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7-Pyrrolidinyl- and 7-Piperidinyl-5-aryl-pyrrolo[2,3-*d*]-pyrimidines—Potent Inhibitors of the Tyrosine Kinase c-Src

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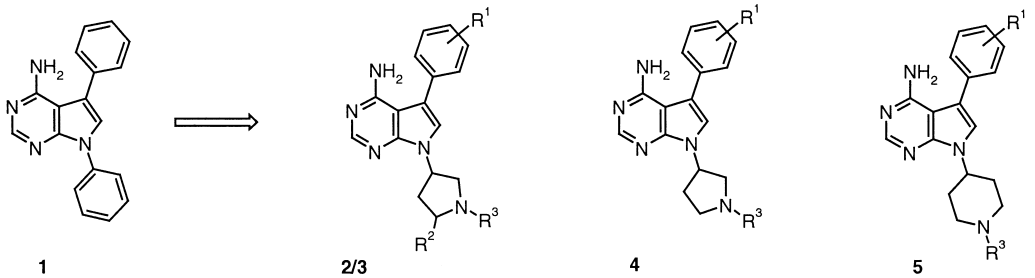
Abstract—7-Heterocyclyl-5-aryl-pyrrolo[2,3-*d*]pyrimidines represent a new class of highly potent and selective inhibitors of the tyrosine kinase pp60$^{c\text{-}Src}$.

In the preceding paper[1] we have described a series of novel 7-substituted 5-aryl-pyrrolo[2,3-*d*]pyrimidines as potent inhibitors of the tyrosine kinase pp60$^{c\text{-}Src}$ (c-Src). c-Src is an essential enzyme for osteoclastic bone resorption[2,3] and synthetic c-Src inhibitors have been shown to interfere with this process both in vitro[4] and in vivo.[5] We report herein on a second optimization strategy for our lead compound **1** (5,7-diphenyl-pyrrolo[2,3-*d*]pyrimidine), which involves replacement of the N^7-phenyl moiety by different types of heterocycles. In analogy to the strategy described previously[1] these heterocyclic N^7-substituents are assumed to occupy the pocket which is usually utilized by the ribose moiety of ATP. Substitution of polar heterocyclic moieties for the N^7-phenyl substituent in **1** might result in additional interactions with polar amino acids within this sugar pocket that are not possible with **1** (Fig. 1).

We have investigated four different types of heterocycles attached to the N^7-position of the pyrrolo-pyrimidine moiety. These include *N*-substituted derivatives of pyrrolidine or piperidine as well as analogues that are derived from 2-ethoxycarbonyl or 2-hydroxymethyl pyrrolidine, respectively.

A representative synthesis of N^7-heterocyclyl-5-aryl-pyrrolo-pyrimidines is outlined in Scheme 1. The preparation of 4-amino-5-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidine **7** was already described in the preceding paper.[1] Attachment of the *N*-Boc-protected heterocycle[6] **6** to **7** was achieved via mesylate displacement in the presence of potassium carbonate and 18-crown-6 in DMF at 55 °C.[7] Removal of the Boc protecting group with 4 N HCl in Et_2O gave **4k** which was subsequentely alkylated with methyl bromoacetate. Treatment of ester **4n** with ammonia in MeOH resulted in amide **4o**.

As illustrated by the data summarized in Table 1, for 2-substituted pyrrolidine derivatives with *trans* stereochemistry the *N*-Boc protected compounds (**2a**, **b** and **3a**, **b**) were always slightly more active than the corresponding free amines (**2c**, **d** and **3c**, **d**). In contrast, the



1 2/3 4 5

Figure 1.

