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Synthesis of benzopentathiepin analogs and their evaluation as inhibitors of the phosphatase STEP

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Abstract

Striatal-enriched protein tyrosine phosphatase (STEP) is a brain specific protein tyrosine phosphatase that has been implicated in many neurodegenerative diseases, such as Alzheimer's disease. We recently reported the benzopentathiepin TC-2153 as a potent inhibitor of STEP in vitro, cells and animals. Herein, we report the synthesis and evaluation of TC-2153 analogs in order to define what structural features are important for inhibition and to identify positions tolerant of substitution for further study. The trifluoromethyl substitution is beneficial for inhibitor potency, and the amine is tolerant of acylation, and thus provides a convenient handle for introducing additional functionality such as reporter groups.

Keywords

Phosphatase; Inhibitor; Alzheimer's Disease; STEP; Redox

Striatal-enriched protein tyrosine phosphatase (STEP) is a neuron-specific phosphatase that regulates key signaling molecules involved in synaptic plasticity, which is critical to maintaining proper cognitive function.¹ Enhanced levels of STEP activity have been observed in several neuropsychiatric diseases and neurodegenerative disorders and result in the dephosphorylation and inactivation of several neuronal signaling molecules, including extracellular signal-regulated kinases 1 and 2 (ERK1/2), proline-rich tyrosine kinase 2 (Pyk2), mitogen-activated protein kinase p38, and the GluN2B subunit of the *N*-methyl-D-aspartate receptor (NMDAR).²

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Supplementary Material

Experimental details including synthetic procedures and analytical characterization of compounds and enzyme assay curves associated with this article can be found, in the online version, at [placeholder].

An increase in STEP likely contributes to the cognitive deficits in Alzheimer's disease (AD). Indeed, AD mice lacking STEP have restored levels of glutamate receptors on synaptosomal membranes and improved cognitive function, results that suggest STEP as a novel therapeutic target for AD.³ We recently identified that 8-(trifluoromethyl)-1,2,3,4,5-benzopentathiepin-6-amine hydrochloride (TC-2153, Figure 1) is a novel inhibitor of STEP.^{4,5,6} The cyclic polysulfide of the benzopentathiepin pharmacophore effects potent inhibition by a redox reversible covalent bond with the catalytic cysteine in the phosphatase active site. This redox reversible mode of inhibition is reminiscent of well-known cellular mechanisms for redox regulation of protein tyrosine phosphatases.^{7,8} Importantly, TC-2153 enhanced the phosphorylation levels of the known STEP substrates GluN2B, Pyk2 and ERK1/2 in rat cortical neurons. Moreover the inhibitor reduced cognitive deficits in Alzheimer's disease (AD) mice as measured by the established novel object recognition and Morris water maze tests.⁴

To date, derivatives of TC-2153 have not been evaluated for STEP inhibition. Given the promising biological properties of TC-2153, we were motivated to define the essential characteristics of the molecule necessary for enzyme inhibition (Figure 1). First, we wished to explore the electronic effects of the inhibitor core to assess the importance of electronics on the redox-reversible inhibition of STEP. Secondly, we wanted to discover sites on the molecule tolerant of substitution to enable modulation of physicochemical properties such as solubility, for the introduction of reporter groups, or for the introduction of functionality to facilitate pull down assays and proteomic analysis.

To prepare the analogs, we first investigated the synthesis sequence for the preparation of TC-2153 as first reported by Kulikov and coworkers (Scheme 1).⁹ Elevated temperatures were required for the S_NAr reactions with sodium dimethyldithiocarbamate when the less activated substrates **2** and **3** containing a simple methyl or proton substituent at the R position were used, with lower temperatures resulting only in a single nucleophilic substitution of the chloride. Consistent with a previous report,¹⁰ treatment of the intermediate nitrodithiolones **4–6** with NaSH yielded little to none of the desired products **7–9** except in the case of the trifluoromethyl substituted derivative **4**, and even then the desired pentathiepin **7** was obtained in only 41% yield with numerous byproducts formed, including nitrotrithiole **10** (37%) and dimerization product **13** (5%).

