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2-Guanidinoquinazolines as new inhibitors of the STAT3 pathway

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Abstract

Synthesis and SAR investigation of 2-guanidinoquinazolines, initially identified in a high content screen for selective STAT3 pathway inhibitors, led to a more potent analog (**11c**) that demonstrated improved anti-proliferative activity against a panel of HNSCC cell lines.

Keywords

STAT3 pathway; Guanidinoquinazolines; Skraup synthesis; Structure–activity relationships; Cancer cell line screening

Despite recent advances in early detection methods and treatment regimens, cancer continues to be a major health threat, responsible for over 25% of deaths annually in the U.S. alone.[1] Head and neck squamous cell carcinomas (HNSCC) are particularly challenging therapeutic targets[2] as evidenced by the fact that the monoclonal antibody Cetuximab (Erbitux), an epidermal growth factor receptor (EGFR) inhibitor,[3] was the only new drug approved for this indication in the last several decades. To address this issue, a wide range of signaling pathways that control cell proliferation have been interrogated as potential therapeutic strategies for HNSCC, including the family of signal transducers and activators of transcription (STATs).[4-6] STAT3 is a tumor promoting transcription factor that

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has been shown to be constitutively activated in numerous cancers, and suppression of STAT3 leads to inhibition of tumor growth in both in vitro and in vivo experiments. In contrast, the related transcription factor, STAT1, activates genes that promote tumor suppression. Therefore, molecules that selectively inhibit STAT3-mediated pathways with no effect on STAT1 pathways, have the potential to be highly effective anti-tumor agents.

Several small organic molecules that inhibit the STAT3 pathway have been reported in the literature.[7] One strategy has been to design molecules that directly target the Src homology 2 (SH2) domain in STAT3 (**1–4**, Fig. 1).[8] Other approaches include focusing on inhibiting kinases operative in the STAT3 pathway, such as Janus activated kinases (JAKs), and identified quinolones, pyridones, and the pyridine carboxamide, sorafenib (**5**, **6** and **7**, respectively, Fig. 1).[9] Additionally, natural products, including STA-21 (**8**), curcumin (**9**), and cucurbitacin Q (**10**), inhibit the STAT3 pathway; however, specific inhibitory mechanisms are still being elucidated (Fig. 2).[5b] Finally, anti-sense oligonucleotides (AZD9150) and decoy nucleotides directed at STAT3 also exhibit promising anti-proliferative activities in cellular assays.[5,10]

By using a high content phenotypic screen (HCS) to identify selective inhibitors of IL-6 induced activation of the STAT3 pathway,[11] we identified the quinazoline 11a (Fig. 3). In Cal33 head and neck tumor cells, 11a inhibited IL-6-induced STAT3 tyrosine phosphorylation and nuclear translocation (IC_{50} = 15.7 μM), but had no effect on IFNγ-induced activation of the STAT1 pathway at 50 μM (Fig. 3). Western blot analysis indicated a 69% decrease in phospho-STAT3 (pSTAT3) levels upon treatment of **11a** at 39.6 μM concentration (Fig. 4, A and B). Unlike the JAK inhibitor **6** that displayed nanomolar potencies against both STAT3 and STAT1 (data not shown),[11] compound **11a** selectively inhibited STAT3 compared to STAT1 and displayed no effects on JAK1/JAK2 as determined by Western blot analysis (Figs. 3 and 4, panels C and D). In addition, **11a** exhibited anti-proliferative activities (IC_{50}'s = 17-37 lM in four HNSCC cell lines (CAL33, FADU, 686 LN, OSC19, Fig. 3). Examination of the literature and PubChem revealed limited examples of biological effects for this chemotype, and Lipinski and Veber parameters fell into the generally desirable ranges (Fig. 3).[12-15] While the specific mode of action of 11a was not determined, its apparent lack of activity in the STAT1 assay likely rules out direct binding to SH2 domains. Furthermore, this hit compound did not exhibit any significant activity against a panel of >80 kinases (data not shown). The promising selectivity for STAT3, the notable anti-proliferative activity and desirable physical properties made this compound an attractive lead structure for further medicinal chemistry optimization, and herein we report the results of these efforts.