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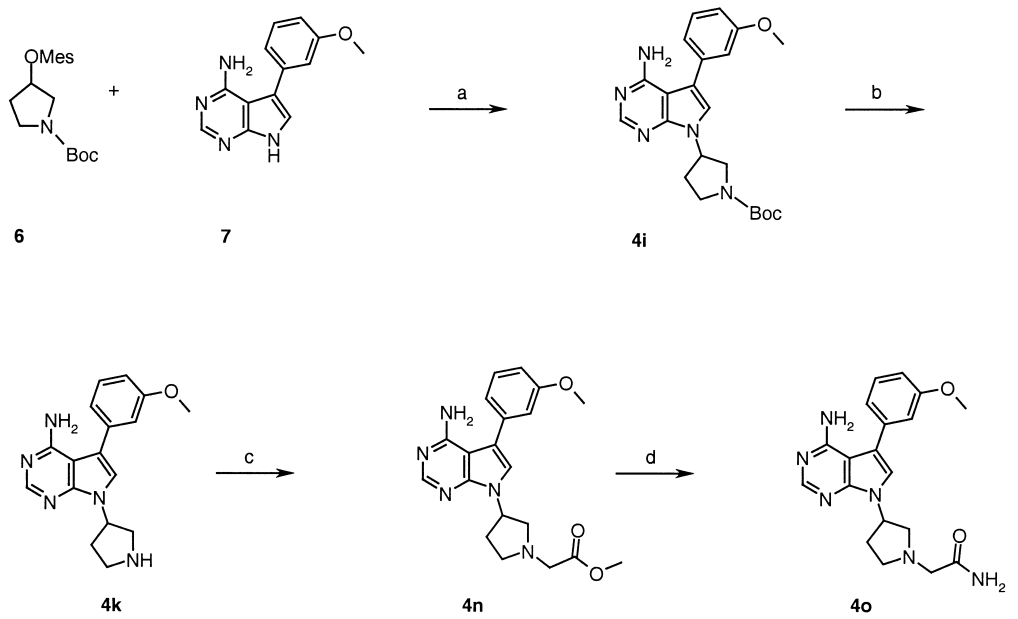
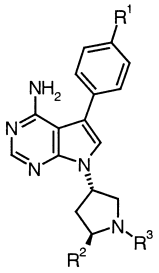
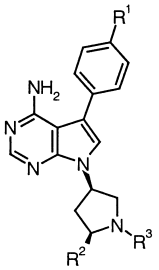


Scheme 1. (a) K_2CO_3, 18-crown-6, DMF, 55 °C, 51%; (b) 4 N HCl/Et_2O, rt, 70%; (c) $BrCH_2CO_2CH_3$, DMF, DIEA, 70%; (d) NH_3, MeOH, sealed tube, 60 °C, 83%.

Table 1. Inhibition of c-Src enzyme activity and cellular activity of inhibitors **2** and **3**

	Compd	R^1	R^2	R^3	c-Src (enzyme)[a] IC_{50} (μM)	c-Src (cell)[b] IC_{50} (μM)
	**2a**	H	$CH_2CO_2CH_2CH_3$	CO_2t-Bu	0.41	n.d.[c]
	2b	OH	$CH_2CO_2CH_2CH_3$	CO_2t-Bu	0.035	0.70
	2c	H	$CH_2CO_2CH_2CH_3$	H	0.60	n.d.[c]
	2d	OH	$CH_2CO_2CH_2CH_3$	H	0.10	n.d.[c]
	3a	H	CH_2OH	CO_2t-Bu	0.13	n.d.[c]
	3b	OH	CH_2OH	CO_2t-Bu	0.024	3.3
	3c	H	CH_2OH	H	0.30	n.d.[c]
	3d	OH	CH_2OH	H	0.095	>5
	**2e**	H	$CH_2CO_2CH_2CH_3$	CO_2t-Bu	5	n.d.[c]
	2f	OH	$CH_2CO_2CH_2CH_3$	CO_2t-Bu	0.30	n.d.[c]
	2g	H	$CH_2CO_2CH_2CH_3$	H	0.015	>5
	2h	OH	$CH_2CO_2CH_2CH_3$	H	0.005	3.3
	3e	H	CH_2OH	CO_2t-Bu	2	n.d.[c]
	3f	OH	CH_2OH	CO_2t-Bu	0.35	n.d.[c]
	3g	H	CH_2OH	H	0.13	n.d.
	3h	OH	CH_2OH	H	0.05	>5

