preferentially couples to the G_q class of G proteins; hence, all compounds of the newly synthesized chemical series were screened using a cell-based functional assay that monitors ligand-mediated changes in intracellular Ca²⁺ levels.²⁸ NT(8–13) (Arg-Arg-Pro-Tyr-Ile-Leu), the active fragment of neurotensin,²⁹ was employed as the positive control. After exploring the stereochemistry at the leucine residue of compound **1**, we turned our attention to the sulfonamido substituent at the 5-position of the indole scaffold. A variety of aryl and arylamino groups were investigated as alternatives to the quinoline-8-sulfonamido moiety, and remarkably all the explored substituents were highly tolerated, giving low-micromolar agonists (Table 1). Interestingly, analogues bearing small groups at the 5-position (cmpds **12**, **13** and **14**) maintained high activity, with compound **12** (R = H) showing also high efficacy ($E_{\max}$ = 112%). These data suggest that bulky aromatic groups in position 5 of the indole core may not be necessary to gain potency and efficacy.

In order to develop robust structure-activity relationships (SARs), we decided to replace the leucine residue in compound **8** with alternative amino acid residues. Clearly, such a drastic change was not tolerated, with all the analogues significantly losing activity or efficacy (Table 2). Notably, this is in line with a similar investigation, previously reported for compound **2**, where the isobutyl group appeared to be preferred.²³ Compound **31**, bearing a phenylalanine residue, retained activity (EC₅₀ = 4 μ M) but not efficacy ($E_{\max}$ = 15%). Only compound **27**,³⁰ in which the carboxylic acid group of leucine was replaced with a bioisosteric tetrazole moiety, had comparable activity and efficacy (EC₅₀ = 5 μ M; $E_{\max}$ = 92%) to hit analogue **8**. In addition, the loss of activity observed for compounds **25** and **26**, in which the carboxylic acid group of leucine was replaced with a methyl ester or alcohol

moiety respectively, showed the importance, in this chemotype, of the acidic functionality for NTSR1 activity. Moreover, compound **32**, enantiomer of **8**, displaying no activity against NTSR1 confirmed that the stereochemistry at the α -carbon of the leucine residue is critical for activity.

To further demonstrate the importance of the leucine residue for NTSR1 activity in this chemical series, an identical SAR investigation, previously generated for compound **8**, was developed for compound **17** ($EC_{50} = 3 \mu M$; $E_{max} = 100\%$). Consistent with data reported in Table 2, replacement of the leucine side chain in compound **17** with alternative amino acid residues was found to be detrimental for activity (Table 3). Again, masking the carboxylic acid of leucine as methyl ester (cmpd **34**) abolished activity. Collectively, these data suggest that the bulky aliphatic isobutyl group and the carboxylic acid functionalities of leucine are essential structural features for NTSR1 agonist activity. Notably, this is not surprising as both neurotensin and several small-molecule NTSR1 agonists reported in the literature (e.g., cmpds **2** and **4**) contain a leucine residue in their chemical structures.

Finally, as a further step of our large program of structural manipulations, we decided to explore the significance, in controlling potency, of substituents at the 6-position of the indole heterocycle (Table 4). Three analogues (cmpds **39**, **40** and **41**) were synthesized and screened against NTSR1. All three compounds showed similar trends in potency and efficacy observed with compounds bearing identical substituents in position 5 of the indole nucleus (cmpds **8**, **17** and **21**). According to these biochemical data we can speculate that aromatic rings placed in either position 5 or 6 of the indole scaffold may partially protrude outside the NTSR1 binding domain, establishing weak intermolecular interactions with the binding site amino acid residues and therefore making only little contribution to activity and efficacy.

In order to confirm our small molecules were indeed acting as agonists at the neurotensin-1 receptor, we screened selected key compounds in the presence and absence of an antagonist. In the presence of antagonist **5** the NTSR1 stimulatory activity of NT(8–13), **7**, **8**, **12**, **17**, **21**, **27**, **39** and **40** was abolished, proving these compounds as robust NTSR1 agonists (Supplementary Material, Figure S1).

In summary, structural optimization of the known indole-based NTSR1 partial agonist **1**, identified at Wyeth, led us to the discovery of NTSR1 agonists with improved potency and efficacy. Indeed, several compounds of this chemical series were found to have single-digit micromolar potency against NTSR1 and full agonist behaviour, showing ~90-fold potency improvement compared to Wyeth compound **1** ($EC_{50} = 178 \mu M$; $E_{max} = 17\%$). Further biological evaluation and potency optimization of our best hit compound **8** are currently underway in our laboratories. As neurotensin is believed to elicit most of its biological functions in the brain, the identification of potent small-molecule agonists with high blood-brain barrier (BBB) permeability would represent an invaluable resource to further investigate the neurotensin system and provide a path forward to developing novel therapeutics for schizophrenia, pain, and drug-addiction disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Molecular structures and activities of several NTSR1 agonists (**1–4**) and antagonists (**5** and **6**) reported in the literature.

