ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Discovery of ML314, a Brain Penetrant Nonpeptidic β-Arrestin Biased Agonist of the Neurotensin NTR1 Receptor

Satyamaheshwar Peddibhotla,[†] Michael P. Hedrick,[‡] Paul Hershberger,[†] Patrick R. Maloney,[†] Yujie Li,[‡] Monika Milewski,[‡] Palak Gosalia,[‡] Wilson Gray,[‡] Alka Mehta,[†] Eliot Sugarman,[†] Becky Hood,[†] Eigo Suyama,[†] Kevin Nguyen,[†] Susanne Heynen-Genel,[‡] Stefan Vasile,[†] Sumeet Salaniwal,[‡] Derek Stonich,[‡] Ying Su,[‡] Arianna Mangravita-Novo,[†] Michael Vicchiarelli,[†] Gregory P. Roth,[†] Layton H. Smith,[†] Thomas D. Y. Chung,[‡] Glen R. Hanson,[§] James B. Thomas,[⊥] Marc G. Caron,[∥] Lawrence S. Barak,*[,∥] and Anthony B. Pinkerton*[,‡]

[†]Conrad Prebys Center for Chemical Genomics at Sanford-Burnham Medical Research Institute, Orlando, Florida 32827, United States

[‡]Conrad Prebys Center for Chemical Genomics at Sanford-Burnham Medical Research Institute, La Jolla, California 92037, United States

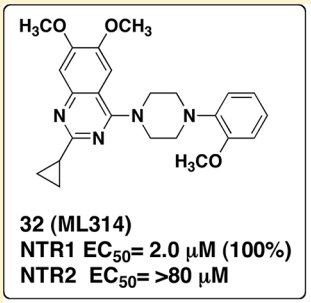
[§]Department of Pharmacology and Toxicology, University of Utah, 260 S. Campus Drive, Salt Lake City, Utah 84112, United States

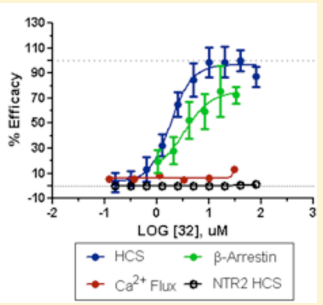
[∥]Duke University Medical Center, Durham, North Carolina 27710, United States

[⊥]RTI International, 3040 E Cornwallis Road, Durham, North Carolina 27709, United States

Supporting Information

ABSTRACT: The neurotensin 1 receptor (NTR1) is an important therapeutic target for a range of disease states including addiction. A high-throughput screening campaign, followed by medicinal chemistry optimization, led to the discovery of a nonpeptidic β-arrestin biased agonist for NTR1. The lead compound, 2-cyclopropyl-6,7-dimethoxy-4-(4-(2-methoxyphenyl)-piperazin-1-yl)quinazoline, **32** (ML314), exhibits full agonist behavior against NTR1 (EC_{50} = 2.0 μM) in the primary assay and selectivity against NTR2. The effect of **32** is blocked by the NTR1 antagonist SR142948A in a dose-dependent manner. Unlike peptide-based NTR1 agonists, compound **32** has no significant response in a Ca^{2+} mobilization assay and is thus a biased agonist that activates the β-arrestin pathway rather than the traditional G_q coupled pathway. This bias has distinct biochemical and functional consequences that may lead to physiological advantages. Compound **32** displays good brain penetration in rodents, and studies examining its in vivo properties are underway.





KEYWORDS: *NTR1, neurotensin, GPCR, SR142948A, quinazoline, agonist, β-arrestin bias, addiction*

Methamphetamine addiction is a major public health concern in the United States.[1] Due to high relapse rates and the difficulty in breaking addiction, there is an urgent need for new medications. Neurotensin (NT) receptors are expressed on dopaminergic neurological pathways associated with reward, and the neurotensin receptor 1 (NTR1) has been implicated as a therapeutic target for the treatment of methamphetamine abuse. In particular, peptide-based NTR1 agonists produce behaviors that are exactly opposite to the psychostimulant effects observed with methamphetamine, such as hyperactivity, neurotoxicity, psychotic episodes, and cognitive deficits. These studies found that in a methamphetamine self-administration rat model (a) the substitution of the peptide NT agonist ($Lys(CH_2NH)$lys-Pro,Trp-*tert*-Leu-Leu-OEt) for methamphetamine did not significantly affect motor activity but dramatically reduced lever pressing associated with the drug, (b) the peptide agonist was not self-administered, and (c) the effects were associated with nucleus accumbens dopamine D1 receptors.[2]

NTR1 is a G protein coupled receptor (GPCR). Two distinct, interdependent paradigms are associated with GPCR signaling. In addition to the well-defined signaling cascades involving heterotrimeric G proteins, recent advances in receptor pharmacology have identified the importance of β-arrestins in regulating alternative biochemical cascades that produce their own unique biological effects.[3] For example, in a mouse model, Allen et al. developed a series of β-arrestin-2 biased agonists for the D(2)R with antipsychotic properties and, most importantly, a

