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Identification of novel protein tyrosine phosphatase sigma inhibitors promoting neurite extension

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ABSTRACT

Protein tyrosine phosphatase sigma (PTPσ) is a potential target for the therapeutic treatment of neurological deficits associated with impaired neuronal recovery, as this protein is the receptor for chondroitin sulfate proteoglycan (CSPG), which is known to inhibit neuronal regeneration. Through a high-throughput screening approach started from 6400 representative compounds in the Korea Chemical Bank chemical library, we identified 11 novel PTPσ inhibitors that can be classified as flavonoid derivatives or analogs, with IC_{50} values ranging from 0.5 to 17.5 μM. Biochemical assays and structure-based active site-docking simulation indicate that our inhibitors are accommodated at the catalytic active site of PTPσ as surrogates for the phosphotyrosine group. Treatments of these compounds on PC-12 neuronal cells led to the recovery of neurite extension attenuated by CSPG treatment, demonstrating their potential as antineurodegenerative agents.

Protein tyrosine phosphatase sigma (PTPσ) is a member of the receptor type IIA protein tyrosine phosphatase (PTP) subfamily, together with leukocyte common antigen receptor (commonly called as LAR) and protein tyrosine phosphatase delta. Like other subfamily members, PTPσ is highly expressed in mammalian neuronal tissues and mediates central nervous system development and regeneration of injured nerves.[1–3] PTPσ, comprising 1948 amino acids, consists of three parts: extracellular, transmembrane and intracellular regions. The extracellular region of PTPσ, containing three immunoglobulin-like modules and repetitive fibronectin domains, recognizes ligands including heparan sulfate proteoglycan (HSPG)[4] and chondroitin sulfate proteoglycan (CSPG).[5] Sulfate chain-dependent binding of HSPG and CSPG to the first immunoglobulin-like domain of PTPσ leads in the opposite direction: PTPσ oligomerization and neurite growth are promoted by HSPG binding, whereas these processes are inhibited by CSPG binding.[4,6] CSPG is known to be highly accumulated at the sites of neural injury and to have inhibitory effects on recovery from neural injury, which is implicated in a variety of neurological diseases.[7] Previous reports indicate the involvement of PTPσ in the CSPG-mediated inhibition of neural regeneration,[5,8] which was further demonstrated in the study with the PTPσ-deficient mouse model.[9] The intracellular region of PTPσ contains two tandem phosphatase domains D1 and D2. Even though both of these phosphatase domains contain a PTP signature motif CX_5R (P-loop), only D1, but not D2, is enzymatically active and capable of dephosphorylating its substrates in vitro and in cells.[10] The catalytic activity of the D1 of PTPσ also plays a key role in controlling neural regeneration, as PTPσ dephosphorylates Trk receptor kinases, and its overexpression interferes with neurite outgrowth in primary sensory neurons.[11] Furthermore, a recent report by Lang et al. exhibited that treatment of a novel PTPσ-specific membrane-permeable peptide facilitates functional recovery from spinal cord injury in rats.[12] Collectively, these results suggest that PTPσ could be an effective target for the development of a drug candidate for the treatment of neurological diseases associated with impaired

Abbreviations: PTPσ, protein tyrosine phosphatase sigma; CSPG, chondroitin sulfate proteoglycan; PTP, protein tyrosine phosphatase; HSPG, heparan sulfate proteoglycan; IC_{50}, half-maximal inhibitory concentration; HTS, high-throughput screening; KCB, Korea Chemical Bank; *p*NPP, *para*-nitrophenylphosphate; NGF, nerve growth factor.

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neuronal regeneration. To date, however, only limited numbers of inhibitory small molecules for PTPσ have been identified,[13–15] and their effects on neuronal cells have yet not been proven at all. Based on virtual screening with docking simulations, we previously reported seven compounds that inhibit the PTPσ activities with half-maximal inhibitory concentration (IC_{50}) values of 5.1–10.7 μM.[15] In this study, we attempted to discover additional PTPσ inhibitors based on high-throughput screening (HTS) using the Korea Chemical Bank (KCB) chemical library.

The search for PTPσ inhibitors was started from the measurement of the phosphotyrosine hydrolysis activity of the protein. To this end, we employed the recombinant tandem PTP domains of human PTPσ (residues 1367–1948) and *para*-Nitrophenylphosphate (*p*NPP), a widely used chromogenic phosphatase substrate that mimics phosphotyrosine. *p*NPP is hydrolyzed to *p*-nitrophenol and inorganic phosphate by PTP proteins, and it is possible to determine the amount of produced *p*-nitrophenol by monitoring the absorbance at 415 nm. We tested the dephosphorylating activity of the catalytic domain of PTPσ using *p*NPP substrate, and calculated the kinetic constants k_{cat}, K_M, and k_{cat}/K_M of PTPσ; these were found to have values of 0.115 s^{-1}, 5.33 mM, and 21.6 s^{-1} M^{-1}, respectively.