Due to the difficulty in obtaining the desired pentathiepin products for analogs lacking electron-deficient substituents, an alternative pathway was explored (Scheme 2). By first reducing the nitro group in dithiolones **5** and **6**, then treating the free anilines **16** and **17** with sodium hydrosulfide in DMSO, we were able to synthesize the desired intermediate benzopentathiepins **8** and **9**. Salt formation with HCl yielded the final inhibitor analogs **18** and **19**. Further optimization of this sequence has been applied to the preparation of tens of grams of **TC-2153**.⁴

Analog **22**, which does not contain an amine, was synthesized using an analogous procedure (Scheme 3). 2-Chloro-1-nitro-4-(trifluoromethyl)benzene, **20**, was treated with sodium dimethyldithiocarbamate at elevated temperature to yield the dithialone **21**. Treatment with NaSH in DMSO yielded the analog **22**.

The amine provides a convenient handle for potentially adding solubilizing functionality or chemical labels, which has the potential of being introduced either by alkylation to maintain the basicity of the amine or by acylation. Acylation of intermediate **7** according to literature precedent with either trifluoroacetic anhydride or acetyl chloride yielded analogs **23** and **24**, respectively (Scheme 4).¹¹ Reductive amination of **7** with acetaldehyde or benzaldehyde provided *N*-ethyl and *N*-benzyl analogs **25** and **26** (Scheme 5).

The inhibitory activity of the analogs was determined against STEP both in the presence and the absence of 1 mM reduced glutathione (GSH) (Table 1).¹² GSH is a ubiquitous reducing agent in cells,¹³ and may interact with the inhibitors because of their polysulfide character. We therefore thought it was prudent to investigate the inhibitor activity under both of these conditions. In the absence of GSH the most potent inhibitor in the series was the simple 7-(trifluoromethyl)-benzopentathiepin **22** (10 ± 1 nM). However, with the absence of the amine substituent, the solubility and thus future utility of **22** is limited. With a benchmark IC_{50} of 24.6 ± 0.8 nM, **TC-2153** remains one of the most potent analogs in this series, along with the other aniline hydrochloride salts **18** (25 ± 7 nM) and **19** (32 ± 3 nM). Trifluoroacetamide derivative **23** also showed good potency (24 ± 1 nM), while the less electron deficient acetamide derivative **24** showed a two-fold decrease in inhibitory activity (49 ± 2 nM). Alkylated anilines **25** and **26**, showed a larger drop in potency. Interestingly, the byproducts generated in the synthesis of TC-2153 (**10** and **27**) also showed activity against STEP though with decreased inhibitory activity.

When GSH was added to the assay mixture, the same trends generally held, with the acylated derivatives performing comparably to the free anilines and the alkylated derivatives having slightly decreased potency. In all cases, there was a decrease in potency by 2–3 orders of magnitude when the GSH reducing agent was present in the assay.

Because the inhibition of STEP by TC-2153 has been shown to be irreversible in the absence of GSH,⁴ we next determined the second order rate of inactivation (k_{inact}/K_i) for all of the inhibitors under these conditions using the progress-curve method (Table 2).^{14,15} These results demonstrate that the methyl (**18**) and unsubstituted (**19**) derivatives are about three times less potent than trifluoromethyl substituted **TC-2153**. Additionally, alkylated derivatives **25** and **26** were also less potent, consistent with the IC_{50} data (Table 1). The K_i values of **23** and **24** indicated that acylation modestly attenuates binding. However, these inhibitors still showed larger k_{inact}/K_i values than TC-2153 because of their much higher k_{inact} values relative to the non-acylated parent structure.

In conclusion, we have prepared and characterized the STEP inhibitory activities of a series of analogs of TC-2153, which has shown promising results in mouse models for AD.⁴ This study establishes that the electron deficient trifluoromethyl group contributes to potency while the amino group does not, though it is important for aqueous solubility. Other modifications upon the structure are also tolerated. Most importantly, acylation of the aniline is accommodated and could provide a site for introducing reporter groups or functionality for pull down and proteomic applications. Finally, the optimized route for the synthesis of these types of analogs enables their rapid preparation through acylation of a common intermediate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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volume. Once the inhibitor stocks were added, the assay plate was allowed to incubate for 10 min at 27 °C with shaking. This was followed by addition of STEP (stock: 1.0 μ M, 50 nM in assay) and another 10 min incubation at 27 °C prior to addition of pNPP substrate.