Received: May 13, 2013
Accepted: July 22, 2013
Published: July 22, 2013

reduced propensity to induce catalepsy-like standard neuroleptic antagonists.[4,5] These studies illustrate how ligand directed signaling bias, in this case favoring β-arrestin, can ameliorate undesirable biological outcomes. Downstream modulators of β-arrestin/GPCR signaling are less well-characterized than their G protein counterparts and, due to their potential as targets for producing new medical therapies, are the subjects of increasing numbers of investigations. Well-recognized β-arrestin partners include the proteins Src, ERK, and Jnk. Their agonist-induced interactions with β-arrestin are associated with clathrin-compartmentalized signaling and the accumulation of ligand activated β-arrestin/GPCR complexes in clathrin-coated pits.[6] The determination as to whether a GPCR ligand is biased toward or against β-arrestin may consequently be evaluated by following these biochemical processes.[7−10]

Although there have been reports[11,12] of NTR1 antagonists, there have been few reports of small molecule nonpeptide agonists for NTR1, even though the tridecapeptide neurotensin was characterized in the 1970s.[13−15] Recently, two known trisubstituted pyrazole antagonists, SR48692 (**1a**) and SR142948A (**1b**), were modified to identify partial agonists[11,12,16,17] such as **2** (Figure 1). Additionally, two simple

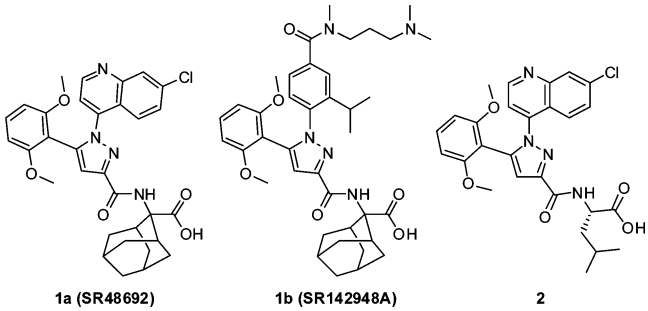


Figure 1. Examples of known pyrazole based NTR1 antagonists **1a** and **1b** and agonist **2**.

leucine derivatives unrelated to **1a**, **1b**, and **2** have shown partial agonist activity for NTR1.[18] However, there have been no reports of in vivo activity associated with nonpeptide agonists. Newly published X-ray crystallography data showing the neurotensin fragment NT (8−13) in a NTR1 binding site may assist the design of nonpeptide agonists and antagonists in the future.[19]

To identify nonpeptide small molecule NTR1 agonists, we carried out high content screening (HCS) of receptor/β-arrestin-GFP complexes based on a β-arrestin conjugated green fluorescent protein (GFP) reporter expressed in a U2OS cell line (see Supporting Information for details). This assay measured the quantification of β-arrestin/GFP puncta. A library of 331,861 compounds from the MLSMR (Molecular Libraries Small Molecule Repository)[20] was tested in this high-content primary screen. This campaign [PubChem AID 493055][21] identified 559 compounds with ≥40% activity of NT (8−13) peptide, which is the prototypical reference agonist, at 20 μM. Liquid samples (499 compounds) were resupplied and tested at 20 μM, and 184 compounds showed >40% activity. The confirmed compounds were tested in a dose response format in the HCS primary assay. Dose response testing of reconstituted dry powders of 122 compounds that had an EC_{50} < 20 μM was performed in a three-assay panel: the NTR1 HCS primary assay to assess potency, and the NTR2 and GPR35 HCS assays to ascertain selectivity against a related and unrelated GPCR. Compound **5**, a quinazoline derivative, was identified as a singleton hit with an EC_{50} of <10 μM against NTR1, with an E_{max} of 85% of the control neurotensin peptide. Subsequent medicinal chemistry optimization relative to the primary β-arrestin assay resulted in the discovery of **32** (ML314), the first nonpeptidic small molecule full agonist against NTR1 (EC_{50} = 2.0 μM) with >20× selectivity over NTR2 and GPR35. In this report we discuss the design and synthesis of SAR analogues that led to the discovery of **32**, as well as its in vitro and in vivo pharmacological profiling.

