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Bone-Targeted Pyrido[2,3-*d*]pyrimidin-7-ones: Potent Inhibitors of Src Tyrosine Kinase as Novel Antiresorptive Agents

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Abstract—The design of bone-targeted pyrido[2,3-*d*]pyrimidin-7-ones as Src tyrosine kinase inhibitors is described. Leveraging SAR from known compounds and using structure-based methods, we were able to rapidly incorporate bone binding components, which maintained, and even increased potency against the target enzyme. Compound **4** displayed a high affinity for hydroxyapatite, a major constituent of bone, and demonstrated antiresoprtive activity in our cell-based assay.

Src is a non-receptor protein tyrosine kinase (PTK) composed of multiple modular domains through which complex signaling is orchestrated. In addition to its oncogenic traits,[1] knockout studies conducted by Soriano in the early 1990s implicated Src as a critical component of osteoclast signaling.[2] Osteoclasts isolated from these animals were functionally impaired, appearing flattened against the bone surface, and devoid of a ruffled border through which resorption takes place.[2–4] Exploiting these observations, a number of inhibitors of both the SH2 domain,[5] as well as the kinase[6] region have been reported to possess antiresorptive activity both in vitro and in vivo. Recently, we reported on the design and biological properties of an inhibitor of the SH2 domain of Src which was also endowed with properties which enabled it to target bone.[7] By targeting this inhibitor to bone, we were able to achieve tissue selectivity by concentrating the molecule to the bone surface and making available to actively resorbing osteoclasts, higher concentrations of the inhibitor. Since that time, we have extended the approach to a series of known Src tyrosine kinase inhibitors. By initially focusing on known inhibitors of the enzyme, we were able to leverage the wealth of existing structural as well as SAR data to develop and validate some initial models, and provide ourselves with 'proof of concept.' In this paper, we describe the design and synthesis of a series of bone-targeted pyrido[2,3-*d*]pyrimidin-7-ones (Fig. 1), a chemotype originally pursued by the Pfizer group,[8–10] which are potent inhibitors of Src tyrosine kinase. The development of a reliable model for the active form of the enzyme was critical in determining the precise location of the bone-targeting group that would maximize both molecular interactions while simultaneously targeting the molecules to bone. The application of this approach to other templates is described elsewhere in this journal.

A model of the catalytically active conformation of Src kinase was constructed using the crystal structures of inactive Src kinase,[11] Lck kinase,[12] and active insulin receptor kinase.[13] The model was validated by docking a diverse series of Src kinase inhibitors found in the literature. Adjustments were then applied until the model could differentiate between active and inactive compounds. Docking studies using the FLO software predicted that compound **3** would bind to Src kinase as

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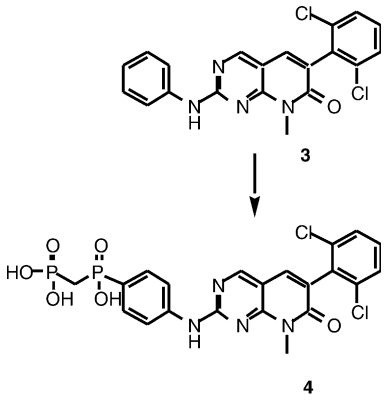
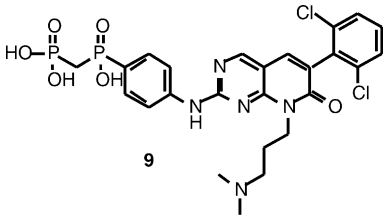


Figure 1. Incorporation of a bone-targeting moiety onto the pyrido[2,3-*d*]pyrimidin-7-one scaffold.

shown in Figure 2b. Relative to AMP-PNP (a non-hydrolyzable derivative of ATP, Fig. 2a), the pyrimidine ring of **3** occupies a similar position to that of the adenosine ring, however, the pyrimidine ring is twisted slightly forming a new series of hydrogen bonds to Met341 and Ser342. Furthermore, the dichloro phenyl substituent projects deep into a hydrophobic pocket not occupied by AMP-PNP. The model indicated that bone-targeting groups might be most successfully attached to the *para*-position of the aniline ring (Fig. 2c) where they would extend into solvent and, thus, have little or no effect on the binding affinity. Based on this information, two compounds incorporating a bone-targeting group in the *para*-position were envisioned, as well as several more which explored other substitution patterns.

