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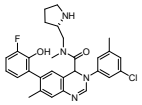

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human sst2 EC_{50} = 0.73 nM

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Discovery of nonpeptide 3,4-dihydroquinazoline-4-carboxamides as potent and selective sst2 agonists

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ABSTRACT

Nonpeptide sst2 agonists can provide a new treatment option for patients with acromegaly, carcinoid tumors, and neuroendocrine tumors. Our medicinal chemistry efforts have led to the discovery of novel 3,4-dihydroquinazoline-4-carboxamides as sst2 agonists. This class of molecules exhibits excellent human sst2 potency and selectivity against sst1, sst3, sst4 and sst5 receptors. Leading compound 3-(3-chloro-5-methylphenyl)-6-(3-fluoro-2-hydroxyphenyl)-N,7-dimethyl-N-{[(2S)-pyrrolidin-2-yl]methyl}-3,4-dihydroquinazoline-4-carboxamide (**28**) showed no inhibition of major CYP450 enzymes (2C9, 2C19, 2D6 and 3A4) and weak inhibition of the hERG channel.

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wide variety of cell types in the CNS and gut and has pleiotropic effects.[1] SS14 acts through both endocrine, paracrine, and nerve pathways to affect its target cells via a family of five receptors: sst1, sst2, sst3, sst4, and sst5.[2, 3] Many of these effects result in the regulation of other hormones, such as growth hormone (GH), glucagon, and insulin. SS14 inhibits the secretion of GH from the pituitary mainly via activation of the sst2 and sst5 receptors.[4]

Acromegaly is a rare disease in which a pituitary adenoma results in the over-production of GH and subsequent elevation of insulin-like growth factor 1 (IGF-1) levels.[5, 6] This loss of homeostasis in the GH axis results in excess tissue growth and other adverse metabolic effects throughout the body. If transsphenoidal surgery to remove the adenoma is unsuccessful or only partially successful, the first-in-line medical treatment for acromegaly is the use of injectable somatostatin peptide analogs (SSAs). Octreotide, lanreotide, and pasireotide are SSAs approved by the FDA for the treatment of acromegaly, carcinoid tumor symptom control, or neuroendocrine tumors.[7, 8] They are most commonly delivered monthly by intramuscular or deep subcutaneous injections which require office visits and often lead to injection site discomfort or pain.[9] In addition to the burden on the patient, SSAs like octreotide have undesirable properties and side effects: octreotide is known to induce receptor phosphorylation, internalization, and desensitization responses, limiting their therapeutic benefits.[10, 11] Pasierotide suppresses insulin secretion in the pancreas and results in hyperglycemia.[12] Furthermore, many acromegaly patients under SSA treatment fail to achieve normalization of IGF-1 levels and/or experience a return of symptoms near the end of their injection cycle. Consequently, an orally bioavailable and selective sst2 agonist that could reduce counter-regulatory activities to improve efficacy is highly desirable.

Figure 1. Selected examples of nonpeptide sst2 agonists

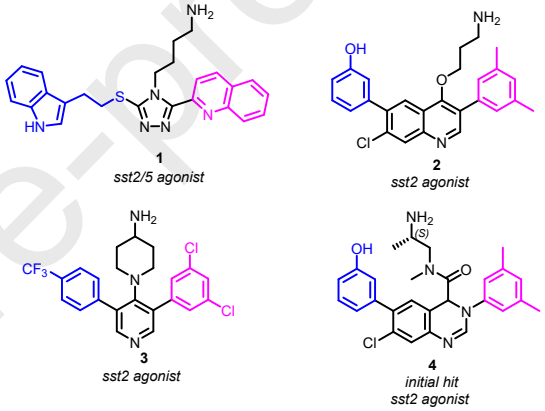
The search for nonpeptide sst2 agonists have been attempted by many groups, and the representative examples are illustrated in **Figure 1**. For instance, the triazole analog **1** was found to be a potent sst2 and sst5 dual agonist.[13] Compound **2** represents another class of molecules reported to be selective sst2 agonists.[14] Most recently, an aminopyridine analog **3** was revealed to be a sst2 agonist.[15] Based on chemical series reported in the literature, we designed and synthesized a small but focused library featuring diverse chemical structures, which led to the discovery of several novel hits. Among them, the 3,4-dihydroquinazoline pharmacophore as exemplified by compound **4** exhibited good potency for sst2. In comparison to literature compound **2**, which contains lipophilic quinoline scaffold substituted with ether linker, the 3,4-dihydroquinazoline carboxamide scaffold appears to be more hydrophilic itself, thus allowed us to have more leeway to use relatively hydrophobic side chains if necessary, maintaining the balance of hydrophobicity and hydrophilicity.