Our initial strategy was to incorporate modest structural modifications onto the 2-guanidinoquinazoline core in order to establish preliminary structure-activity relationships. Using established synthetic procedures,[16] the dihydroquinolines **13** were generated through the treatment of the substituted anilines **12** with acetone under modified Skraup conditions (Scheme 1).[17] Conversion to the guanidines **14** occurred by reaction with cyano-guanidine under aqueous acidic conditions.[18] The final products, dihydropyrimidinyl-aminoquinazolines **11a-d**, were formed via thermal cyclodehydrations using mesityl oxide in DMSO. The structure of **11b** was confirmed by X-ray analysis (Scheme 1).[16] In this

subset of analogs (Table 1), it was apparent that structural modification was tolerated and modulated the biological profile; the C6-methyl (**11b**) and C6-,C8-dimethyl (**11c**) analogs exhibited improved potency (4- and 30-fold, respectively) while maintaining selectivity versus STAT1 compared to the original hit **11a**. Unlike **11a**, **11b** and **11d**, which failed to achieve ≥50% inhibition of IFNγ-induced STAT1 activation at 50 μM, **11c** exhibited an IC_{50} of 5.9 μM for STAT1 but still maintained a good selectivity index (Table 1).

Efforts to examine a simplified pharmacophore focused on the preparation of 2-aminoquinazolines of general structure **17** (Scheme 2). The pivotal 2-chloro intermediate **16** was prepared in three steps[19] and subjected to amination under microwave conditions to provide the corresponding quinazoline derivatives **17a–i**. All of these compounds were devoid of activity and therefore established the importance of quinazoline substitutions as well as the C2-linked nitrogenous heterocycle (Table 2).[11]

To investigate substituent effects on the pyrimidine, we also synthesized pyrimidinones of general structure **19**. The reaction of guanidines **14** with substituted acetoacetic esters (Scheme 3)[20,21] afforded the dihydropyrimidones **19a–t**. Representative compounds in this series and their activities are shown in Table 3. Several substitutions (e.g., entries **19a**, **b**, **g**, **h**) slightly improved potency and selectivity compared to the original hit (**11a**), whereas other substitution patterns (e.g., **19e**, **o**, **p**) completely abolished activity. With the exception of **19p**, all 5,6-dimethylpyrimidinones (**19b**, **19f**, **19l**, **19n**) demonstrated inhibition of pSTAT3 at less than 10 lM concentration and maintained at least a 3-fold selectivity against pSTAT1. All new compounds were fully characterized, and all tested compounds had LC/MS/ELSD purities exceeding 91%.

The ability of the most potent analogs to inhibit the proliferation of a panel of HNSCC cell lines is shown in Table 4. While a strict correlation between STAT3 inhibition and cell growth inhibition potency was not evident, compound **11c**, which exhibited the most potent activity in our STAT3 assays, also displayed the most potent anti-proliferative effects.