The first-round PTPσ inhibitor screening was performed with a KCB chemical library comprising 6400 representative compounds that were selected from 200,000 compounds (Fig. 1; Stage 1).

In this round, 147 compounds were selected as initial hits (hit ratio of 2.3%); these compounds showed higher than 30% inhibition of PTPσ enzymatic activity at a concentration of 20 μM. Among these compounds, we found that 34 of 147 (especially 18 of 57 exhibiting >50% inhibition for PTPσ) could be classified as 1,2-benzenediol derivatives. Our initial hits contained a few 1,2,3-benzenetriol derivatives showing distinguishing PTPσ inhibitory activity. Moreover, at least two cases have been reported that 1,2,3-benzenetriol derivatives function as PTP inhibitors.[16,17] We therefore selected 33 numbers of 1,2,3-benzenetriol derivatives in the full KCB library, and performed second screening with these focused compounds (Fig. 1; Stage 2). Four flavonoid derivatives and five analogs showing at least 80% inhibition of PTPσ were discovered; IC_{50} values of these compounds were determined, finding them to be in the range of 0.5–17.5 μM (Table 1).

A specific chemical structure was determined based on these data (Fig. 1; Stage 3); the structure contains two aromatic rings with five substitutions denoted by X^1–X^5. This structure was applied to a further search for compounds containing the structure in the library. Additional 22 compounds were selected, and the ensuing third in vitro screening led to the discovery of two more inhibitory chemicals that interfered with the PTPσ activity at a level of more than 80% and showed IC_{50} values less than 7 μM (Table 2).

Collectively, we determined 11 actual PTPσ inhibitors showing IC_{50} values less than 17.5 μM.

Based on the screening results, we carried out a structure–activity relationship analysis of the novel PTPσ inhibitors we found. In the entire set of inhibitors, positions X^1, X^2, and X^3 are occupied by

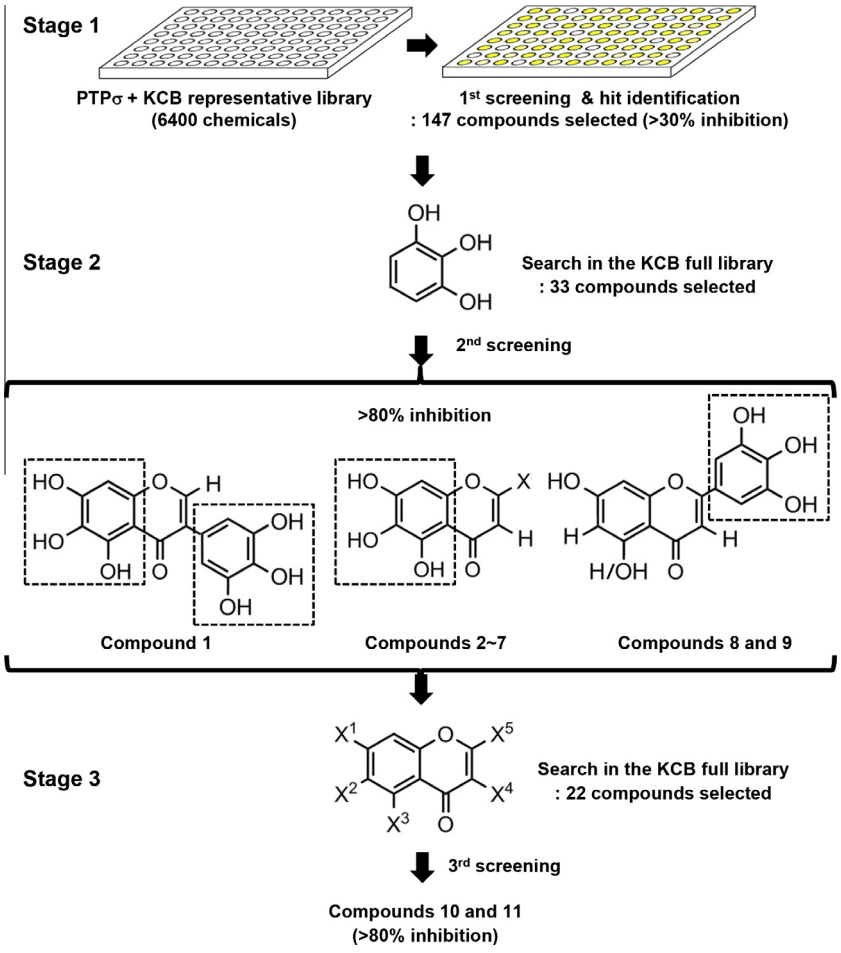

Figure 1. Overall flow of PTPσ inhibitors screening. Stage 1: A representative 6400 KCB library was screened for PTPσ inhibition, yielding 147 compounds as first hits. Stage 2: 33 numbers of 1,2,3-benzenetriol derivatives were selected and screened, yielding nine compounds with IC_{50} values in the range of 0.5–17.5 μM. Stage 3: 22 compounds additionally selected based on the specific chemical structure were screened, yielding two more compounds with IC_{50} values of 4.3 μM and 6.6 μM.