The synthesis of 3,4-dihydroquinazoline-4-carboxamides is illustrated in Scheme 1. In this sequence, nucleophilic replacement to fluoro-benzaldehyde **I** generated azide intermediate **II** which underwent a multi-component Ugi reaction with 1-(2,2-dimethoxyethyl)-2-isocyanobenzene to afford 3,4-dihydroquinazoline precursor **III**.[16] The azide group was subsequently reduced with methyldiphenylphosphine followed by an acid-mediated cyclization to yield **IV**. Removal of the indole protecting group furnished 3,4-dihydroquinazoline-4-carboxylic acid **V**. This compound can either directly couple with a protected secondary amine or a primary amine followed by alkylation to produce amide **VI**. The top amide bond was constructed at the final

Alternatively, azide **II** can react with an iso-nitrile with R^4 group pre-installed to generate intermediate **VII**, which was treated with methyldiphenylphosphine to give 3,4-dihydroquinazoline-4-carboxylic amide **VIII**. Alkylation of this compound gave bromo-substituted 3,4-dihydroquinazoline-4-carboxamide **VI**. Subsequent Suzuki coupling followed by removal of protecting group ultimately produced final compound **IX**.

The activation of sst2 by an agonist results in the decrease of intracellular cyclic adenosine monophosphate (cAMP) in functional cell-based assays.[17] Breifly, Chinese hamster ovary (CHO-K1) cells stably expressing the human sst2 receptor were treated with NKH477, a soluble analog of forskolin, to induce the production of cAMP. Upon agonist activation, the level of intracellular cAMP is decreased in a concentration-dependent manner, which allows the measurement of the potency of the compound (EC_{50}) to guide the study of structure activity relationships (SAR).

As illustrated in **Table 1**, the initial hit **4** showed an EC_{50} of 38 nM in the functional assay, however, removing the *N*-methyl group on the amide completely suppressed its ability to reduce cAMP production (**5**). Deletion of the chloro-substitution on the *7* position of the 3,4-dihydroquinazoline core also decreased agonist activity more than 10-fold (**6**). Extending the length of the alkyl group adjacent to the top primary amine enhanced sst2 potency about 3-fold (EC_{50}=11 nM) (**7**). Interestingly, agonist potency was further improved when the 7-chloro group was replaced by a methyl group (**8**). In an effort to reduce the total number of proton donors and flexible bonds, the top primary amine was replaced with a pyrrolidine fragment, resulting in compound **9**, which also improved potency to 1.3 nM. We subsequently carried out a rapid SAR campaign on substituent R^6 and found that halogens are well tolerated and that Cl is preferred (compound **10-11**).