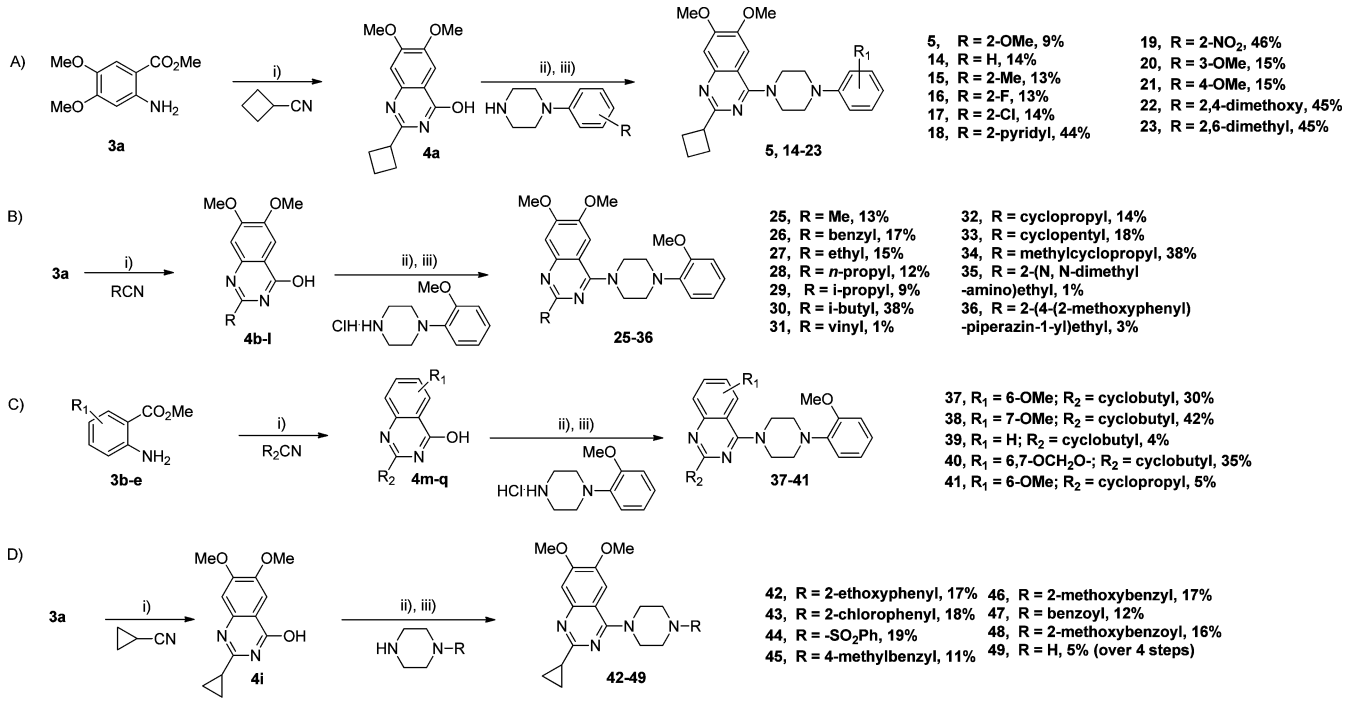
Analogues were synthesized from substituted methyl anthranilates in three steps using previously reported methods (Scheme 1).[22,23] Acid-catalyzed condensation of methyl 2-amino-4,5-dimethoxybenzoate (**3a**) with cyclobutane carbonitrile afforded the 4-hydroxyquinazoline intermediate **4a**. Chlorination of **4a** followed by amination under microwave heating with various substituted aryl piperazines provided the primary hit compound **5** and quinazoline analogues **14**−**23** (Scheme 1A).

For a second point of diversity **3a** was condensed with a range of substituted nitriles to afford 4-hydroxyquinazolines (**4b**−**4l**), which were converted to the corresponding quinazolines (**25**−**36**) by sequential chlorination and amination with 1-(2-methoxyphenyl)-piperazine hydrochloride (Scheme 1B). 2-(2-Chloroethyl)-6,7-dimethoxyquinazolin-4-ol (**4h**) under the above conditions resulted in the 2-vinyl analogue (**31**) via elimination and compound **36** via double addition (Scheme 1B). Using the above three-step route, differentially substituted methyl anthranilates (**3b**−**3e**) were converted to compounds **37**−**41** via 4-hydroxyquinazolines (**4m**−**q**) (Scheme 1C). Similarly, 2-cyclopropyl-6,7-dimethoxyquinazolin-4-ol (**4i**) was subjected to chlorination and then treated with diverse 1-substituted piperazines to access quinazolines **42**−**49** (Scheme 1D). All final compounds were obtained in moderate yields and high purity needed to support SAR studies.

Compound **5**, the singleton screening hit (EC_{50} = 5.9 μM and E_{max} ∼85% of the NT(8−13) peptide response), was resynthesized (Scheme 1A) and was confirmed equipotent to the purchased material (Table 1; entry **5**). A small set of commercially available 2-phenylquinazolines (Table 1, entries **6**−**13**) tested inactive except for the 2-methoxyphenylpiperazine derived analogue **6**, which displayed partial agonist activity (EC_{50} = 20 μM). In general we saw full agonist activity for all compounds regardless of potency with some exceptions, although in no case was the E_{max} below ∼70%. To expand the SAR around the singleton hit **5**, a focused set of analogues was prepared to investigate the effect of various substituents and their positions on the aryl piperazine moiety. This revealed that, irrespective of the electronic nature, all substituents that were smaller or comparable in size to the 2-OMe group retained activity within 2−4 fold of compound **5** (Table 2; entries **14**−**17**). Much larger 2-nitro and basic 2-pyridyl groups were far less potent than **5** (>6 fold; Table 2; entries **18**, **19**). Altering the position of the methoxy group, and disubstitution, also led to a significant loss of potency (>4−13 fold; Table 2; entries **20**−**23**). We next investigated the role of substituents at the 2-position of the quinazoline ring occupied by the cyclobutyl ring in **5** (Table 3, entry **5**). 2-H and 2-Me analogs incurred complete loss of activity (Table 3, entry **24**−**25**); however, the 2-benzyl analog (Table 3, entry **26**) was 2.3× less active than compound **5**. Combined with the fact that 2-phenyl quinazolines (Table 1, entries **6**−**13**) were mostly inactive, we speculated that a methylene linker between the 2-substituent and the quinazoline

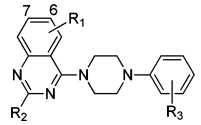
dx.doi.org/10.1021/ml400176n | ACS Med. Chem. Lett. 2013, 4, 846−851

Scheme 1. Synthesis of NTR1 Agonists 32 and Analogues[a]



[a]Conditions:[22] (i) alkyl carbonitrile, 4 M HCl (1,4-dioxane), 100 °C, 15 h; (ii) $POCl_3$, reflux, 15 h; (iii) piperazine derivative (1.5 equiv), K_2CO_3 (3 equiv), 1,4-dioxane, microwave, 80 °C, 1.5 h, 1−46% overall yield over three steps.

