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Squaric Acids: A New Motif for Designing Inhibitors of Protein Tyrosine Phosphatases

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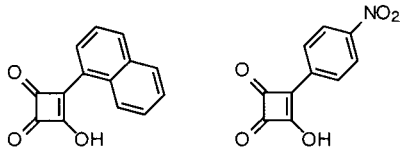
ABSTRACT



Protein tyrosine phosphatases (PTPases) are important targets in medicinal chemistry. These enzymes play a role in a number of human diseases, including type II diabetes and infection by *Yersinia pestis*, the causative agent of bubonic plague. Derivatives of squaric acids such as 2-aryl-1-hydroxycyclobut-1-ene-3,4-diones represent a new class of monoanionic inhibitors for PTPases.

Protein phosphorylation and dephosphorylation are two key chemical events that regulate the catalytic and signaling activity of a wide variety of proteins. These two reactions are catalyzed by protein kinases and phosphatases, respectively. Within the general class of phosphatases, protein tyrosine phosphatases (PTPases) in particular have been found to play central roles in a number of human diseases.[1] For example, PTP1B is a potential target for the treatment of type II diabetes.[2] This PTPase dephosphorylates the insulin receptor and thus downregulates both its kinase activity and its ability to promote uptake of glucose. As a result, inhibitors of PTP1B may help to restore insulin sensitivity to patients with type II diabetes by maintaining the receptor in its activated state.

Several phosphatases, including PTP-α and the Cdc25 family, are potential targets for anticancer therapies. It is believed that dephosphorylation reactions catalyzed by PTP-α activate the Src family of kinases,[3] while Cdc25B activates cyclin-dependent kinases.[4] CD45 is a PTPase that regulates antigen receptor signaling in T and B cells.[5] Therefore, inhibitors of this phosphatase may be useful for treating disorders of the immune system, including autoimmune diseases and inflammation. Finally, a number of pathogenic bacteria employ PTPases that are vital to their pathogenicity.[6] These bacteria use a type III secretion system to inject a phosphatase into host cells, where it disrupts host signal transduction pathways. Examples include *Yersinia pestis*, the causative agent of bubonic plague, and *Salmonella typhimurium*.

Because of the potential importance of PTPases in medicinal chemistry, there has been significant interest in developing nonhydrolyzable mimics of phosphotyrosine that can serve as the basis for designing PTPase inhibitors.[7] A number of examples have been reported in the literature,

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including difluoromethylenephosphonates,[8] 4′-*O*-[2-(2-fluoromalonyl)]-tyrosine,[9] 2-(oxalylamino)-benzoic acids,[10] difluoromethylenesulfonic acids,[11] *O*-carboxylmethyl salicylic acids,[12] and α-ketocarboxylic acids.[13] Although many of these motifs can be used to develop potent inhibitors, one of the current efforts in the field is to design effective mimics of phosphotyrosine that have a reduced negative charge in order to improve bioavailability. As part of our efforts in this direction, we report on our discovery that derivatives of squaric acid can serve as mimics of phosphate esters, and that these compounds can be used to design effective inhibitors of PTPases.

Figure 1 shows a comparison of the structures of 2-phenyl-1-hydroxycyclobut-1-ene-3,4-dione and phenyl phosphate.

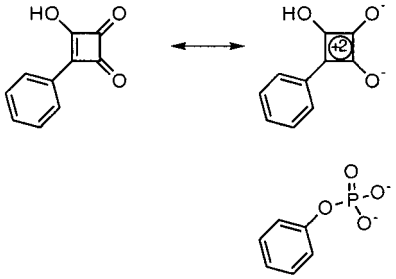

Figure 1. Comparison of the structure of a squaric acid derivative with a phosphate ester.

The squaric acid derivative has a resonance structure that places a negative charge on each of the two carbonyl oxygen atoms and provides an aromatic cyclobutenyl ring system that contains 2 π electrons. Therefore, this resonance structure represents a good electrostatic mimic for the phosphate group. Deprotonation of the hydroxycyclobutenedione gives a monoanion with an increased charge density on the oxygen atoms.[14,15]

Squaric acids have found a variety of uses in bioorganic and medicinal chemistry. Squaric acid itself is an inhibitor of glyoxylase I,[16] and semisquaric acid (3-hydroxy-3-cyclobutenedione) is an inhibitor of pyruvate dehydrogenase and transketolase.[17] Recently, Sekine and co-workers have used a diamide of squaric acid as a replacement for one of the phosphate diester linkages in an oligodeoxynucleotide.[18] Beaulieu and co-workers have used squaric acid as a replacement for phosphate in a peptide-based ligand for an SH2 domain.[19] Other derivatives of squaric acid serve as high-affinity ligands for exitatory amino acid receptors[20] and antagonists of the NMDA (*N*-methyl-D-aspartate) receptor.[21] In our current studies, we have examined eight simple 2-aryl-1-hydroxycyclobut-1-ene-3,4-diones (Figure 2) and six monoamides of squaric acid (Figure 3) for their ability to inhibit the *Yersinia* PTPase (YopH) and PTP1B. We have used two different methods to synthesize the 2-aryl-1-hydroxycyclobut-

