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α-Bromoacetophenone Derivatives as Neutral Protein Tyrosine Phosphatase Inhibitors: Structure–Activity Relationship

Gulnur Arabaci, Tian Yi, Hua Fu, Mary E. Porter, Kirk D. Beebe and Dehua Pei*

Department of Chemistry and Ohio State Biochemistry Program, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA

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Abstract—A series of α-haloacetophenone derivatives was tested for inhibition of protein tyrosine phosphatases SHP-1 and PTP1B. The results show that the bromides are much more potent than the corresponding chlorides, whereas the phenyl ring is remarkably tolerant to modifications. Derivatization of the phenyl ring with a tripeptide Gly-Glu-Glu resulted in a potent, selective inhibitor against PTP1B.

Protein tyrosine phosphatases (PTPs) are a diverse family of enzymes that catalyze the hydrolysis of phosphotyrosine (pY) residues in proteins. Together with protein tyrosine kinases, they control the level of intracellular tyrosine phosphorylation and regulate a variety of cellular functions.[1] Malfunction of PTPs can lead to human diseases and conditions.[2] Therefore, inhibitors against PTPs provide potential therapeutic agents as well as useful probes for studying their physiological functions. For example, specific inhibitors of PTP1B are expected to provide effective treatment for type II diabetes.[3]

Virtually all of the reported PTP inhibitors contain a negatively charged, nonhydrolyzable pY mimetic such as phosphonates,[4] malonates,[5] aryl carboxylates,[6] or cinnamates[7] as the core structure. The poor membrane permeability of these inhibitors may compromise their potential for further development. We reported previously[8] that several α-bromoacetophenone derivatives act as fairly potent PTP inhibitors, by covalently alkylating the conserved catalytic cysteine in the PTP active site (Fig. 1). Because they are neutral, these agents readily diffuse into human B cells and inhibit the intracellular PTPs. In an attempt to improve both the potency and selectivity of these molecules as PTP inhibitors, we undertook an SAR study of the haloacetophenone core and report herein the initial findings of this study.

Figure 2 shows the compound series used in this study. Compounds **1**, **6**, and **13** were synthesized as previously described.[8] Compounds **9**, **15**, and **16** were prepared from the corresponding methyl ketones by treatment with Br_2 in acetic acid. Compound **14** was obtained by treating 2-acetylpyridine with pyridine hydrobromide perbromide in acetic acid.[9] Compound **20** was prepared from 2-biphenylcarboxylic acid (**22**), which was first protected as the methyl ester and then acetylated by Friedel–Crafts reaction to give ketone **23** (Scheme 1). Hydrolysis of the methyl ester by NaOH followed by bromination with Br_2 resulted in acid **20**, which was further converted into its *N*-hydroxysuccinimide ester **24** using dicyclohexylcarbodiimide (DCC). Treatment of a support-bound tripeptide Gly-Glu-Glu (on Wang resin) with **24** followed by cleavage with trifluoroacetic acid afforded compound **21**. Peptidyl derivatives **18** and **19** were similarly prepared from acid **6**. The rest of compounds were obtained from commercial suppliers.

The SAR study was performed with the catalytic domain of phosphatase SHP-1, SHP-1(ΔSH2).[10] All of the compounds exhibited time-dependent inactivation of SHP-1, consistent with the mechanism in Figure 1. Their potency is described by an equilibrium constant K_I, which represents the dissociation constant of the noncovalent enzyme–inhibitor complex (E∗I), and the first-order rate constant (k_{inact}) for conversion of the E∗I

*Corresponding author. Tel.: +1-614-688-4068; fax: +1-614-292-1532; e-mail: pei@chemistry.ohio-state.edu

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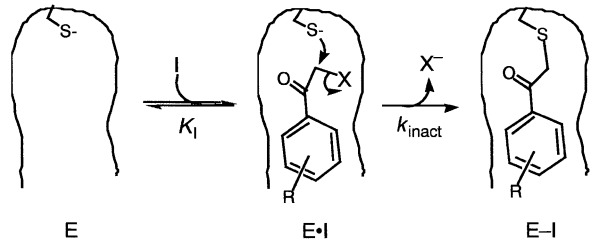


Figure 1. Mechanism of inhibition of PTPs by α-haloacetophenone derivatives. X = Br or Cl.