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15. General protocol for determination of k_{inact}/K_i values: Assay wells contained a mixture of the inhibitor (800, 400, 200, 100, 50, 0 nM) and 745 μ M of pNPP ($K_M = 745 \mu\text{M}$) in 1 $\times$ buffer. Aliquots of STEP (final concentration = 10 nM) were added to each well to initiate the assay. Hydrolysis of pNPP was monitored for 30 min at 405 nm. To determine the inhibition parameters, time points for which the control ($[I] = 0$) was linear were used. A k_{obs} was calculated for each inhibitor concentration via a nonlinear regression of the data according to the equation $P = (v_i/k_{\text{obs}})(1 - e^{-(k_{\text{obs}}t)})$ (where P = product formation, v_i = initial rate, t = time (s)) using Prism v5.01 (GraphPad). Because k_{obs} varied hyperbolically with $[I]$, nonlinear regression was performed to determine the second-order rate constant, k_{inact}/K_i , using the equation $k_{\text{obs}} = k_{\text{inact}}[I]/([I] + K_i(1 + [S]/K_M))$.

Figure 1.
Proposed SAR study of TC-2153.

Scheme 1. Original synthesis of benzopentathiepin compounds^a^aReagents and conditions: (a) sodium dimethyldithiocarbamate, DMSO, rt or 150 °C; (b) NaSH, DMSO, rt.

Scheme 2. Synthesis of less electron deficient analogs **18** and **19**^a^aReagents and conditions: (a) NH_4Cl , Zn^0 , EtOH, H_2O , rt; (b) SnCl_2 , HCl, H_2O , EtOH, 40 °C; (c) NaSH, DMSO, rt; (d) HCl, ether, rt.

Scheme 3. Synthesis of TC-2153 analog **22**^a^aReagents and conditions: (a) sodium dimethyldithiocarbamate, DMSO, 200 °C; (b) NaSH, DMSO, rt.

Scheme 4. Synthesis of TC-2153 analogs **23** and **24**^a^aReagents and conditions: (a) AcCl or TFAA, CH₂Cl₂, °C to rt.

Scheme 5. Synthesis of TC-2153 analogs **25** and **26**^a^aReagents and conditions: (a) RCHO , AcOH , EtOH , 0°C ; (b) NaCNBH_3 , 0°C

Table 1

Inhibition of STEP *in vitro* in the presence and absence of GSH¹⁰

Entry	Compound	R ¹	R ²	IC ₅₀ (nM) ^{a,b}	IC ₅₀ ' (μM) ^{a,c}
1	TC-2153	CF ₃	NH ₃ Cl	24.6 ± 0.8 ^d	8.8 ± 0.4 ^d
2	22	CF ₃	H	10 ± 1	17 ± 2
3	18	CH ₃	NH ₃ Cl	25 ± 7	17 ± 1
4	19	H	NH ₃ Cl	32 ± 3	33 ± 4
5	23	CF ₃	NHCOCF ₃	24 ± 1	15 ± 2
6	24	CF ₃	NHAc	49 ± 2	24 ± 1
7	25	CF ₃	NHEt	59 ± 9	>50
8	26	CF ₃	NHBn	78 ± 3	27 ± 5
9	27	--	-	33 ± 1	20 ± 1
10	10	--	-	145 ± 6	34 ± 4

^a Assays were performed in duplicate (mean ± S.D.).^b Assays contained no GSH.^c Assays contained 1 mM GSH.^d Assays were performed in quadruplicate (mean ± S.D.); see reference 6.

Table 2Second order rates of inactivation of TC-2153 analogs¹³

Entry	Compound	$K_{\text{inact}}/K_i(\text{M}^{-1}\text{S}^{-1})$	K_i (nM)	K_{inact} (S^{-1})
1	TC-2153	153,000 $\pm$ 15,000	115 $\pm$ 10	0.0176 $\pm$ 0.0007
2	22	135,000 $\pm$ 18,000	109 $\pm$ 14	0.0147 $\pm$ 0.0007
3	18	41,000 $\pm$ 5,300	116 $\pm$ 14	0.0048 $\pm$ 0.0002
4	19	52,200 $\pm$ 8,000	183 $\pm$ 25	0.0095 $\pm$ 0.0006
5	23	161,000 $\pm$ 26,000	235 $\pm$ 33	0.0378 $\pm$ 0.0027
6	24	193,000 $\pm$ 49,000	123 $\pm$ 29	0.0236 $\pm$ 0.0022
7	25	53,500 $\pm$ 8,800	230 $\pm$ 34	0.0123 $\pm$ 0.0009
8	26	33,200 $\pm$ 2,400	175 $\pm$ 11	0.0058 $\pm$ 0.0002
9	27	138,000 $\pm$ 23,000	124 $\pm$ 19	0.0171 $\pm$ 0.0011
10	10	98,000 $\pm$ 20,000	210 $\pm$ 38	0.0205 $\pm$ 0.0018

^a Assays were performed in quadruplicate (mean $\pm$ S.D.) with no GSH added.