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3-Thio-1,2,4-triazoles, novel somatostatin sst_2/sst_5 agonists

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Abstract—Novel 3-thio-1,2,4-triazoles have been obtained via a solution-phase parallel synthesis strategy, affording potent non-peptidic human somatostatin receptor subtypes 2 and 5 agonists.

1. Introduction

Somatostatin (somatotropin release-inhibiting factor, SRIF) is a cyclic peptidic hormone that was originally isolated from the hypothalamus of rats and characterized by Brazeau in 1973.[1] SRIF is widely distributed throughout the body: It is mainly expressed not only in the central and peripheral nervous system and in the gastrointestinal tract, but also in the immune systems, the kidney, the retina, and the vessel walls.[2,3] Somatostatin has important regulatory effects on a variety of endocrine and exocrine functions such as inhibition of growth hormone (GH)[1] and gastrin secretion,[4] and inhibition of the pancreatic secretion of insulin and glucagon.[5] SRIF has been shown to inhibit cell proliferation[6] and can also act as a neurotransmitter.[7] The biological effects of SRIF are mediated through five distinct G protein-coupled receptor subtypes (sst_{1-5})[8], which have been cloned and characterized. If several receptors mediate the antiproliferative activity of SRIF, then only sst_2 and sst_3 have been reported to induce apoptosis, and to be involved in angiogenesis.[9] In the brain, sst_2 and sst_5 exert a predominant role in the inhibition of GH, prolactine, and thyroid-stimulating hormone (TSH) release. In the stomach, sst_2 inhibits gastric acid release and several gastric peptides, such as gastrin, histamine, and ghrelin.[10] In the pancreas, sst_5 is responsible for the regulation of insulin secretion, whereas sst_2 mediates the inhibition of glucagon release. In the intestine, sst_2 has a predominant role in the inhibition of fluid and electrolyte secretion.[11,12] The function of sst_4 remains largely unknown.

Because of its wide range of physiological functions, somatostatin may play an important role in the treatment of numerous human diseases. However, its very short half-life in the circulation and its lack of selectivity have led to the preparation of peptidic and also non-peptidic analogs worldwide.[13] Detailed structure–activity relationship (SAR) studies have shown that Trp[8] and Lys[9] residues are essential for biological activity. These residues are part of the tetrapeptide, Phe[7]-Trp[8]-Lys[9]-Thr[10], that comprises the critical β-II-turn of SRIF (**1**, Fig. 1). On the basis of these observations, numerous analogs have been prepared[14], and more recently, potent (K_i = 50 pM to 200 nM) and receptor subtype-selective compounds have been identified in combinatorial libraries.[15] The design and synthesis of peptidomimetics of SRIF utilizing a β-turn structure have been extensively investigated using heterocyclic scaffolds, such as, for example, 1,4,7-thiadiazonane-3,6-dione (**2**, Fig. 1),[16] imidazopyrazines (**3**, Fig. 1),[17] tetrahydro-β-carbolines,[18] 4,1-benzoxazepines (**4**, Fig. 1)[19], and several alternative displays of Trp and Lys side chains.

To find a new heterocyclic scaffold susceptible to be considered as an amide isostere, we focused our chemical strategy on the synthesis of 3-thio-1,2,4-triazoles. This template, having three points of diversity, can be found in several biologically active compounds.[20,21] It can be obtained through a short, robust, and high-yielding three-step synthesis, well described in solution,[22,23] as well as in solid-phase chemistry[24] and which is suitable for parallel synthesis.[25]

Keywords: Somatostatin agonist; Somatostatin subtypes 2 and 5 agonist; 3-Thio-triazole.
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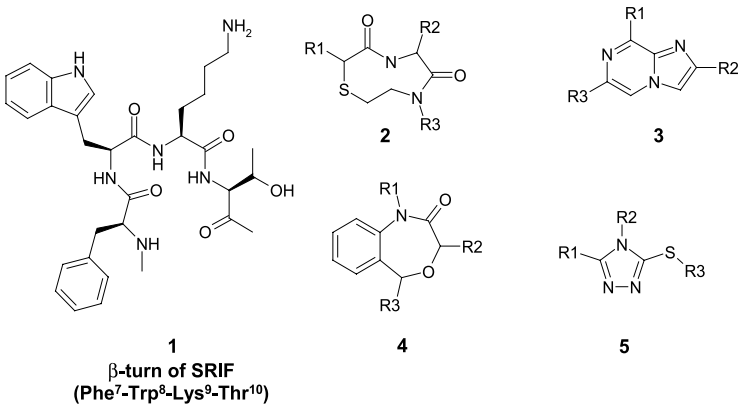