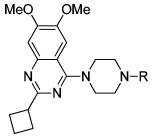
Table 1. SAR of Quinazoline Agonists of NTR1, Compounds 6−13



compound	R_1	R_2	R_3	EC_{50}[a] (μM)	E_{max}[a] (%)
NT(8−13)				0.001 (>4)	100.0
5	6,7-di-OMe	cyclo-butyl	2-OMe	5.9 ± 0.5 (10)	85.3
6	6,7-di-OMe	phenyl	2-OMe	20 ± 11 (5)	78.0
7	6,7-di-OMe	phenyl	H	>80	
8	6,7-di-OMe	phenyl	2-F	>80	
9	6,7-di-OMe	phenyl	4-F	>80	
10	6,7-di-OMe	phenyl	2-Cl	>80	
11	6-OEt, 7-OMe	phenyl	2-OMe	>80	
12	6-OEt, 7-OMe	phenyl	H	>80	
13	6-OEt, 7-OMe	phenyl	2-F	>80	

[a]HCS NTR1 potency measured relative to the EC_{100} (100 nM) of the NT(8−13) peptide control average ± SEM (n = 4 unless otherwise noted); E_{max} was calculated as the % of the response obtained with NT(8−13) peptide. None of the compounds from this series showed activity in the NTR2 (>80 μM) and GPR35 (>40 μM) counterscreens.

Table 2. SAR of Quinazoline-Based Agonists of NTR1, Compounds 14−23



compound	R	EC_{50}[a] (μM)	E_{max}[a] (%)
NT(8−13)		0.001 (>4)	100.0
5	2-methoxyphenyl	5.9 ± 0.5 (10)	85.3
14	phenyl	12 ± 1.4	77.0
15	*o*-tolyl	15 ± 3.2	916
16	2-fluorophenyl	18 ± 1.0 (3)	97.2
17	2-chlorophenyl	12 ± 2.5	70.5
18	pyridin-2-yl	26 ± 2 (6)	91.4
19	2-nitrophenyl	75 ± 1 (2)	100.0
20	3-methoxyphenyl	17 ± 1.0 (2)	74.9
21	4-methoxyphenyl	46 ± 18 (3)	100.0
22	2,4-dimethoxyphenyl	23 ± 0.4 (6)	103.3
23	2,6-dimethylphenyl	61 ± 5.2	100.0

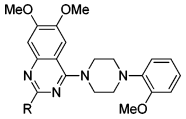
[a]HCS NTR1 potency measured relative to the EC_{100} (100 nM) of the NT(8−13) peptide control average ± SEM (n = 4 unless otherwise noted); E_{max} was calculated as the % of the response obtained with NT(8−13) peptide. None of the compounds from this series showed activity in the NTR2 (>80 μM) and GPR35 (>40 μM) counterscreens.

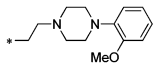
might improve potency and hence generated analogs with alkyl, branched alkyl and vinyl substituents (Table 3, entries **27**−**31**). The 2-isopropyl and 2-vinyl derivatives were found to be equipotent to compound **5** (Table 3, entries **29** and **31**). This result prompted the synthesis of the 2-cyclopropyl analogue, compound **32** (ML314, Table 3), which is a full agonist (EC_{50} = 2.0 μM) with 3-fold improvement in activity over **5**.

This met the probe criteria established for the NIH's MLPCN (Molecular Libraries Probe Production Centers Network) program.[24] The larger 2-cyclopentyl (Table 3, entry **33**) was equipotent, and the extended 2-methylcyclopropyl analogue (entry **34**) was 2.4× less potent than **5**. Furthermore,

dx.doi.org/10.1021/ml400176n | ACS Med. Chem. Lett. 2013, 4, 846−851

Table 3. SAR of Quinazoline-Based Agonists of NTR1, Compounds 24−36



Compound	R	EC_{50} (μM)[a]	E_{max} (%)[a]
NT(8-13)	-	0.001 (>4)	100.0
5	-cyclobutyl	5.9 ± 0.5 (10)	85.3
24	-H	>80	-
25	-Me	>80	-
26	-CH_2Ph	14 ± 3.4	81.9
27	-ethyl	43 ± 4	100.0
28	*n*-propyl	17 ± 2	100.0
29	*i*-propyl	8 ± 1.0	100.0
30	*i*-butyl	15 ± 1.5	96.3
31	-vinyl	7.0 ± 1.0	100.0
32 ML314	-cyclopropyl	2.0 ± 0.1 (8)	104.7
33	-cyclopentyl	5.8 ± 1.5	92.8
34	methylcyclopropyl	14 ± 1.2	96.9
35	-$CH_2CH_2NMe_2$	>80	-
36		26 ± 14 (2)	74.3

[a]HCS NTR1 potency measured relative to the EC_{100} (100 nM) of the NT(8−13) peptide control average ± SEM (n = 4 unless otherwise noted); E_{max} was calculated as the % of the response obtained with NT(8−13) peptide. None of the compounds from this series showed activity in the NTR2 (>80 μM) and GPR35 (>40 μM) counterscreens.