In summary, 6-ethoxy-4-methylquinazoline-2-amino-dihydro-trimethylpyrimidine **11a**, a structurally novel, selective inhibitor of STAT3-mediated signaling, was further optimized through three rounds of SAR studies. Modest structural changes established the preferred quinazoline substitutions. Most notably, a 20-fold improvement in STAT3 inhibition, while maintaining selectivity over STAT1, was obtained with the 6,8-dimethyl substituted analog **11c**. More substantial changes to the dihydropyrimidine moiety led to complete loss of activity; however, substitution of the quinazoline with dihydropyrimidinones also retained potency and selectivity. The most promising lead structures, **11b**, **11c**, and **19a** exhibited low-micromolar potency against STAT3, at least a 2-fold selectivity over STAT1, and anti-proliferative activity in the 1–50 μM range. Additional SAR iterations will be needed to increase the activity of these compounds to the sub-micromolar level; however, **11b**, **11c**, and **19a** represent attractive tool compounds to investigate a kinase- and STAT1-independent downregulation of the STAT3 pathway. Studies on the mechanism of action of this class of compounds as well as the biological data and medicinal chemistry of other STAT3 HCS hits will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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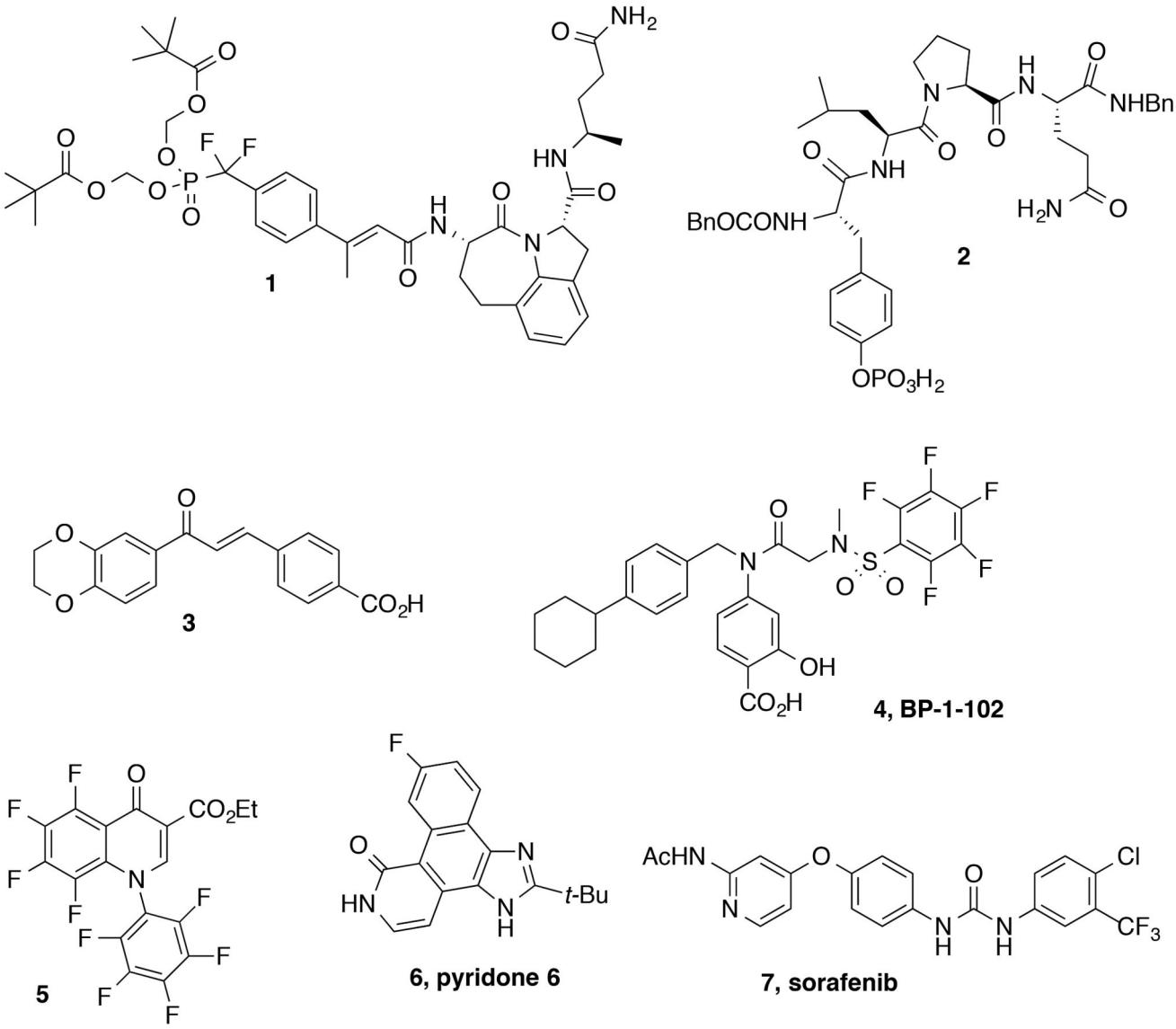


Figure 1.
SH2 targeted phosphopeptide mimetics and JAK inhibitors of the STAT3 pathway.