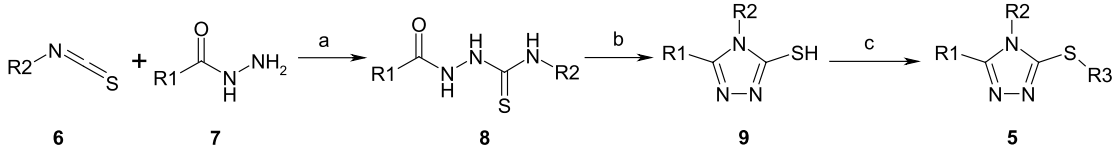
Figure 1. Structure of the β-turn (**1**) and β-turn mimetic scaffolds (**2**–**5**).

A novel series of 3-thio-1,2,4-triazoles (**5**, Fig. 1) has been prepared and its binding profile to somatostatin receptors has been evaluated.

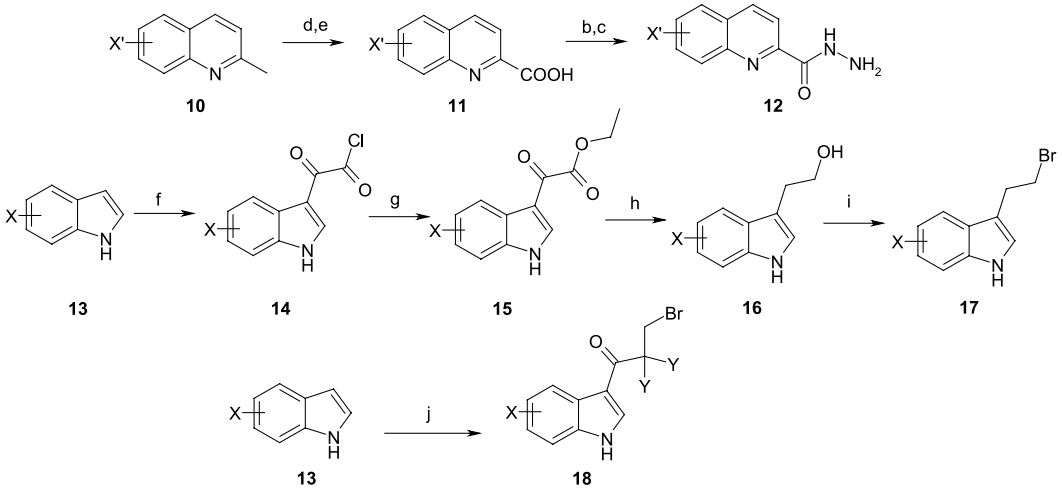
2. Chemistry

3-Thio-1,2,4-triazole derivatives **5** were prepared according to Scheme 1.[26] Condensation of isothiocyanates **6** with acyl hydrazides **7** afforded hydrazinecarbothioamides **8**. 3-Mercapto-1,2,4-triazoles **9** were obtained by base-catalyzed cyclization, allowing introduction of the third diversity element by subsequent S-alkylation. The highly basic, non-nucleophilic polymer-supported BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine on polystyrene)[27,28] was used as the deprotonating reagent, allowing a wide panel of alkylating agents (i.e., benzylbromides, α-bromoketones, but also alkylbromides and 3-(2-bromoethyl)indoles) to introduce the R3 group.

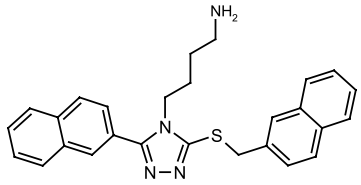
In addition, to increase the structural diversity of the 3-thio-1,2,4-triazoles obtained by this strategy, efforts were focused on the synthesis of original and diverse starting materials (Scheme 2). Thus, isothiocyanates **6** were prepared in-house from primary amines.[26] Acyl hydrazides



Scheme 1. Synthetic route for 5-sulfanyl-4*H*-1,2,4-triazoles **5**. Reagents and conditions: (a) **6** (1.1 equiv), DCM, 25 °C, 18 h; (b) NaOH 1 M (1.5 equiv), EtOH, dioxane, 85 °C, 4 h; (c) PS-BEMP (3 equiv), 25 °C, 10 min, R3-Br (1 equiv), 25 °C, 4 h.