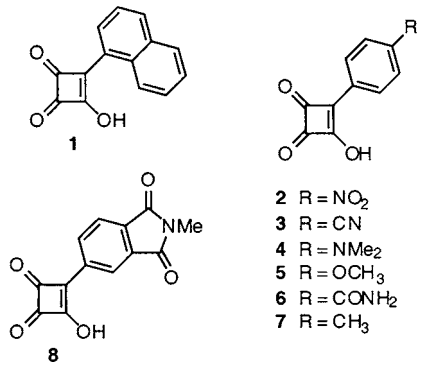

Figure 2. Structures of 2-aryl-1-hydroxycyclobut-1-ene-3,4-diones.

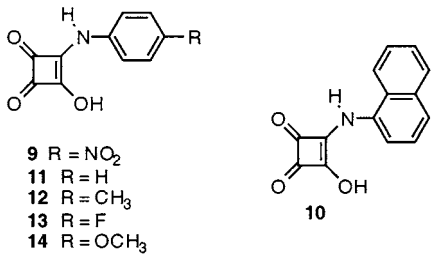

Figure 3. Structures of amides of squaric acid.

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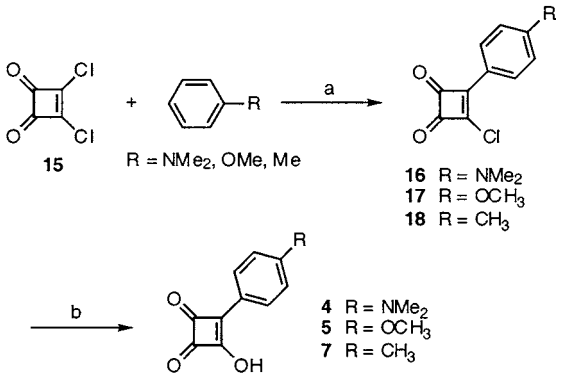
1-ene-3,4-diones. Compounds **4**, **5**, and **7** that contain electron-donating groups on the aromatic ring were prepared using a Friedel−Crafts procedure as outlined in Scheme 1.[22]

Scheme 1[a]

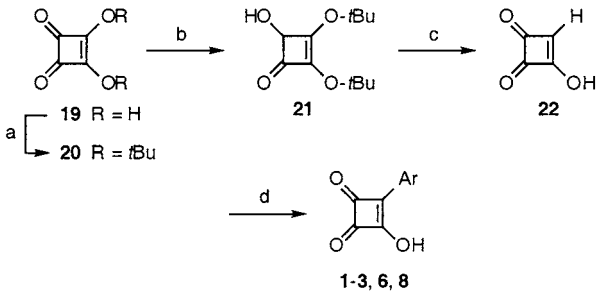


[a] Reagents: (a) $AlCl_3$, CH_2Cl_2, reflux. (b) AcOH, HCl, H_2O, reflux.

The dichloride of squaric acid (**15**)[23] was reacted with anisole or toluene in the presence of aluminum trichloride to give compounds **17** and **18**. Dimethylaniline is sufficiently electron rich that it reacts with **15**, upon heating at 120 °C via microwave irradiation and in the absence of the Lewis acid catalyst, to give compound **16**. Hydrolysis of the monochlorides **16**−**18** was accomplished using aqueous HCl in acetic acid to give the final squaric acid derivatives.

For electron-poor aromatic rings, the Friedel−Crafts procedure was not successful. As a result, we employed an alternate synthesis that is shown in Scheme 2. Squaric acid

Scheme 2[a]

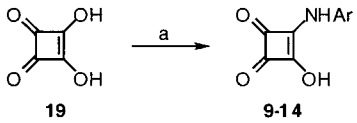


[a] Reagents: (a) *t*BuOH, $CH(OMe)_3$, reflux. (b) $LiAl(tBuO)_3H$, THF, −48 °C. (c) F_3CCO_2H. (d) $ArN_2^+Cl^-$, NaOAc, H_2O.

19 was first converted to the di-*tert*-butyl ester **20** using trimethylorthoformate as a dehydrating agent in refluxing *tert*-butyl alcohol.[24] Next, one of the carbonyl groups of this diester was reduced to the corresponding alcohol **21** with $LiAl(tBuO)_3H$.[25] Trifluoroacetic acid was used to both deprotect the *tert*-butyl esters and promote dehydration to give semisquaric acid **22**. Finally, compound **22** was coupled to the appropriate aryl diazonium chloride to give the desired squaric acid derivatives bearing electron-poor aromatic rings (compounds **1**−**3**, **6**, and **8**).[26]

The amides of squaric acid (Figure 3) were prepared using a simple one-step procedure (Scheme 3).[27] Squaric acid and

Scheme 3[a]

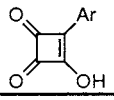


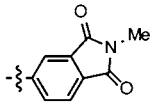
[a] Reagents: (a) $ArNH_2$, H_2O, reflux.

the appropriate aromatic amines were heated at reflux in water. Upon cooling of the reaction mixtures, the corresponding amides (compounds **9**−**14**) precipitated from solution and were isolated by simple filtration.