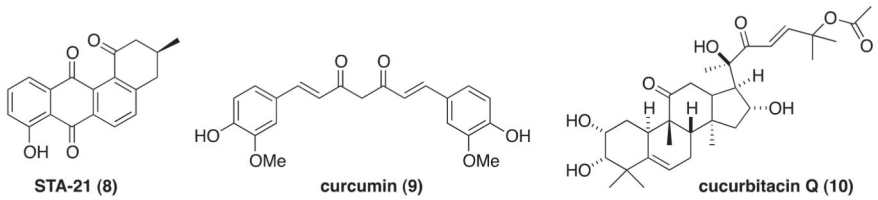


Figure 2.
Natural product STAT3 inhibitors.

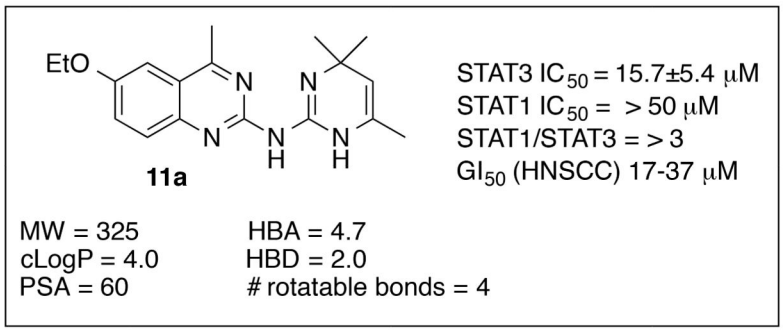


Figure 3.
Guanidinoquinazoline hit **11a**.

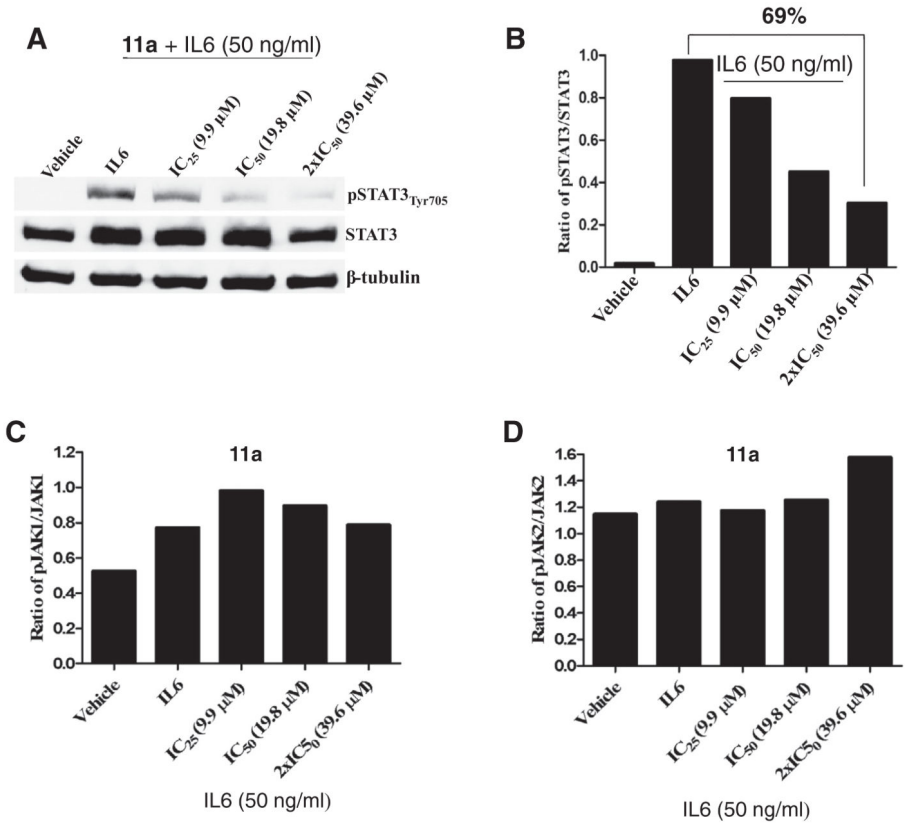
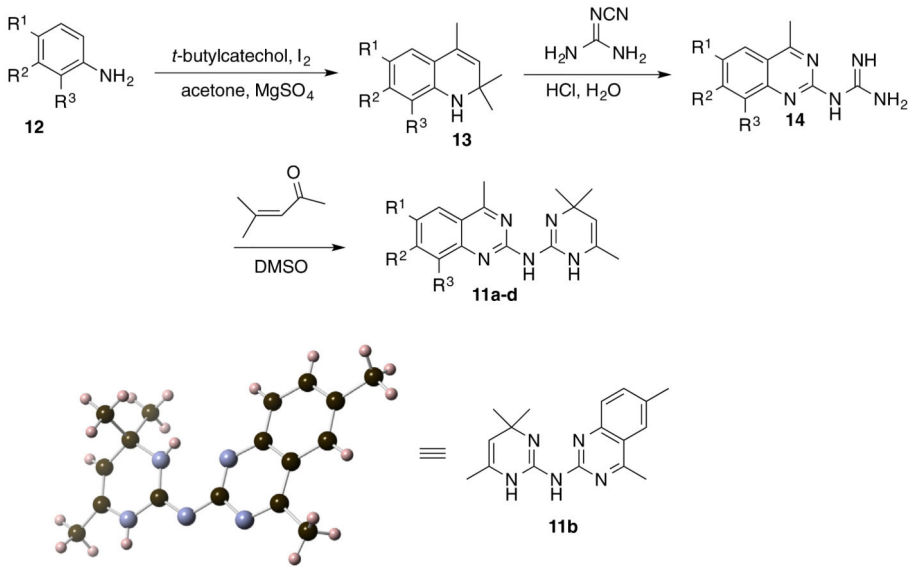