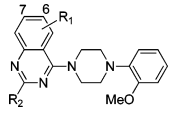
incorporation of basic nitrogen containing substituents (Table 3, entries **35**, **36**) resulted in significantly diminished activity.

We also investigated the importance of 6- and 7-OMe groups. Thus, systematic removal of 7-OMe, 6-OMe, and both 6- and 7-OMe groups (Table 4, entries **37**−**39**), revealed that 6-OMe is key to activity and its removal leads to a ∼4−6 fold loss in activity (Table 4, entries **38** and **39**). This trend was maintained in the corresponding 2-cyclopropyl-6-methoxy analogue of **32** (Table 4, entry **41**). When the 6- and 7-OMe groups were joined as part of a dioxolane ring (Table 4, entry **40**), the potency dropped by over 6-fold compared to **5**.

Having the more potent and full agonist **32** in hand, we investigated the aryl piperazine moiety with the goal of further improving the potency. Replacement of the 2-OMe group with the larger 2-OEt or an electronegative 2-Cl led to >3−10 fold loss in potency compared to **32** (Table 5, entries **42** and **43**). This was a more pronounced effect than that observed for the primary hit compound **5**. Replacement of the *N*-arylpiperazine group in **32** with benzenesulfonyl, substituted benzyl, or benzoyl groups led to significant erosion of activity (>10−40 fold; Table 5, entries **44**−**49**), demonstrating the importance of a 2-methoxyphenyl group in this region of the molecule.

Compound **32** was further profiled in an orthogonal NTR1 activation assay based on DiscoveR$_x$ β-arrestin complementation and a downstream functional assay measuring Ca^{2+} Flux (ChanTest). The agonist activity of **32** in the primary NTR1 HCS assay was confirmed in the DiscoveR$_x$ β-arrestin assay

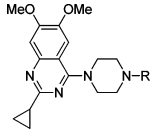
Table 4. SAR of Quinazoline-Based Agonists of NTR1, Compounds 37−41



compound	R_1	R_2	EC_{50}[a] (μM)	E_{max}[a] (%)
NT(8−13)			0.001 (>4)	100.0
5	6,7-di-OMe	cyclobutyl	5.9 ± 0.5 (10)	85.3
37	6-OMe	cyclobutyl	10 ± 1.6	101.9
38	7-OMe	cyclobutyl	30 ± 0 (3)	111.5
39	H	cyclobutyl	23 ± 3.9	100.0
40	6,7-OCH_2O-dioxolane	cyclobutyl	34 ± 17 (3)	87.0
41	6-OMe	cyclopropyl	4.1 ± 0.5	95.7

[a]HCS NTR1 potency measured relative to the EC_{100} (100 nM) of the NT(8−13) peptide control average ± SEM (n = 4 unless otherwise noted); E_{max} was calculated as the % of the response obtained with NT(8−13) peptide. None of the compounds from this series showed activity in the NTR2 (>80 μM) and GPR35 (>40 uM) counterscreens.

Table 5. SAR of Quinazoline-Based Agonists of NTR1, Compounds 42−49



compound	R	EC_{50}[a] (μM)	E_{max}[a] (%)
NT(8−13)		0.001 (>4)	100.0
32	2-methoxyphenyl	2.0 ± 0.1 (8)	104.7
42	2-ethoxyphenyl	6.1 ± 0.4	98.4
43	2-chlorophenyl	20 ± 2.2	100.0
44	benzenesulfonyl	>80	
45	4-methylbenzyl	25 ± 3.2	100.0
46	2-methoxybenzyl	35 ± 4.4	100.0
47	benzoyl	45 (1)	100.0
48	2-methoxybenzoyl	68 ± 5.5 (3)	100.0
49	H	>80	

[a]HCS NTR1 potency measured relative to the EC_{100} (100 nM) of the NT(8−13) peptide control average ± SEM (n = 4 unless otherwise noted); E_{max} was calculated as the % of the response obtained with NT(8−13) peptide. None of the compounds from this series showed activity in the NTR2 (>80 μM) and GPR35 (>40 uM) counterscreens.

(EC_{50} = 3.41 μM; E_{max} = 86.6%). Compound **32** was inactive (EC_{50} = >80 uM, Figure 2A, B) in the NTR1 Ca^{2+} Flux assay. In contrast, the known[16] partial agonist **2** showed activity (EC_{50} = 0.75 μM) and only partial agonism (E_{max} < 80%) in the NTR1 HCS assay. Interestingly, **2** was inactive in the DiscoveR$_x$ β-arrestin assay (EC_{50} > 33 μM) but showed evidence of traditional G_q signaling in the Ca^{2+} flux assay (EC_{50} < 156 nM, E_{max} = 63%). The latter result was consistent with the published data for **2** (EC_{50} = 67 nM, E_{max} = 54%).[16,17] Thus, **32** appears to be a biased agonist operating via the β-arrestin pathway rather than the traditional G_q coupled pathway. Specificity of binding of **32** to NTR1 was evaluated by the ability of the antagonist **1b** to block the response of **32** in the HCS assay (Figure 2C). Previous studies have eported the inhibition of binding of [^{125}I]-NT peptide to the NTR1 receptor by **1b** (IC_{50} = 0.24 nM).[25] Preincubation with **1b** inhibited compound **32** (EC_{100} = 10 μM)