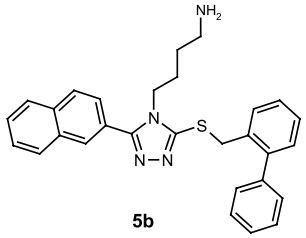


Scheme 2. Preparation of starting materials. Reagents and conditions: (a) CS_2 (10 equiv), polymer supported *N*-cyclohexylcarbodiimide (1.1 equiv), DCM, 25 °C, 3 h; (b) $TMSCHN_2$ 2 N in hexane (2 equiv), DCM/MeOH (1:1); (c) $H_2N–NH_2$, H_2O (10 equiv), MeOH, 25 °C, 60 h; (d) SeO_2 (6 equiv), dioxane, 80 °C, 3 h; (e) $NaClO_2$ (9 equiv), NaH_2PO_4 (8 equiv), H_2O, *t*-BuOH, 2-methylbut-2-ene, 25 °C, 4 h; (f) $(COCl)_2$ (1.3 equiv), Et_2O, 25 °C, 3 h; (g) EtOH, Et_3N (1.2 equiv), 78 °C, 2 h; (h) $LiAlH_4$ 1 M in THF (3 equiv), 66 °C, 2 h; (i) CBr_4 (1.2 equiv), PPh_3 (1.2 equiv), DCM, 25 °C, 2 h; (j) Me_2AlCl (1.5 equiv), $BrCH_2C(Y)_2C(O)Cl$ (1.5 equiv), DCM, 0 °C, 2 h.



5a

K_i sst$_2$ = 1100 +/- 77 nM
K_i sst$_5$ = 480 +/- 62 nM



5b

K_i sst$_2$ = 920 +/- 80 nM
K_i sst$_5$ = 85 +/- 11 nM

Figure 2. Structure of **5a** and **5b**.

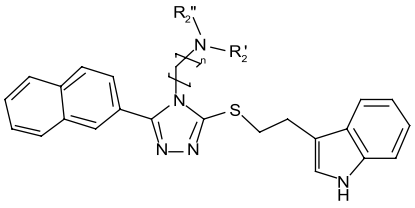
7 were obtained from corresponding carboxylic acids or esters,[29] which, in the case of substituted quinolines, were prepared by two-step oxidation of appropriate 2-methyl quinolines **10**.[30] Alkylating agents were prepared from commercially available alcohols and particularly, substituted 3-(2-bromoethyl)indoles **17** were obtained by acylation of appropriate substituted indoles **13**, reduction of corresponding α-cetoesters **15**, and alcohol bromination.[31] 3-Bromo-1-(indol-3-yl)propan-1-one **18** were obtained by Friedel–Crafts acylation of indoles **13**.[32]

3. Results and discussion

More than 700 individual 3-thio-1,2,4-triazole compounds have been rapidly prepared by parallel synthesis methods. UV purity of the compounds presented here, determined by LC/MS,[33] was in the range 80–99%. Competitive inhibition of [^{125}I-Tyr^{11}]SRIF-14 (Perkin-Elmer) binding to membranes isolated from CHO-K1 cells stably expressing each human SRIF receptor subtype was measured in 96-well plates, as previously described.[17,18] Compounds were first tested at 10 μM. Inhibition constants (K_i) were determined for compounds eliciting more than 70% inhibition at 10 μM.

First hits (**5a**, **5b**, Fig. 2) were found to bind to the sst$_2$ and sst$_5$ receptor subtypes with micromolar and submi-

Table 1. Inhibition constants (K_i) of selected 3-thio-1,2,4-triazoles on human sst$_2$/sst$_5$ receptors