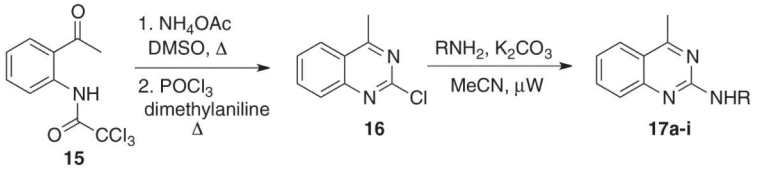
Figure 4.

Inhibition of STAT3 phosphorylation using Western blot analysis of compound **11a** versus vehicle in interleukin 6 (IL6, 50 ng/mL)-stimulated CAL33 cells (A & B). Compound **11a** did not show any effects on pJAK1/JAK1 (C) or pJAK2/JAK2 (D).

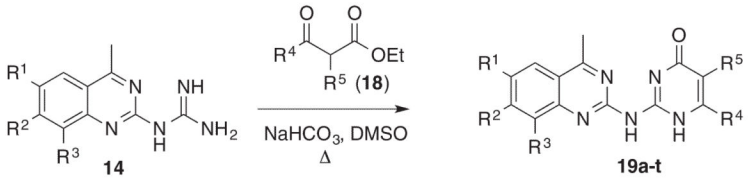


Scheme 1.

Preparation of 2-guanidinoquinazolines **11a–d** and X-ray structure of **11b** (CCDC 1020633).



Scheme 2.
Preparation of 2-aminoquinazolines (**17a–j**).



Scheme 3.

Preparation of 2-aminoquinozoline dihydropyrimidones (**19a–t**).

Table 1

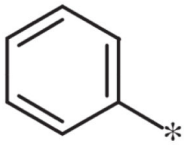
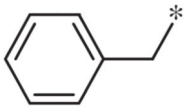
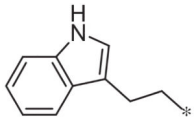
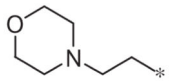
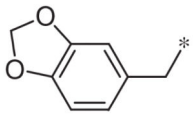
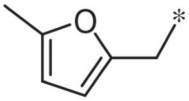
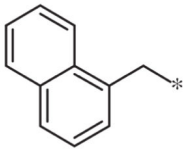
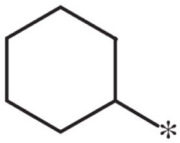
STAT3 and STAT1 Activity of 2-guanidinoquinazolines **11a–d**

Compd#	R^1	R^2	R^3	STAT3 IC_{50}[11] (μM)	STAT1 IC_{50}[11] (μM)	SI[a] (STAT1/STAT3)
11a	OEt	H	H	15.7 ± 5.4	>50	>3
11b	Me	H	H	7.9 ± 2.8	>50	>6
11c	Me	H	Me	0.8 ± 0.6	5.9 ± 0.9	>7
11d	H	OEt	H	20.7 ± 3.0	>50	>2

[a] Selectivity index ratio.