dx.doi.org/10.1021/ml400176n | ACS Med. Chem. Lett. 2013, 4, 846−851

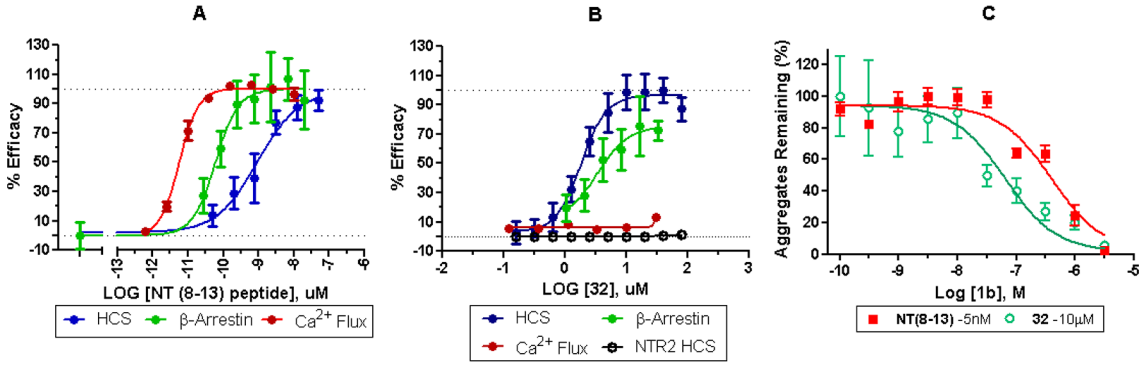


Figure 2. (A) Dose response of the NT(8−13) peptide in the NTR1 HCS, β-arrestin, and Ca^{2+} Flux assays. (B) Compound **32** (ML314) in the NTR1 HCS, β-arrestin, and Ca^{2+} Flux assays, and the NTR2 HCS assay. Percent efficacy in each assay is measured relative to the NT peptide control. (C) Inhibitory dose response of NTR1 antagonist **1b** in the presence of an EC_{100} concentration (10 μM) of compound **32**; data points/curves represent the average of all dose response experiment runs.

mediated NTR1 activation with an IC_{50} of 50.1 nM, demonstrating that **32** is inhibited from forming aggregates by **1b** in a dose-dependent manner. This result supports the mechanistic hypothesis that **32** acts via NTR1 binding.

A PubChem analysis of compound **5** (CID1230852) displayed very low cross reactivity, with NTR1 being the only receptor activity <10 μM in over 600 assays.[26] In addition to NTR2 and GPR35, compound **32** was tested across a range of GPCRs using functional high content or β-arrestin based screens and was found to have no cross reactivity.[27] Compound **32** was also submitted to the Psychoactive Drug Screening Program (PDSP)[28] for testing in a GPCR binding assay panel (~40 receptors) and was found moderately promiscuous at 10 μM, with K_is < 10 μM on seven receptors.[29] A follow-up study at Panlabs/Ricerca in their lead profiling panel confirmed activity in only two of those receptors (MOR, 86% at 10 μM and σ_1 69% at 10 μM), In addition, **32** showed moderate binding across a range of adrenergic receptors (α_{1a}, α_{1B}, α_{1D}, α_{2A} 63−100% at 10 μM) in the Panlabs panel. Other analogues were not examined for their binding cross reactivity.

In vitro pharmacology screening (Table 6) was also conducted for **32**. Consistent with its aqueous solubility, **32** exhibited high permeability in the Parallel Artificial Membrane Permeability Assay (PAMPA) with increasing pH of the donor compartment.[30] When incubated with an artificial membrane that models the blood−brain barrier (BBB), **32** was highly permeable. Compound **32** was highly plasma protein bound and exhibited very high plasma stability but was metabolized rapidly when incubated in vitro with human and mouse liver homogenates. The low metabolic stability may be associated with the presence of several unsubstituted aryl and alkyl positions and Ar-OMe ethers susceptible to oxidation, hydrolysis, conjugation, and other metabolic reactions. Compound **32** had a >15-fold window for toxicity (LC_{50} = 30 μM) toward human hepatocytes.

Table 6. Summary of in Vitro ADME/T Properties of NTR1 Agonist 32

aqueous solubility (μM) in pION buffer; pH 5.0/6.2/7.4		>297/21.4/1.2
aqueous solubility (μM) in 1× PBS, pH 7.4		1.1
PAMPA permeability, P_e (× 10^{-6} cm/s), donor pH: 5.0/6.2/7.4, acceptor pH: 7.4		1163/2145/2093
BBB-PAMPA permeability, P_e (× 10^{-6} cm/s), donor pH: 7.4, acceptor pH: 7.4		399
plasma protein binding (% bound)	human 1 μM/10 μM	99.5/99.2
	mouse 1 μM/10 μM	99.7/98.9
plasma stability (% remaining at 3 h) human/mouse		100/99.6
hepatic microsome stability (% remaining at 1 h) human/mouse		1.4/0.2
toxicity toward Fa2N-4 immortalized human hepatocytes over 24 h, LC_{50} (μM)		29.6