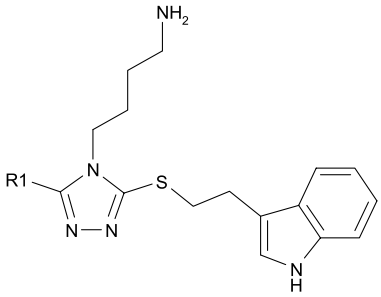


5c-f

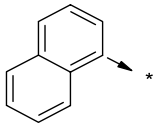
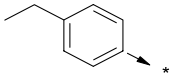
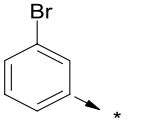
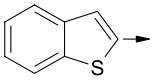
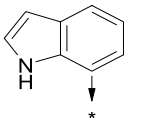
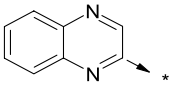
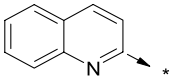
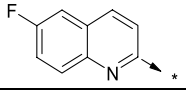
Compound	*n*	R2′	R2″	K_i (nM)[a]	
				sst$_2$	sst$_5$
5c	4	H	H	100 ± 21	27 ± 7.4
5d	3	H	H	640 ± 68	66 ± 11
5e	5	H	H	610 ± 170	70 ± 14
5f	4	Me	Me	2800 ± 470	1300 ± 150

[a] Data represent means ± SEM of 3–5 experiments.

Table 2. Inhibition constants (K_i) of selected 3-thio-1,2,4-triazoles on human sst$_2$/sst$_5$ receptors



5g-n

Compound	R1	K_i (nM)[a]	
		sst$_2$	sst$_5$
5g		150 ± 25	410 ± 73
5h		430 ± 72	26 ± 5.5
5i		250 ± 69	51 ± 5.6
5j		160 ± 20	58 ± 1.7
5k		220 ± 9.0	64 ± 9.9
5l		32 ± 3.7	56 ± 18
5m		12 ± 2.9	10 ± 3.5
5n		12 ± 0.9	7.8 ± 1.8

[a] Data represent means ± SEM of 3–5 experiments.
[*] Attachment point.

cromolar affinities, respectively. The introduction of an indole moiety at the R3 position of **5**, leading to **5c**, markedly increased the activity (Table 1). Shortening the basic chain of **5c** (compound **5d**), extending it by one carbon (**5e**,) as well as introducing rigidity in this part of the molecule by replacing the linear carbon chain by cyclohexyl, piperidine, or 1,3-dioxane rings (data not shown), caused a decrease in affinity (Table 1). Replacement of the primary amine by a dimethylamino group (**5f**) also resulted in a drop in binding potency.

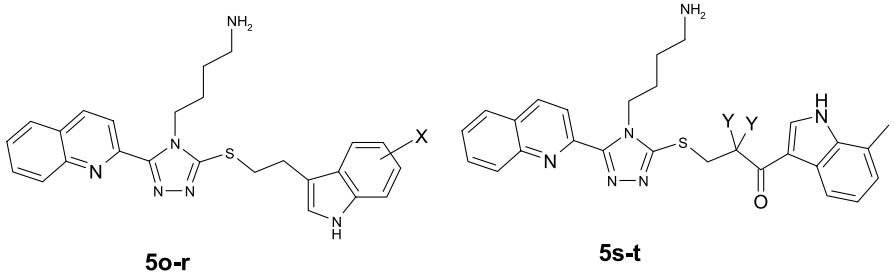
Interestingly, the position of the naphthyl ring attachment seemed to be critical for the sst_2/sst_5 affinity ratio. Binding affinities of 2-naphthyl compounds (**5c**) toward sst_2 and sst_5 receptor subtypes showed a 4-fold selectivity for sst_5, whereas 1-naphthyl compound **5g** (Table 2) was three times more potent toward sst_2 receptor subtype. Replacing the naphthyl moiety by a substituted phenyl ring, by a benzothiophene or an indole ring did not increase affinity (**5h–k**, Table 2). On the contrary, replacing naphthyl by quinoxaline or quinoline resulted in a significant increase in activity (**5l, 5m**, Table 2). Substitution on the quinoline moiety preserved this potency but did not increase it significantly (**5n**).

To confirm the key role of the indole feature in sst_2 and sst_5 binding affinity, 26 substituted ethyl indole bromides were prepared and introduced on the triazole scaffold at the R3 position. Among them, 7-methyl (**5o**, Table 3), 6-fluoro (**5p**), 7-chloro (**5q**), and 5-chloro (**5r**) indole increased sst_2 and sst_5 affinity up to six times regarding the unsubstituted indole ring (**5m**, Table 2). Introduction of a carbonyl group on the ethyl chain at the R3 position (**5s**, Table 3) retained potency and even increased it in the sst_5 receptor subtype case, while adding a dimethyl group (**5t**) caused a drop in activity, suggesting that steric hindrance has to be avoided on this part of the molecule and that the R3 side-chain displays an optimal flexibility pattern in compounds **5o** and **5s.**