Figure 2. Molecular structures of compounds **1**, **7** and **8** (SR-12062). The structure-activity relationship (SAR) investigation is highlighted in compound **1**.

Scheme 1.

Synthesis of compound 8. Reagents and conditions: (a) Quinolin-3-ylboronic acid, $\text{Pd}(\text{PPh}_3)_4$, K_2CO_3 , dioxane, H_2O , 80 °C (MW), 30 min, 68%. (b) i) NaH , DMF, 0 °C, 30 min; ii) Ethyl 2-bromoacetate, rt, 16 h, 72%. (c) $\text{LiOH} \cdot \text{H}_2\text{O}$, THF, H_2O , rt, 4 h, 98%. (d) L-Leucine methyl ester hydrochloride, HATU, DIPEA, DMF, rt, 16 h, 92%. (e) $\text{LiOH} \cdot \text{H}_2\text{O}$, THF, H_2O , rt, 4 h, 95%.

Table 1SAR of 5-substituted indoles **1**, **7–24** against NTSR1

Cmpd	R	EC ₅₀ (μM) ^a	E _{max} (%) ^a
NT(8–13)	-	4.16 × 10 ⁻⁵	100
1	-	> 100	-
7		8.99 ± 3.14	64.80 ± 19.13
8		2.05 ± 0.96	99.44 ± 3.03
12	H	6.65 ± 5.35	112.55 ± 20.71
13	Br	9.99 ± 2.40	87.29 ± 0.78
14	NH ₂	25.82 ± 4.06	57.58 ± 9.29
15		9.44 ± 2.79	81.81 ± 15.77
16		15.38 ± 4.31	73.78 ± 8.76
17		3.26 ± 0.80	100.84 ± 14.06

Cmpd	R	EC ₅₀ (μM) ^a	E _{max} (%) ^a
18		9.39 ± 3.87	78.02 ± 14.67
19		55.07 ± 4.50	38.18 ± 10.58
20		4.96 ± 1.39	74.33 ± 11.51
21		3.24 ± 0.46	90.94 ± 15.74
22		7.72 ± 1.97	78.07 ± 12.86
23		24.20 ± 17.73	55.91 ± 4.64
24		2.12 ± 0.74	79.40 ± 10.63

^a NTSR1 potency was measured relative to the EC₁₀₀ (100 nM) of the NT(8–13) peptide control average ± SEM (*n* = 4 unless otherwise indicated). E_{max} was calculated as the % of the response obtained with the NT(8–13) peptide.

Table 2SAR of 5-(quinoline-3-yl)indoles **25–33** against NTSR1

Cmpd	R	EC ₅₀ (μM) ^a	E _{max} (%) ^a
NT(8–13)	-	4.16 × 10 ⁻⁵	100
25		> 100	-
26		> 100	-
27		5.17 ± 1.09	92.35 ± 4.15
28		> 100	-
29		> 100	-
30		> 100	-

Cmpd	R	EC ₅₀ (μM) ^a	E _{max} (%) ^a
31		4.33 ± 3.23	14.94 ± 20.97
32		> 100	-
33		> 100	-

^aNTSR1 potency was measured relative to the EC₁₀₀ (100 nM) of the NT(8–13) peptide control average ± SEM (*n* = 4 unless otherwise indicated). E_{max} was calculated as the % of the response obtained with the NT(8–13) peptide.

Table 3SAR of 5-(naphthalen-1-yl)indoles **34–38** against NTSR1

Cmpd	R	EC ₅₀ (μM) ^a	E _{max} (%) ^a
NT(8–13)	-	4.16 × 10 ⁻⁵	100
34		> 100	-
35		> 100	-
36		> 100	-
37		> 100	-
38		> 100	-

^a NTSR1 potency was measured relative to the EC₁₀₀ (100 nM) of the NT(8–13) peptide control average ± SEM (*n* = 4 unless otherwise indicated). E_{max} was calculated as the % of the response obtained with the NT(8–13) peptide.

Table 4SAR of 6-substituted indoles **39–41** against NTSR1

Cmpd	R	EC ₅₀ (μM) ^a	E _{max} (%) ^a
NT(8–13)	-	4.16 × 10 ⁻⁵	100
39		3.28 ± 1.36	75.87 ± 7.11
40		2.85 ± 1.28	93.40 ± 20.24
41		7.46 ± 3.61	96.95 ± 6.74

^aNTSR1 potency was measured relative to the EC₁₀₀ (100 nM) of the NT(8–13) peptide control average ± SEM (*n* = 4 unless otherwise indicated). E_{max} was calculated as the % of the response obtained with the NT(8–13) peptide.