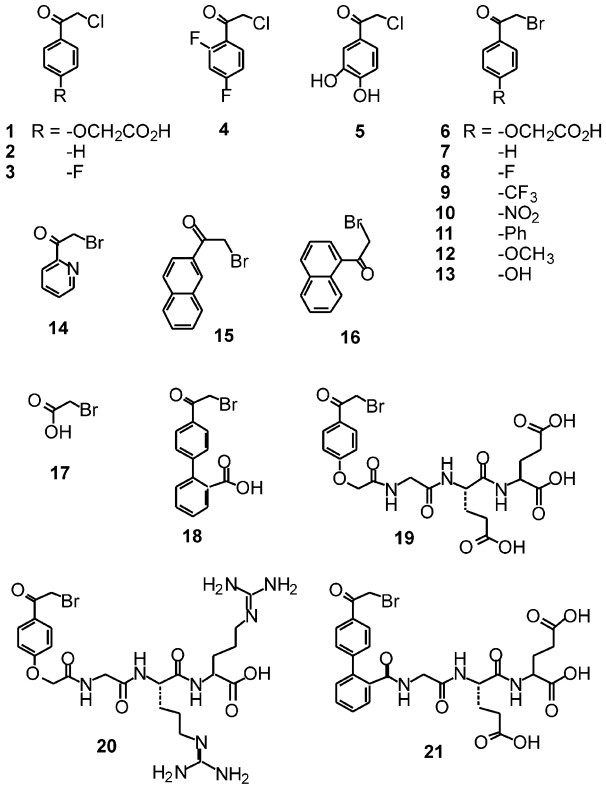
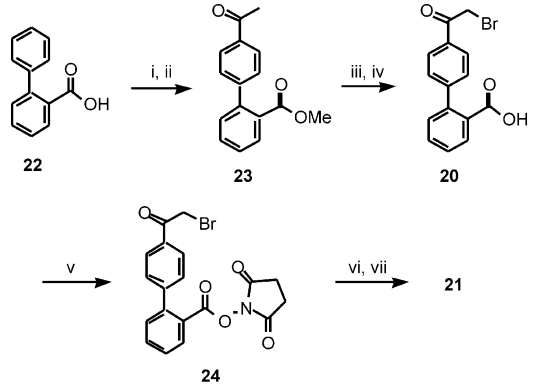


Figure 2. Structures of PTP inhibitors used in this work.



Scheme 1. (i) $MeOH/H_2SO_4$; (ii) CH_3COCl, $AlCl_3$, 84% (2 steps); (iii) $NaOH/H_2O$; (iv) $Br_2/CHCl_3$, 75% (2 steps); (v) NHS, DCC, 91%; (vi) H_2N-GEE-resin; (vii) 9:1 TFA/H_2O.

complex into the covalent adduct, E–I. The K_I and k_{inact} values were determined using *p*-nitrophenyl phosphate as substrate as previously described.[8]

We noted previously[8] that α-bromoacetophenone **6** is 13-fold more potent than the corresponding chloride **1** against the catalytic domain of SHP-1 (Table 1). To determine whether this is a general trend, we tested four other α-chloroacetophenones (**2**–**5**) containing various substituents on the phenyl ring and the corresponding bromides (**6**–**8**). The overall potency of the bromides (k_{inact}/K_I) are consistently 13- to 20-fold more potent than the corresponding chlorides. This difference is primarily due to the higher affinity of the bromides to the PTP active site (lower K_I values), whereas the k_{inact} values are remarkably similar for all of the compounds. We speculate that the larger α-bromoacetyl group is perhaps a closer match (in size) to the phosphate group.

We next examined the effect of phenyl substitution on inhibitor potency. From the comparison between compounds **6**–**16** and α-bromoacetic acid (**17**), it is clear that an aromatic ring is essential for high-affinity binding to the PTP active site (Table 2). However, the exact nature of the aromatic ring is less critical. Attachment of either electron-withdrawing (**8**–**10**) or electron-donating groups (**6** and **11**–**13**) to the *para* position causes only small changes (<6-fold) in the overall potency (k_{inact}/K_I). Even the addition of a fused ring (**15** and **16**) or substitution of a ring nitrogen (**14**) has little effect. The only exception is substitution at the *ortho* position, which substantially decreases the inhibitor potency as a

Table 1. Comparison of α-bromo and chloro derivatives

Compd	K_I, (μM[a])	k_{inact}, (min^{-1})[a]	k_{inact}/K_I, $(M^{-1}min^{-1})$
1	2500 ± 600[b]	1.8 ± 0.4[b]	710[b]
2	480 ± 25	0.21 ± 0.04	430
3	380 ± 48	0.22 ± 0.07	580
4	17,500 ± 2300	2.1 ± 0.1	120
5	1400 ± 990	0.59 ± 0.24	420
6	193 ± 38[b]	1.8 ± 0.3[b]	9300[b]
7	81 ± 2	0.58 ± 0.02	7100
8	56 ± 1	0.51 ± 0.03	9100

[a]Values are means ± SD from three independent sets of experiments.
[b]Data from ref 8.

Table 2. Effect of substitution on the phenyl ring

Compd	K_I, (μM)[a]	k_{inact}, (min^{-1})[a]	k_{inact}/K_I, $(M^{-1}min^{-1})$
6	193 ± 38[b]	1.8 ± 0.3[b]	9300[b]
7	81 ± 2	0.58 ± 0.02	7100
8	56 ± 1	0.51 ± 0.03	9100
9	14 ± 1	0.37 ± 0.01	26,000
10	195 ± 150	2.9 ± 1.5	14,800
11	14 ± 8	0.59 ± 0.16	41,000
12	128 ± 10[b]	2.4 ± 0.2[b]	18,000[b]
13	43 ± 10[b]	0.40 ± 0.10[b]	9300[b]
14	173 ± 19	1.3 ± 0.2	7500
15	25 ± 9	0.48 ± 0.04	20,000
16	92 ± 4	0.86 ± 0.03	9400
17	77,000 ± 14,000[b]	1.4 ± 0.2[b]	18[b]

[a]Values are means ± SD from three independent sets of experiments.
[b]Data from ref 8.

result of reduced binding affinity (compare compounds **3** and **4** in Table 1). The reason behind the reduced potency is not entirely clear but does not appear to be steric in origin, since a fluorine atom is not substantially larger than a hydrogen atom.