Binding affinities of the most potent compounds **5o**, **5p**, **5q**, and **5s** toward subtypes 1, 3, and 4 receptors (Table 4) revealed a 10- to 2100-fold selectivity for sst_5 and 20- to 400-fold selectivity for sst_2. In a functional assay based on the inhibition of forskolin-induced intracellular accumulation of adenosine cyclic 3′,5′-monophosphate (cAMP) in CHO-K1 cells expressing human sst_2 or sst_5 receptors,[34] derivatives **5** displayed the characteristics of agonists. Compound **5s** was the most potent agonist, with EC_{50} values of 4.0 and 2.3 nM on cells expressing the human sst_2 and sst_5 receptors, respectively (Table 5).

In conclusion, a series of diverse 3-thio-1,2,4-triazoles was prepared, and binding affinities to human somatostatin receptor subtypes 2 and 5 were determined. Higher affinities were obtained with the butylamine group at the R2 position and indole at the R3 position, and we hypothesize that this template might be mimicking the Trp[8]-

Table 3. Inhibition constants (K_i) of selected 3-thio-1,2,4-triazoles on human sst_2/sst_5 receptors



5o-r **5s-t**

Compound	X	Y	K_i (nM)[a]	
			sst_2	sst_5
5o	7-Me		1.8 ± 0.6	2.1 ± 1.1
5p	6-F		2.7 ± 0.7	1.7 ± 1.1
5q	7-Cl		4.0 ± 0.7	8.4 ± 3.4
5r	5-Cl		5.6 ± 0.4	2.3 ± 1.0
5s		H	2.0 ± 0.6	0.38 ± 0.06
5t		Me	23 ± 7.8	9.3 ± 3.8

[a] Data represent means ± SEM of 3–5 experiments.

Table 4. Binding affinities (K_i, nM) of selected 3-thio-1,2,4-triazoles to human sst_{1-5} receptors

Compound	K_i (nM)[a]				
	sst_1	sst_2	sst_3	sst_4	sst_5
5o	130 ± 16	1.8 ± 0.6	41 ± 17	140 ± 53	2.1 ± 1.1
5p	58 ± 10	2.7 ± 0.7	100 ± 26	74 ± 29	1.7 ± 1.1
5q	180 ± 15	4.0 ± 0.7	97 ± 19	130 ± 38	8.4 ± 3.4
5s	530 ± 100	2.0 ± 0.6	170 ± 57	800 ± 240	0.38 ± 0.06
SRIF-14	0.44 ± 0.06	0.083 ± 0.019	0.26 ± 0.05	0.71 ± 0.07	0.15 ± 0.03

[a] Data represent means ± SEM of 3–5 experiments.

Table 5. EC_{50} values (nM) for inhibition of forskolin-induced cAMP accumulation, by selected 3-thio-1,2,4-triazoles

Compound	EC_{50} (nM)[a]	
	sst_2	sst_5
5o	22 ± 14	34 ± 9.0
5p	14 ± 3.7	44 ± 9.4
5r	6.3 ± 2.1	52 ± 13
5s	4.0 ± 1.5	2.3 ± 0.15
SRIF-14	0.10 ± 0.028	0.19 ± 0.065

[a] Results are expressed as means ± SEM of three experiments performed in triplicate (compound concentration ranging from 0.1 nM to 10 μM).

Lys[9] residue of the β-turn. These novel non-peptidic sst_2 and sst_5 preferential ligands were shown to be agonists.

Studies are in progress with BN82945 (**5o**) and BN83010 (**5s**), to evaluate the therapeutic potential of this new class of non-peptidic somatostatin receptor ligands.

Acknowledgment

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33. Mass spectra were acquired on a single quadrupole electrospray mass spectrometer (Micromass, Platform model) and HPLC retention times were acquired on an HPLC system (HP 1100) equipped with a photodiode array UV detector.
34. CHO-K1 confluent cells expressing the human sst_2 or sst_5 receptor were washed twice with RPMI 1640 containing 0.2% BSA at 37 °C and incubated for 5 min with 0.5 mM isobutylmethylxanthine at 37 °C. Then, the cells were incubated for 5 min at 37 °C with 1 μM forskolin and increasing concentrations of sst_2 or sst_5 agonist. Intracellular cyclic AMP were determined using a fluorescent competitive immunoassay (Catch Point, Molecular Devices).