Small molecule somatostatin receptor subtype 4 agonists are novel candidates for the treatment of neuropathic pain and depression

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Introduction

Somatostatin is a cyclic neuropeptide that regulates the neuroendocrine system. Our group discovered its analgesic, anti-inflammatory and antidepressant effects mediated by the G_i protein-coupled somatostatin 4 receptor (sst_4) independently of endocrine alterations [1]. Sst_4 is highly expressed in glutamatergic neurons of pain- and mood-regulating brain regions, its activation leads to neuronal inibition by intracellular cAMP decrease. Due to its short half-life and endocrine side-effects, somatostatin cannot be used as an analgesic/antidepressant drug, but synthetic, orally active small molecule sst_4 agonists could have important drug developmental potentials [2, 3]. Our patented 4-phenethylamino-7H-pyrrolo[2,3-d]pyrimidine compounds (**Fig. 1**) synthesized by Vichem Ltd. were tested *in silico* for sst_4 binding, *in vitro* on sst_4 activation, and *in vivo* in mouse models of neuropathic pain, anxiety and depression-like behavior.

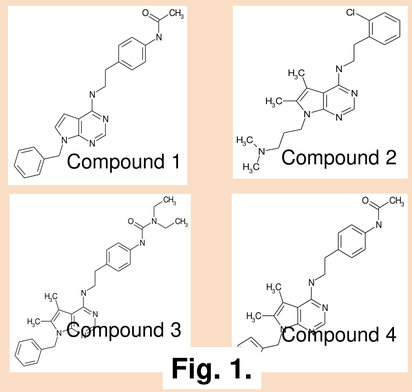


Fig. 1.

Methods

1. Modelling sst_4 binding: Structures were built in the Maestro program. Gasteiger-Marsilli partial charges were assigned in AutoDock Tools, then ligand structures were docked on the extracellular region of sst_4 by AutoDock 4.2.6. After 10 docking runs, ligand conformations were ranked by interaction energy values. Rank 1 was analyzed and selected as representative for each ligand.

2. G-protein activation assay: Membrane fractions prepared from CHO cells stably expressing sst_4 was incubated with GDP, GTPγ35S isotope and the test compound. Radioactivity was measured with TriCarb β-counter. G-protein activation was given as percentage over specific GTPγ^{35}S binding observed in the absence of the agonists.

3. cAMP inhibition assay: Human sst_4-expressing cells were treated with rolipram as a negative control, forskolin as a positive control or varying concentrations of the test compounds for 30 min at 37° C. The chemiluminescent signal corresponding to AMP concentration was detected and data were expressed as cAMP accumulated in the presence of the compounds as percentage of the forskolin response.

4. β-arrestin assay as an indicator of receptor desensitization: Human sst_4-expressing cells were treated with the test compounds for 90 min at 37°C. Agonist mediated β-arrestin2 interaction was detected by luminescence.

5. Neuropathic pain model: Traumatic mononeuropathy was induced in 12-week-old male NMRI mice by 1/3-1/2 ligation of the right sciatic nerve in deep aenesthesia (**Fig. 2.**). The mechanonociceptive threshold of the paws was measured by dynamic plantar aesthesiometry **(Fig. 3.)**, mechanical hyperalgesia on the 7th postoperative day was determined as % decrease of the threshold compared to the pre-operative control values. One hour after oral treatment (100 microg/kg), hyperalgesia values were determined and the antihyperalgesic effect was calculated: (pretreatment mechanical hyperalgesia – posttreatment mechanical hyperalgesia)/ pretreatment mechanical hyperalgesia*100**.**

Fig. 2.

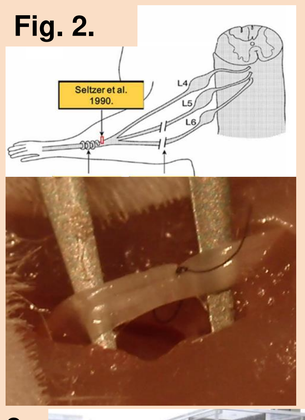


Fig. 3.

6. Depression-like behavior: Immobility time in the Tail Suspension Test (TST) referring to depression-like behavior was measured in the last 4-min of the 6-min period **(Fig. 4.).**

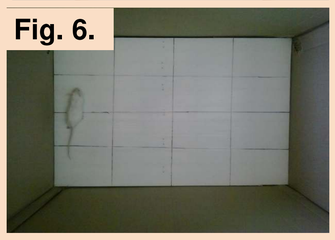
Fig. 4.

7. Anxiety test: In the Elevated plus maze (EPM) mice were placed into the central platform and the time spent in the open arms was determined during the 5-min experimental period **(Fig. 5.).**

Fig. 5.