The syntheses of compounds in Table 1 have been detailed elsewhere[14] and are based on previously published approaches to this class of compounds.[9,10] Briefly, the appropriately substituted anilines were coupled by either an SNAr displacement of the methyl sulfone **1**, or through a palladium mediated coupling with amine **2** and a substituted halobenzene. Removal of the protecting groups then yielded compounds **3**–**8**. We synthesized one additional compound, **9**, which substituted a 3-dimethyaminopropyl group in place of the methyl group at N8.[15] Previous SAR data had demonstrated that this was accompanied by a 6-fold increase in potency against the target protein (Scheme 1).



Compounds were screened for their ability to inhibit Src kinase using scitillation proximity assay (SPA) technology as developed by Amersham and described in detail elsewhere.[14] For the purpose of comparison, in our assay, compound **3** had an $IC_{50} = 0.016$ μM which compares favorably to 0.02 μM reported in the literature.[9] Interestingly, compound **4**, which incorporates the phosphinylmethylphosphonic acid (PCP) in the *para*-position, was 8-fold more potent than the parent compound **3**. While not immediately clear from where this increased potency derives, the hydroxyl of Tyr318

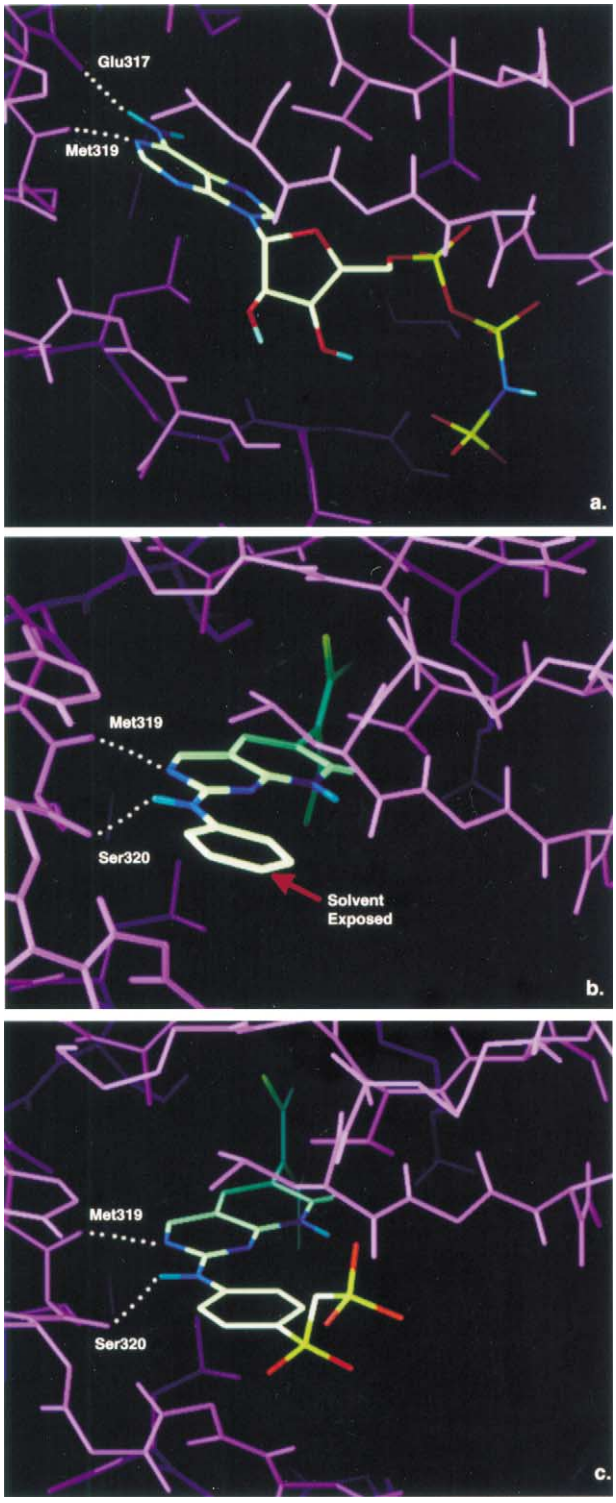
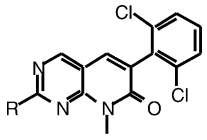
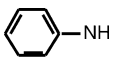
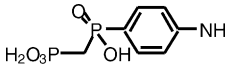
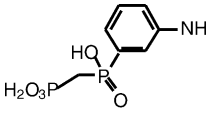
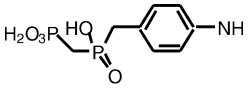
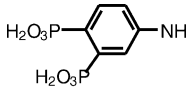
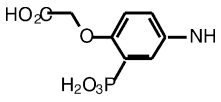