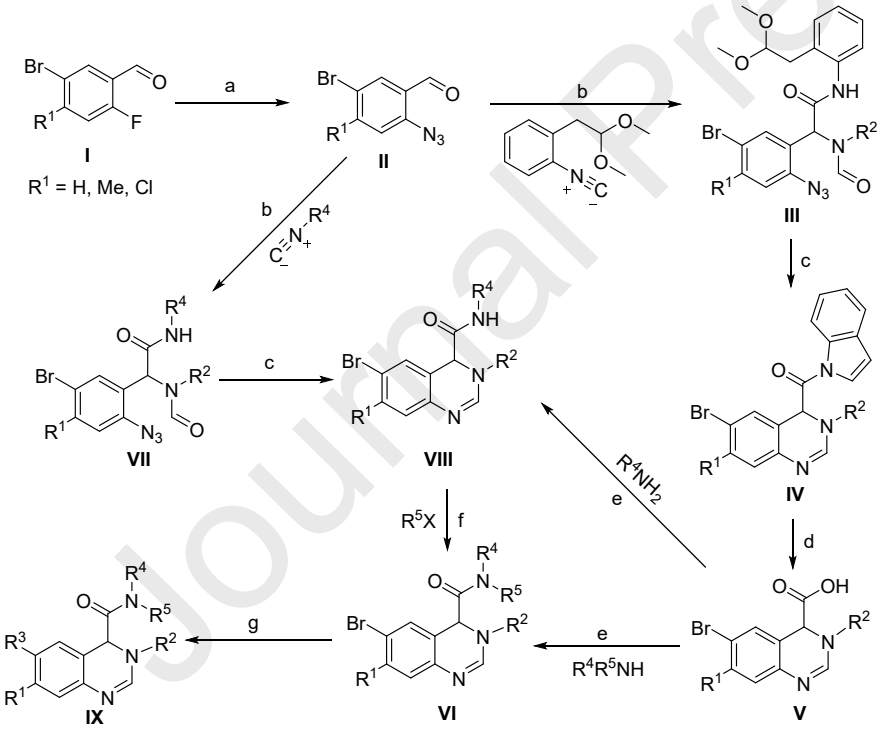


With these results, we examined the impact of a 6-aryl substituent to 3,4-dihydroquinazoline pharmacophore. The original phenol group pharmacophore could potentially pose a safety risk in development, although many approved drugs contain phenol motifs.[18] These results are summarized in **Table 2**. Initially, it was revealed that truncating the hydroxyl group significantly diminished sst2 agonist activity (**12**). Our SAR efforts then mainly became focused on identifying suitable alternatives. We attempted to use a substituted phenyl without hydrogen bond donors (compounds **13-16**), however, only nitrile (**13**, EC_{50}=5.4 nM) was tolerated whereas Cl and CF_3 groups dramatically reduced agonist potency. Introducing heterocycles, i.e. pyrazole, substantially reduced potency as well (**17**). Notably, a fluoro-substitution to the *meta* position of the cyano group boosted agonist potency to 1.3 nM (**18**). Replacing the cyano group with fluoro or methoxy group did not further improve sst2 activity (**19-20**). We next examined whether the phenol group could be replaced by its isosteric groups,

as [illegible] tors carboxylic amide derivative **22** exhibited comparable potency. We also synthesized compounds **25-30** which included electron-withdrawing groups, or replaced the phenyl ring with a pyridine. Compound **26** had EC_{50}=0.12 nM, demonstrating significant potency improvement compared to analog **10**. Moving fluoro from the *meta* to *para* position of the phenol ring had no impact on sst2 receptor affinity (compound **27,** EC_{50}=0.2 nM). Interestingly, compound **28**, bearing a 3-fluoro-2-hydroxyphenyl group, was found to be potent as well, exhibiting an EC_{50}=0.73 nM. Furthermore, adding a fluoro *para* to the hydroxyl of phenol motif reduced sst2 functional activity (compound **29**, EC_{50}=2.1 nM). Replacing the fluoro group on compound **26** with a cyano group (**30**) was found to be tolerable. Compound **31**, bearing a combination of fluoro and carboxylic amide, was as potent as compound **26**. However, shifting the fluoro substitution from the *meta* to *para* position of the carboxamide (**32**) did not yield the beneficial results as observed in compound **27**.

After we identified compounds with excellent sst2 activity, we examined their selectivity against the other sst receptor subtypes in assays similar to the sst2 assay previous described here. The results of selected examples are summarized in **Table 3**. Generally, this class of molecules showed very weak or no sst1, sst4 and sst5 activity, but moderate sst3 activity in some cases. Since compounds **18**, **26**, **27**, **28,** and **31** exhibited the most selective profile, they were chosen to be examined in a hERG inhibition binding assay and CYP450 enzyme inhibition binding (IC_{50}<1 μM), while **18**, **28,** and **31** are moderate inhibitors. The latter two were selected for further evaluation in in vitro screening CYP450 inhibition assays (2C9, 2C19, 2D6 and 3A4). Compound **31** was found to be a moderate 3A4 inhibitor (IC_{50}=1.0 μM) whereas compound **28** showed little inhibition even at the top concentration tested (10 μM). Finally, the two stereoisomers of compound **28** was separated by chromatography, affording compound **28-a** and **28-b**, which exhibited sst2 agonist activity of 0.11 nM and 16 nM, respectively. The more potent isomer **28-a** showed EC_{50} of 0.54 nM in rat sst2 functional assay. Subsequently, compound **28-a** was orally administered to male SD rats at 10 mg/Kg dose, however, less than 5% bioavailability was achieved. Although compound **28-a** is not suitable to further development as an oral agent, it is 50-fold more potent than literature compound **2** in our functional assay, suggesting it can better serve as a tool compound in mechanistic or other proof of concept studies. The absolute stereochemistry of **28-a** was not determined.

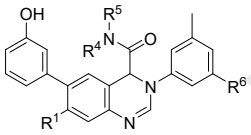
In summary, we report a novel 3,4-dihydroquinazoline-4-carboxamide series that demonstrated superior sst2 potency and selectivity over the other sst receptor subtypes in cell based functional cAMP assays. Although leading compound **28** did not advance to the development stage due to limited oral exposure in male SD rats, the SAR knowledge obtained from this series facilitated the design of nonpeptide sst2 compounds with better ADME profile, and these results will be reported in the near future.