8. Spontaneous locomotor activity test: Mice were placed in a brightly lit Open Field (OF) area. Their spontaneous locomotion in this novel environment was recorded by a video camera and evaluated during a 5-min period **(Fig. 6.).**

Fig. 6.



Summary and conclusion

Our small molecule sst_4 agonists exert analgesic effect in traumatic neuropathy model after a single oral administration, as well anxiolytic and anti-depressant-like actions. Based on these results lead selection and optimalization will be initiated, which can open new directions in the pharmacotherapy of chronic neuropathic pain and common co-morbidities.

Results

1. Our compounds bind to the high affinity binding pocket of the sst_4 receptor
Compounds 1-4 bind to the region called the high affinity binding pocket characterized earlier with the reference sst_4 agonists NNC-269100 and J-2156 on the basis of the interaction energy values. Our new compounds have greatly overlapping binding sites with the reference compounds on sst_4.

Fig. 7. shows **(A)** the 3D high affinity binding pocket of Compound 2 on the sst_4 receptor, and **(B)** color-coded binding pattern hydropobic pocket (green), aromatic-aromatic interactions (blue), H-bonds (yellow).

Fig. 7.

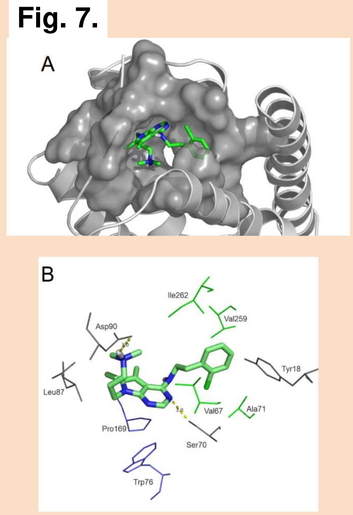
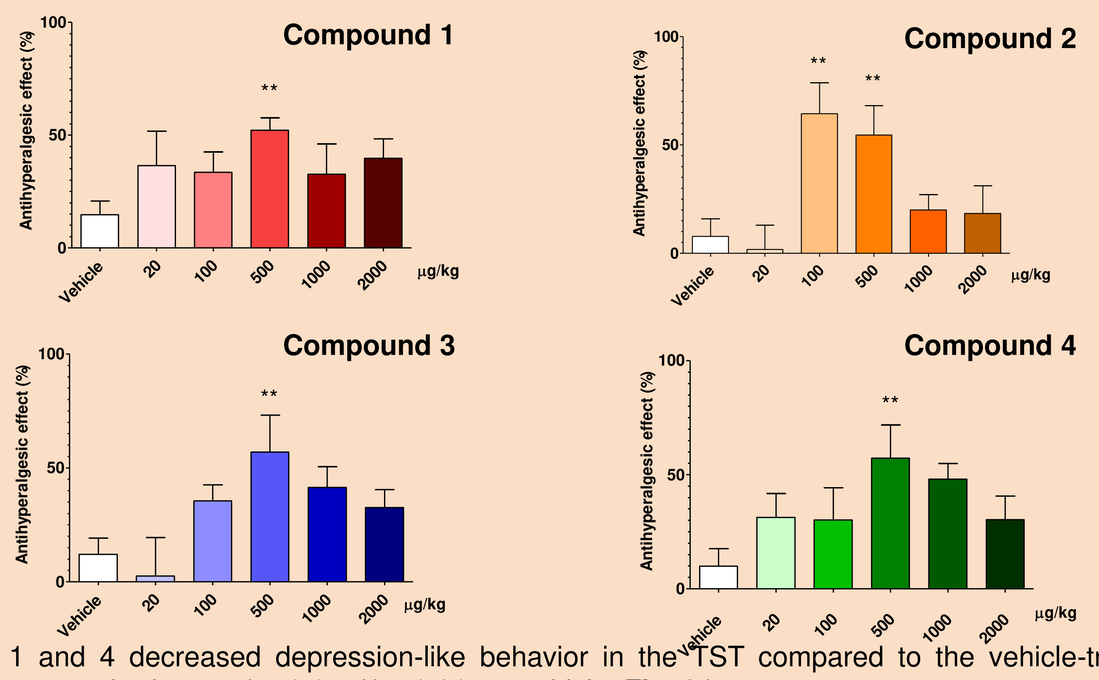


Table 1	EC_{50} GTP-γ-[35S] Assay	Emax GTP-γ-[35S] Assay	EC_{50} cAMP Assay HitHunter	Emax cAMP Assay HitHunter (% Inhibition of forskolin)	EC_{50} β-Arrestin assay 0000PathHunter	E_{max} β-Arrestin assay PathHunter (% of J-2156)
Compound 1	85 nM	**242.7±26%**	0.06 nM	**94 %**	ND (not detectable)	ND
Compound 2	32 nM	213±9%	0.005 nM	91 %	ND	ND
Compound 3	**20 nM**	220±7%	0.02 nM	87 %	ND	ND
Compound 4	25 nM	228.7±9%,	**0.002 nM**	91 %	ND	ND