Table 2

Inhibitory potencies of 2-aminoquinazolines (**17a–j**)

Compd#	R	STAT3[11]	STAT1[11]
		IC_{50} (μM)	IC_{50} (μM)
17a		>30	>30
17b		>30	>30
17c		>30	>30
17d		>30	>30
17e		>30	>30
17f		>30	>30
17g		>30	>30
17h		>30	>30

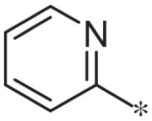
Compd#	R	STAT3[11]	STAT1[11]
		IC_{50} (μM)	IC_{50} (μM)
17i		>30	>30

Table 3

Inhibitory potencies of 2-amino-quinazolinyl-dihydropyridimidones **19a–t**

Compd#	R^1	R^2	R^3	R^4	R^5	STAT3 IC_{50}[11] (μM)	STAT1 $IC_5{}_0$[11] (μM)	SI[b] (STAT1/STAT3)
19a	Me	H	Me	Me	H	5.0 ± 1.8	12.1 ± 0.9	>2
19b	Me	H	Me	Me	Me	6.4 ± 1.6	>50	>7
19c	Me	H	Me	Me	Allyl	14.3 ± 0.9	>50	>3
19d	Me	H	Me	Ph	H	11.2 ± 2.1	>50	>4
19e	OEt	H	H	Me	H	>50	>50	–
19f	OEt	H	H	Me	Me	11.7 ± 5.7	>50	>4
19g	OEt	H	H	Ph	H	3.4 ± 0.6	>50	>14
19h	OEt	H	H	Me	Bn	2.7 ± 2.5	>50	>18
19i	OMe	H	H	Me	H	34.9 ± 2.7	>50	>1
19j	OMe	H	H	Me	Bn	34.7[a]	50	>1
19k	H	Me	H	Me	H	19.1 ± 1.3	39.7 ± 1.6	>1
19l	H	Me	H	Me	Me	10.5 ± 0.2	35.2 ± 1.1	>3
19m	Me	Me	H	Me	H	24.7 ± 0.5	>50	>2
19n	Me	Me	H	Me	Me	10.4 ± 1.3	>50	>4
19o	$-OCH_2O-$		H	Me	H	>50	>50	–
19p	$-OCH_2O-$		H	Me	Me	>50	>50	–
19q	$-OCH_2O-$		H	Ph	H	13.0 ± 1.4	>50	>3
19r	$-OCH_2O-$		H	Me	Bn	>50	>50	–
19t	H	OMe	H	Me	H	33.9 ± 1.8	>50	>1

[a] Results from a single experiment.

[b] Selectivity index ratio.

Table 4

Growth inhibition of selected analogs on HNSCC cell lines 686LN, CAL33, FADU and OSC19

Compd#	686LN IC_{50} (μM)	CAL33 IC_{50} (μM)	FADU IC_{50} (μM)	OSC19 IC_{50} (μM)
11a	37.5 ± 11.1	29.4 ± 5.9	18.8 ± 5.3	17.0 ± 4.6
11b	49.3[a]	19.7 ± 0.5	11.0 ± 0.1	12.8 ± 0.9
11c	2.4 ± 0.6	1.2 ± 0.3	1.9 ± 0.1	3.2 ± 0.4
19a	18.0 ± 10.4	12.9 ± 0.9	30.9 ± 14.9	11.2[a]
19g	37.8[a]	11.1[a]	17.9[a]	19.0[a]
19h	43.7 ± 0.7	>50	27.4 ± 13.3	>50
6	>5	1.3 ± 0.4	2.4 ± 0.6	7.7 ± 4.7

[a]Results from a single experiment.