Compound **32** was profiled for its mouse pharmacokinetics and showed hepatic clearance and moderate exposure after intraperitoneal dosing (clearance 81 mL/min/kg, V_{dss} 6.22 L/kg, $t_{1/2}$ 1.93 h after a 2 mg/kg intravenous dose, C_{max} 763 ng/mL, $t_{1/2}$ 2.58 h, AUC 1223 ng·h/mL after a 10 mg/kg intraperitoneal dose) (see Supporting Information for details). However, **32** displayed excellent brain penetration, with brain levels of 924 ng/mL and 1506 ng/mL at 1 h after a 10 mg/kg or 30 mg/kg intraperitoneal dose (brain/plasma 1.3 or 1.6, respectively).

In conclusion, a high-throughput screen of the MLSMR collection using a NTR1 β-arrestin recruitment HCS assay identified a singleton partial agonist, quinazoline **5**, which was optimized via medicinal chemistry to discover a potent full agonist **32**. Compound **32** was >20× selective over NTR2 and was inhibited from forming ligand activated β-arrestin/NTR1 complexes by antagonist **1b** in a dose-dependent manner. Compound **32** had no significant activity in a Ca^{2+} flux assay, in contrast to previously known agonists, and is therefore an apparent biased agonist operating via the β-arrestin pathway rather than the traditional G_q coupled pathway. Compound **32** displayed moderate pharmacokinetics and showed good BBB penetration in mice. Although **32** displayed some promiscuity in binding assays to other GPCRs, it showed no functional activity at other receptors tested. We are currently investigating its apparent signaling bias and associated physiological consequences. Current efforts to optimize **32** further and improve its in vivo profile are underway.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, annotated ^{1}H NMR and HRMS data for the described compounds, as well as graphical depictions of the PK and in vivo data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*L.S.B.: phone, 01-919-684-6245. E-mail: L.Barak@cellbio.duke.edu. A.B.P.: phone, 01-858-795-5320. E-mail: apinkerton@sanfordburnham.org.

Author Contributions

S.P., M.P.H., and P.H. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by NIH grants 1 R03 MH089653-01 to L.S.B., 5P30DA029925 (M.G.C., L.S.B.), R01 DA031833 to G.R.H., and an NIH Molecular Libraries grant (U54 HG005033-03) to the Conrad Prebys Center for Chemical Genomics at the Sanford-Burnham Medical Research Institute, one of the comprehensive centers of the NIH Molecular Libraries Probe Production Centers Network (MLPCN).

■ REFERENCES

(1) Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings; NSDUH Series H-44, HHS Publication No. (SMA) 12−4713. Substance Abuse and Mental Health Services Administration: Rockville, MD, 2012. http://www.samhsa.gov/data/NSDUH/2011SummNatFindDetTables/ Index.aspx (accessed Nov 5, 2012).

(2) Hanson, G. R.; Hoonakker, A. J.; Alburges, M. E.; McFadden, L. M.; Robson, C. M.; Frankel, P. S. Response of limbic neurotensin systems to methamphetamine self-administration. *Neuroscience* **2012**, *203*, 99−107.

(3) DeWire, S. M.; Ahn, S.; Lefkowitz, R. J.; Shenoy, S. K. Beta-arrestins and cell signaling. *Annu. Rev. Physiol.* **2007**, *69*, 483−510.

(4) Allen, J. A.; Yost, J. M.; Setola, V.; Chen, X.; Sassano, M. F.; Chen, M.; Peterson, S.; Yadav, P. N.; Huang, X.; Feng, B.; Jensen, N. H.; Che, X.; Bai, X.; Frye, S. V.; Wetsel, W. C.; Caron, M. G.; Javitch, J. A.; Roth, B. L.; Jin, J. Discovery of *β*-Arrestin-Biased Dopamine D2 Ligands for Probing Signal Transduction Pathways Essential for Antipsychotic Efficacy. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 18488−18493.

(5) Rajagopal, S.; Rajagopal, K.; Lefkowitz, R. J. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat. Rev. Drug Discovery* **2010**, *9*, 373−386.

(6) Lefkowitz, R. J.; Shenoy, S. K. Transduction of Receptor Signals by *β*-Arrestins. *Science* **2005**, *308*, 512−517.

(7) Kenakin, T. The potential for selective pharmacological therapies through biased receptor signalling. *BMC Pharmacol. Toxicol.* **2012**, *13*, 3.

(8) Barak, L. S.; Peterson, S. Modeling of bias for the analysis of receptor signaling in biochemical systems. *Biochemistry* **2012**, *51*, 1114−25.

(9) Barak, L. S.; Ferguson, S. S.; Zhang, J.; Caron, M. G. A beta-arrestin/green fluorescent protein biosensor for detecting G protein-coupled receptor activation. *J. Biol. Chem.* **1997**, *272*, 27497−500.

(10) Whalen, E. J.; Rajagopal, S.; Lefkowitz, R. J. Therapeutic potential of *β*-arrestin and G protein-biased agonists. *Trends Mol. Med.* **2011**, *17*, 126−139.