Figure 2. (a) AMP-PNP docked into the model of Src tyrosine kinase illustrating critical hydrogen bonds; (b) compound **3** docked in the same model illustrating the solvent exposed *para*-position on the aniline ring; (c) incorporation of phosphinylmethylphosphonic acid (PCP) moiety onto the aniline ring.

or the carbonyl oxygen of Ser342 may be in close enough proximity to the oxygens of the phosphinyl phosphorous to form a hydrogen bond although this needs to be confirmed crystallographically. Compound **5**, which places the PCP moiety in the *meta*-position was predicted to be less active due to unfavorable steric interactions with the protein and so not surprisingly, demonstrated reduced activity relative to **4**. Likewise, compound **6**, which inserts a methylene between the aromatic ring and the PCP group was also found to be less active. Compounds **7** and **8**, both of which incorporate bone binding components which we have successfully employed in the context of SH2 inhibitors, were found to be considerably less potent against the enzyme. This is presumably due to the *meta* phosphonic acid which positions a full negative charge adjacent to hydrophobic surface created by Gly344. Finally, **9**, which was predicted to be the most potent analogue, had an $IC_{50} = 0.3$ nM.[16]

Table 1. Target compounds and their corresponding IC_{50}s against Src[a]



Compd	Substituted aniline (R)	Src IC_{50} (μM)
3		0.016
4		0.002
5		0.077
6		0.075
7		1.3
8		0.20

[a]Values are the mean of two experiments.

To evaluate the affinity of compounds such as **4** to bind to bone, a hydroxapatite adsorption chromatography assay was developed.[7] Compounds which have no affinity for hydroxyapatite typically have $K' < 0.1$ while those which display high affinity, such as the drug alendronate, have $K' > 3.5$. Compound **4** gave a K' value of 2.4, while the parent **3**, exhibited a K' value close to 0 demonstrating that **4** clearly exhibits bone-targeting properties.