The data in Table 2 suggest that it may be difficult to further improve the inhibitor potency by modifying the structure of the phenyl ring. We thus decided to assess the possibility of appending additional binding domains to the *para* (or *meta*) position of the phenyl ring. Our rationale is that the additional binding domain would interact with the substrate-binding surfaces near the active site. These interactions should increase both the binding affinity and selectivity of the inhibitor. As a proof of principle, we attached a tripeptide, Gly-Glu-Glu, to the carboxyl group of inhibitor **6** to produce the peptidyl bromoacetophenone **19** (Fig. 2). This tripeptide, when attached to the *para* position of cinnamic acid, results in a highly potent inhibitor against PTP1B ($K_I = 79$ nM).[7a] As expected, compound **19** is a highly potent inactivator of PTP1B, with a K_I of 2.8 μM, a k_{inact} of 1.2 min^{-1}, and an overall inhibition constant (k_{inact}/K_I) of 4.3×10^5 M^{-1} min^{-1} (Table 3). The corresponding values of inhibitor **13** against PTP1B are 42 μM, 0.57 min^{-1}, and 1.4×10^4 M^{-1} min^{-1}, respectively.[8] Compound **19** also shows drastically improved potency (25-fold) against SHP-1, when compared to the parent molecule (**6**). In contrast, attachment of a positively charged tripeptide Gly-Arg-Arg to inhibitor **6** decreased the overall potency by 1.5-fold (compared **6** and **20** in Table 3). It is known that PTP1B and SHP-1 prefer substrates that contain acidic residues N-terminal to the pY and disfavor pY peptide containing positively charged residues at these positions.[11]

To determine whether it is possible to generate inhibitors with selectivity toward a particular PTP, we attached Gly-Glu-Glu to acid **18** via a rigid biphenyl linker and tested the resulting compound (**21** in Fig. 2) against both PTP1B and SHP-1. While inhibitor **21** has a K_I value of 9.9 μM and a k_{inact}/K_I value of 3.6×10^4 M^{-1} min^{-1} against PTP1B, it does not show saturation at 200 μM and has a k_{inact}/K_I value of 666 M^{-1} min^{-1} against SHP-1 (Fig. 3 and Table 3). This represents a 54-fold selectivity toward PTP1B.

In summary, we have shown that α-bromoacetophenone provides an effective, neutral pY mimetic inhibitor of

Table 3. Effect of derivatization with peptides

Compd	K_I, (μM)[a]	k_{inact}, (min^{-1})[a]	k_{inact}/K_I, ($M^{-1}min^{-1}$)
6	193±38[b]	1.8±0.3	9300[b]
18	220±64	1.0±0.2	4550
19	29±4	6.6±0.4	229,000
19 (PTP1B)	2.8±0.5	1.2±0.1	429,000
20	760±150	4.8±0.6	6300
21	>200	>0.13	666
21(PTP1B)	9.9±1.0	0.36±0.01	36,000

[a]Unless indicated otherwise, values are means±SD of SHP1 from three experiments.
[b]Data from ref 8.

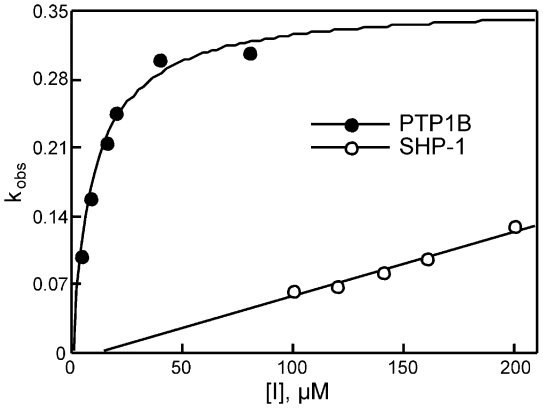


Figure 3. Comparison of the inactivation kinetics of PTP1B and SHP-1 by **21**. The apparent inactivation rate k_{obs} was determined as described.[8] The lines represent the best fits to the data according to equation: $k_{obs} = k_{inact} \cdot [I]/(K_I + [I])$.

PTPs. While perturbation of the electronic properties of the phenyl ring does not significantly improve their overall potency against PTPs, attachment of a proper peptidyl moiety to the *para* position can substantially improve both the potency and the selectivity toward a given PTP. It should be possible to develop membrane permeable and metabolically stable inhibitors by attaching peptidomimetics or small molecules to the *para* position of α-bromoacetophenone. Furthermore, since the covalent PTP inhibitor complex can be photolytically cleaved to regenerate the PTP activity,[8] these molecules may provide a novel class of photolytic switches for controlling cellular signaling processes.

Acknowledgements

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