(11) Gully, D.; Labeeuw, B.; Boigegraiin, R.; Oury-Donat, F.; Bachy, A.; Poncelet, M.; Steinberg, R.; Suaud-Chagny, M. F.; Santucci, V.; Vita, N.; Pecceu, F.; Labbe-Jullio, C.; Kitabgi, P.; Soubrie, P.; Le Fur, G.; Maffrand, J. P. Biochemical and Pharmacological Activities of SR 142948A, a New Potent Neurotensin Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 802−812.

(12) Gully, D.; Canton, M.; Boigegraiin, R.; Jeanjean, F.; Molimard, J.-C.; Poncelet, M.; Gueudet, C.; Heaulme, M.; Leyris, R.; Brouard, A.; Pelaprat, D.; Labbe-Jullio, C.; Mazellai, J.; Soubrie, P.; Rostene, W.; Kitabgi, P.; Le Fur, G. Biochemical and pharmacological profile of a potent and selective nonpeptide antagonist of neurotensin receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 65−69.

(13) Carraway, R.; Leeman, S. E. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J. Biol. Chem.* **1973**, *238*, 6854−6861.

(14) Carraway, R.; Leeman, S. E. Characterization and radioimmunoassayable neurotensin in the rat. *J. Biol. Chem.* **1976**, *251*, 7045−7052.

(15) Griebel, G.; Holsboer, F. Neuropeptide receptor ligands as drugs for psychiatric diseases: the end of the beginning? *Nat. Rev. Drug Discovery* **2012**, *11*, 462−478.

(16) Thomas, J. B.; Navarro, H.; Warner, K. R.; Gilmour, B. The identification of nonpeptide neurotensin receptor partial agonists from the potent antagonist SR48692 using a calcium mobilization assay. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1438−1441.

(17) Thomas, J. B.; Runyon, S. P. Compounds Active at the Neurotensin Receptor. Compounds active at the neurotensin receptor. World Patent WO 2011/156557 A2, December 15, 2011.

(18) Fan, Y.; Lai, M. H.; Sullivan, K.; Popiolek, M.; Andree, T. H.; Dollings, P.; Pausch, M. H. The identification of neurotensin NTS1 receptor partial agonists through a ligand-based virtual screening approach. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5789−5791.

(19) White, J. F.; Noinaj, N.; Shibata, Y.; Love, J.; Kloss, B.; Xu, F.; Gvozdenovic-Jeremic, J.; Shah, P.; Shiloach, J.; Tate, C. G.; Grisshammer, R. Structure of the agonist-bound neurotensin receptor. *Nature* **2012**, *490*, 508−513.

(20) NIH's Molecular Libraries Small Molecule Repository (MLSMR) collection. http://mli.nih.gov/mli/compound-repository/mlsmr-compounds (accessed May 3, 2013).

(21) National Center for Biotechnology Information. PubChem BioAssay Database; 493055, Source = Sanford-Burnham Center for Chemical Genomics, Sanford, Burnham Medical Research Institute, San Diego, CA. http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=493055 (accessed May 3, 2013).

(22) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D. H.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine Kinase Inhibitors. 8. An Unusually Steep Structure−Activity Relationship for Analogues of 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a Potent Inhibitor of the Epidermal Growth Factor Receptor. *J. Med. Chem.* **1996**, *39*, 267−276.

(23) Sirisoma, N.; Pervin, A.; Zhang, H.; Jiang, S.; Willardsen, J. A.; Anderson, M. B.; Mather, G.; Pleiman, C. M.; Kasibhatla, S.; Tseng, B.; Drewe, J.; Cai, S. X. Discovery of *N*-(4-Methoxyphenyl)-*N*,2-dimethylquinazolin-4-amine, a Potent Apoptosis Inducer and Efficacious Anticancer Agent with High Blood Brain Barrier Penetration. *J. Med. Chem.* **2009**, *52*, 2341−2351.

(24) ML314 has been declared a probe via the Molecular Libraries Probe Production Centers Network (MLPCN) and is available through the network, see: http://mli.nih.gov/mli/.

(25) Schaeffer, P.; Laplace, M. C.; Bernat, A.; Prabonnaud, V.; Gully, D.; Lespy, L.; Herbert, J. M. SR142948A is a potent antagonist of the cardiovascular effects of neurotensin. *J. Cardiovasc. Pharmacol.* **1998**, *31*, 545−50.

(26) Other activities <10 μM: inhibition of mycobacterium TB H37Rv (3.90 μM, AID: 1626), PAX8 cytotoxicity (0.26 μM, AID: 687029), inhibition of HCV growth (3.16 μM, AID: 651820).

(27) Other GPCRs include GPR55, APJ, k-opiod, CCR6, and CXCR6.

(28) National Institute of Mental Health's Psychoactive Drug Screening Program, Contract No. HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda, MD, USA.

(29) Follow up studies revealed K_is of 0.4 μM (5-HT1A) 1.0 μM (5-HT3), 0.93 μM (5-HT7), 1.42 μM (H1) 1.54 μM (MOR), 2.54 μM (PBR), and 0.41 μM (sigma1).

(30) Avdeef, A.; Nielsen, P. E.; Tsinman, O. PAMPA-a drug absorption in vitro model 11. Matching the in vivo unstirred water layer thickness by individual-well stirring in microtitre plates. *Eur. J. Pharm. Sci.* **2004**, *22*, 365−374.