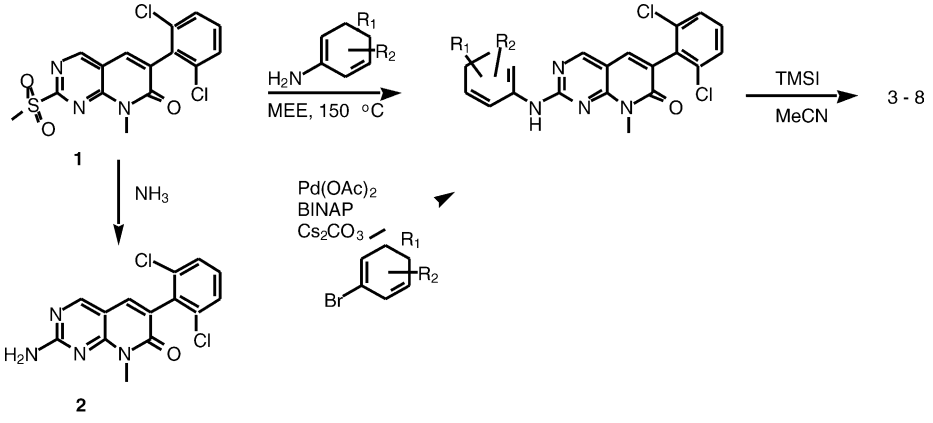
Because Src has been implicated in the regulation of osteoclast functional activity, compounds **4**, **5**, and **9** were assayed for their ability to inhibit rabbit-osteoclast mediated resorption of dentine slices (Table 2).[17] Compounds **4**, **5**, and **9**, inhibited resorption of dentine slices within an IC_{50} range from 4 to 0.8 μM. Importantly, the most potent inhibitor in the Src kinase assay, compound **9**, was functionally the most potent molecule as well. Furthermore, none of the compounds demonstrated any signs of toxicity as monitored by the presence of tartrate resistant acid phosphatase (TRAP)-positive cells and surrounding fibroblasts.

In summary, we have employed structure-based meth-

Table 2. Inhibition of rabbit osteoclast-mediated resorption of dentine[a]

Compd	Inhibition of resorption IC_{50} (μM)	TRAP staining	Fibroblasts
4	3	Normal	Normal
5	4	Normal	Normal
9	0.8	Normal	Normal

[a]Values are the average of two experiments.



Scheme 1. Two different approaches to the incorporation bone-targeting groups onto the pyrido[2,3-*d*]pyrimidine scaffold.

ods for the incorporation novel bone-targeting groups onto the pyrido[2,3-*d*]pyrimidine scaffold. Compound **4**, in which the PCP moiety was installed in the *para*-position, demonstrated increased potency relative to the parent molecule **3**, and exhibited robust affinity for hydroxapatite, a major constituent of bone. This should be useful, not only in making these compounds tissue selective, but also by making available to actively resorbing osteoclasts higher concentrations of the inhibitor. In support of these hypotheses, compounds **4**, **5**, and **9**, were functionally active in inhibiting resorption by active osteoclasts.

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15. Spectroscopic data are included for the two most potent compounds (**4** and **9**). ^{1}H (300 MHz), ^{31}P (121 MHz) and ^{19}F (282 MHz) spectra were obtained at 300 K on a Bruker ARX-300 instrument with DMSO as solvent. Low resolution mass spectra were obtained on a Micromass Model PLT II. Both compounds were purified using RP HPLC (CH_3CN/H_2O). **4:** ^{1}H NMR: δ 10.46 (s, 1H), 8.89 (s, 1H), 7.92–7.99 (m, 3H), 7.71–7.84 (m, 2H), 7.58 (d, $J=7.5$ Hz, 2H), 7.46 (dd, $J=8.9$ and 7.14 Hz, 1H), 3.70 (s, 3H), 2.43–2.27 (m, 2H); ^{31}P NMR: δ 33.3, 23.3; ^{19}F NMR: δ −70.7; LRMS (ES^-): $(M-H)^-$ 553. **9:** ^{1}H NMR: δ 10.44 (s, 1H), 8.90 (s, 1H), 7.90 (s, 1H), 7.86–7.70 (m, 4H), 7.56 (d, $J=7.4$ Hz, 2H), 7.40 (dd, $J=8.30$ and 7.19 Hz, 1H), 4.27–4.33 (m, 2H), 3.03–3.12 (m, 2H), 3.42 (s, 6H), 2.43–2.26 (m, 2H), 1.97–2.04 (m, 2H); ^{31}P NMR: δ 32.5, 20.3; ^{19}F NMR: δ −69.8; LRMS (ES^-): $(M-H)^-$ 623.
16. The IC_{50} for compound **9** was determined using a LANCE assay which will be described in a subsequent manuscript. For comparison, compound **4** had an $IC_{50}=3.5$ nM in this assay.
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