(a) NaN_3, DMAc; (b) R^2NH_2, HCO_2H, MeOH; (c) (1) $MePPh_2$; (2) AcOH, TFA, toluene; (d) NaOH, THF; (e) HATU, TEA, DMF; (f) NaH, THF; (g) (1) cat. Pd, $R^3B(OR)_2$; (2) TFA

Scheme 1. Synthesis of 3,4-dihydroquinazoline-4-carboxamides

Table 1. SAR studies on R^1, R^4, R^5 and R^6

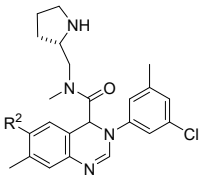


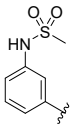
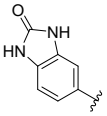
Compa[q]	R^1	R^4	R^5	R^6	sst2 pEC_{50}±SEM[b]	sst2 Avg EC_{50} (nM)
4	Cl	Me		Me	7.4 ± 0.1	38
5	Cl	H		Me	< 6[c]	>1000
6	H	Me		Me	< 6[c]	>1000
7	Cl	Me		Me	8.0 ± 0.1	11
8	Me	Me		Me	8.4 ± 0.2	4.2
9	Me	Me		Me	8.9 ± 0.1	1.3
10	Me	Me		Cl	9.5 ± 0.2	0.33

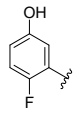
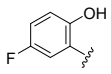
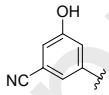
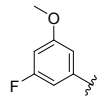
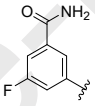
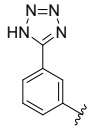
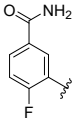
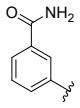
11	Me	Me		F	9.0 ± 0.1	0.98

[a]Purity of compounds is more than 95% as determined by LCMS or ^{1}H NMR analysis. [b]pEC_{50} is average of two or more independent measurements. [c]For EC_{50} values >1000 nM, the pEC_{50} is reported as < 6.

Table 2. SAR studies on aromatic ring R^2



Compd[a]	R^2	sst2 pEC_{50}±SEM[b]	sst2 Avg EC_{50} (nM)	Compd[a]	R^2	sst2 pEC_{50}±SEM[b]	sst2 Avg EC_{50} (nM)
12		7.3 ± 0.0	56	**23**		6.9 ± 0.1	130
13		8.3 ± 0.1	5.4	**24**		8.3 ± 0.2	4.6

14		7.9 ± 0.4	11	**25**		8.1 ± 0.1	8.1
15		7.3 ± 0.0	53	**26**		9.9 ± 0.2	0.12
16		7.4 ± 0.0	39	**27**		8.5 ± 0.1	0.20
17		6.8 ± 0.1	170	**28**		9.1 ± 0.0	0.73
18		8.9 ± 0.2	1.3	**29**		8.7 ± 0.0	2.1
19		8.4 ± 0.0	3.7	**30**		9.4 ± 0.1	0.36
20		8.2 ± 0.1	6.1	**31**		9.8 ± 0.1	0.15
21		< 6.0[c]	>1000	**32**		8.1 ± 0.0	7.8
22		8.1 ± 0.0	7.6				

[a]Purity of compounds is more than 95% as determined by LCMS or ^{1}H NMR analysis. [b]pEC_{50} is average of two or more independent measurements. [c]For EC_{50} values >1000 nM, the pEC_{50} is reported as < 6.

Table 3. sst sub-type selectivity and hERG inhibition of selected compounds

Compd[a]	sst pEC_{50}±SEM[b]					hERG pIC_{50}±SEM[b]
	sst1	sst2	sst3	sst4	sst5	
18	<6[c]	8.9 ± 0.2	8.4 ± 0.0	6.2 ± 0.0	5.7 ± 0.1	5.8 ± 0.0
26	<6[c]	9.9 ± 0.2	8.1 ± 0.1	6.6 ± 0.0	<6[c]	6.2 ± 0.1
27	<6[c]	9.7 ± 0.1	7.2 ± 0.0	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1
28	<6[c]	9.1 ± 0.0	6.8 ± 0.2	6.1 ± 0.3	<6[c]	5.3 ± 0.2
31	<6[c]	9.8 ± 0.1	8.1 ± 0.1	<6[c]	<6[c]	5.6 ± 0.2

[a]Purity of compounds is more than 95% as determined by LCMS or ^{1}H NMR analysis. [b]pEC_{50} and pIC_{50} are average of two or more independent measurements. [c]For EC_{50} values >1000 nM, the pEC_{50} is reported as < 6.

Ack

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Supplementary data

Supplementary data including the synthesis of selected compound can be found, in the online version, at

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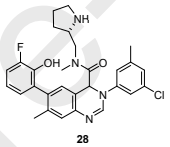

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