2. Table 1. summarizes the results of the sst_4 activation assays
All compounds proved to be potent and effective sst_4 agonists in the G-protein activation assay.
Compond 3 was the most potent (EC_{50}: 20 nM) and Compound 1 had the highest efficacy (242.7 ± 26%).
They all showed a robust inhibitory effect on the forskolin-stimulated cAMP production. Compound 4 was the most potent agonist in this test (0.002 nM) and Compound 1 had the highest efficacy (94%).
In contrast, none of our 4 compounds displayed detectable β-arrestin-2 recruitment in the PathHunter assay. Since β-arrestin-2 plays a role in the desensitization of G-protein coupled receptors, these findings suggest that long-term administration of our compounds is not likely to cause tolerance.

3. All compounds showed dose-dependent **antihyperalgetic effect** in the traumatic mononeuropathy model becoming significant in 500 µg/kg. Compound 2 had significant effect already in 100 µg/kg (n=7-19; one-way ANOVA + Bonferroni's multiple comparison test; *p<0.05, **p<0.01 vs. vehicle; **Fig. 8.).**

Fig. 8.



Compounds 1 and 4 decreased depression-like behavior in the TST compared to the vehicle-treated controls (n=6-7; unpaired t-test; *p<0.05, **p<0.01 vs. vehicle; **Fig. 9**.)
Neither Compound 1 nor 4 in 100 µg/kg altered the time spent in open arms determined in the EPM, but 500 µg/kg of Compound 2 and 4 showed anxiolytic actions (n=5-12; unparired t-test; *p<0.05, **p<0.01 vs. vehicle; **Fig. 10**.).
Only Compound 4 decreased the locomotor activity in 500 µg/kg compared to the vehicle control which may refer to sedative actions (n=6; unparired t-test; *p<0.05, **p<0.01 vs. Vehicle; **Fig. 11**.).

Fig. 9.

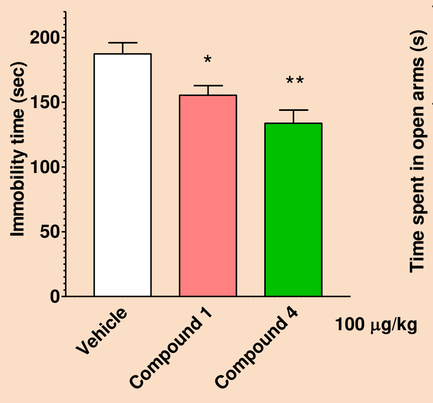


Fig. 10.

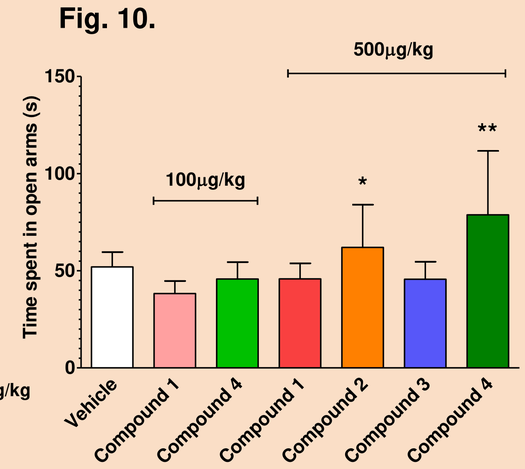
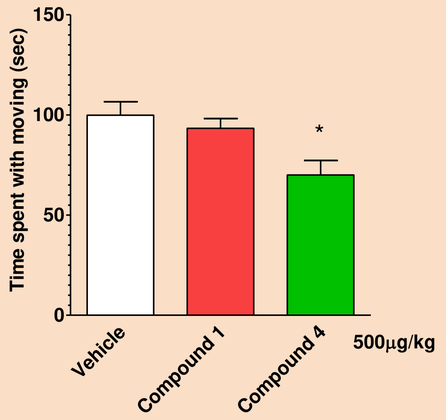


Fig. 11.



References
1. Szolcsanyi et al. 1998. Release of somatostatin and its role in the mediation of the anti-inflammatory effect induced by antidromic stimulation of sensory fibres of rat sciatic nerve. Br. J. Pharmacol. 123, 936–942.
2. Sándor et al., 2006. Analgesic effects of the somatostatin sst_4 receptor selective agonist J-2156 in acute and chronic pain models. European Journal of Pharmacology 539, 71–75.
3. Scheich et al., 2016. Somatostatin receptor subtype 4 activation is involved in anxiety and depression-like behavior in mouse models. Neuropharmacology. 101:204-15.

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