The squaric acid derivatives were assayed against two PTPases, the *Yersinia* PTPase (pH 5.5) and PTP1B (pH 7.0), using *p*-nitrophenyl phosphate as the substrate. The 3-aryl-4-hydroxy-3-cyclobutene-1,2-diones are good inhibitors of the *Yersinia* PTPase, with IC_{50} values that range from 47 to 260 μM (Table 1). Strong electron-withdrawing groups such as nitro (**2**) and cyano (**3**) on the phenyl ring increase the activity of the inhibitors. Lineweaver−Burk analysis of compound **3** against this PTPase confirms that it is a

Table 1. Inhibition of PTPases by 3-Aryl-4-hydroxy-3-cyclobutene-1,2-diones[a]



cmpd	Ar	IC_{50} (μM) *Yersinia* PTPase pH = 5.5	*Yersinia* PTPase pH = 7.0	PTP1B pH = 7.0
1	1-naphthyl	47 ± 5	80 ± 10	400 ± 30
2	*p*-$C_6H_4NO_2$	56 ± 8		380 ± 30
3	*p*-C_6H_4CN	68 ± 10		350 ± 40
4	*p*-$C_6H_4NMe_2$	100 ± 10		230 ± 90
5	*p*-$C_6H_4OCH_3$	190 ± 20		
6	*p*-$C_6H_4CONH_2$	230 ± 30		
7	*p*-$C_6H_4CH_3$	240 ± 15		
8		260 ± 30		

[a] Average of two measurements.

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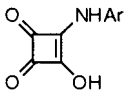
reversible competitive inhibitor.[28] The larger naphthyl group in compound **1** is also beneficial to potency. The activity of this inhibitor is somewhat sensitive to pH; its IC_{50} value increases by a factor of approximately 2 when the pH of the assay solution is raised from 5.5 to 7.0.[29] Assays of compounds **1**−**4** against PTP1B show that they are moderate inhibitors against this PTPase, with IC_{50} values that range from 230 to 400 μM.

The data are consistent with a structural model in which the squaric acid binds in the active site of the PTPase in a conformation that mimics the binding of aryl phosphate ester substrates.[30] The anion of the squaric acid could form an electrostatic interaction with Arg-409 (*Yersinia* PTPase numbering). This residue along with Cys-403 and Asp-356 make up the key catalytic residues of the enzyme.[31] The aromatic portion of the inhibitor could then make hydrophobic contacts with Phe-229. The larger aromatic ring of inhibitor **1** may strengthen these hydrophobic contacts and lead to better inhibition. In addition, favorable electrostatic interactions between Phe-229 and the electron-poor aromatic rings of compounds **2** and **3** may result in the enhanced activity of these inhibitors when compared to their electron-rich counterparts.

The amides of squaric acid (Table 2) have IC_{50} values that range from 120 μM to 4.2 mM, and they are generally less potent than the 3-aryl-4-hydroxy-3-cyclobutene-1,2-diones. However, similar factors appear to influence the activity of both sets of compounds. Both strong electron-withdrawing groups such as nitro (**9**) and larger aromatic groups such as 1-naphthyl (**10**) are beneficial to the activity of the amides. In contrast, electron-donating groups such as methoxy (compounds **5** and **14**) are detrimental to binding in both series of inhibitors.

Table 2. Inhibition of PTPases by Amides of Squaric Acid[a]



compound	Ar	IC_{50} (μM) *Yersinia* PTPase pH = 5.5
9	*p*-$C_6H_4NO_2$	120 ± 20
10	1-naphthyl	350 ± 40
11	phenyl	780 ± 60
12	*p*-$C_6H_4CH_3$	1200 ± 200
13	*p*-C_6H_4F	1400 ± 800
14	*p*-$C_6H_4OCH_3$	4200 ± 500

[a] Average of two measurements.

In summary, we have demonstrated that squaric acid is an effective pharmacophore for the development of PTPase inhibitors. These compounds provide a good electrostatic mimic of pTyr and bear a reduced negative charge at neutral pH when compared to many of the other dianionic pTyr analogues that have been reported to date. The inhibitors show good activity for such simple and low-molecular weight compounds that are likely to interact only with residues that are proximal to the catalytic site of the PTPases. We are currently working to improve the potency and specificity of these compounds by elaborating them with functional groups that are designed to make contacts with the secondary binding site and other distal regions of the active site cleft.

Acknowledgment. This research was supported by a grant from the NIH NIGMS (GM057327). We thank Professor Matthew Zimmt for helpful discussions.

Supporting Information Available: Full experimental details and NMR spectral data, a Lineweaver−Burk plot for compound **3**, and electron density calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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