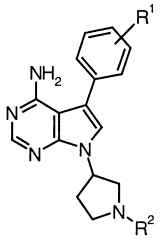
[a]Inhibition of c-Src enzyme activity in the liquid-phase tyrosine phosphorylation assay, c-Src concentration: 830 ng/mL, IC_{50} values are the mean of 2 experiments carried out in duplicate, individual data points in each experiment were within a 3-fold range with each other.
[b]Inhibition of c-Src mediated phosphorylation of Fak in IC8.1 fibroblasts.[5]
[c]Not determined.

free amines (**2g**, **h** and **3g, h**) proved to be more potent inhibitors of c-Src in the *cis*-pyrrolidine series. However, despite the fact that nanomolar potency was achieved for enzyme inhibition in some cases, none of the pyrrolidine derived analogues exhibited submicromolar inhibition of c-Src in cells.

In order to investigate the role of the substituent at the 2-position of the pyrrolidine ring we prepared the racemic derivatives **4a**–**d** (Table 2). Rather surprisingly, a substituent at this position is not required for low nanomolar activity.

Methylation of the 4-OH or 3-OH substituent on the 5-phenyl ring as in compounds **4e**, **f** and **4i**, **k** proved to be less favorable for c-Src inhibition, resulting in a 10–50-fold loss in potency in comparison with **4c**, **d** and **4g**, **h**, respectively. These 2-unsubstituted analogues were then further modified by alkylation at the pyrrolidine nitrogen with bromoacetic acid methyl ester. These *N*-sub-

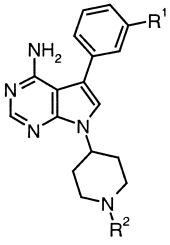
Table 2. Inhibition of c-Src enzyme activity, cellular activity, and selectivity profile of inhibitors **4**



Compd	R^1	R^2	c-Src[a] (enzyme) IC_{50} (μM)	c-Src[b] (cell) IC_{50} (μM)	EGF-R[c] (enzyme) IC_{50} (μM)	v-Abl[d] (enzyme) IC_{50} (μM)
4a	H	CO_2t-Bu	0.18	>5	8.3	n.d.[e]
4b	H	H	0.23	n.d.[e]	13.4	n.d.[e]
4c	4-OH	CO_2t-Bu	0.008	0.40	n.d.[e]	n.d.[e]
4d	4-OH	H	0.037	2.7	2.67	2.8
4e	4-OMe	CO_2t-Bu	0.40	0.4	n.d.[e]	n.d.[e]
4f	4-OMe	H	0.30	n.d.[e]	n.d.[e]	n.d.[e]
4g	3-OH	CO_2t-Bu	0.005	3.1	2.2	0.058
4h	3-OH	H	0.006	0.80	1.53	0.29
4i	3-OMe	CO_2t-Bu	0.27	4.4	2.1	2.4
4k	3-OMe	H	0.053	>5	1.12	1.4
4l	3-OH	$CH_2CO_2CH_3$	0.003	0.7	0.74	0.054
4m	3-OH	CH_2CONH_2	0.004	0.4	0.4	0.15
4n	3-OMe	$CH_2CO_2CH_3$	0.050	>5	0.83	0.59
4o	3-OMe	CH_2CONH_2	0.038	>5	0.44	0.7

[a]Inhibition of c-Src enzyme activity in the liquid-phase tyrosine phosphorylation assay, c-Src concentration: 830 ng/mL, IC_{50} values are the mean of 2 experiments carried out in duplicate, individual data points in each experiment were within a 3-fold range with each other.
[b]Inhibition of c-Src mediated phosphorylation of Fak in IC8.1 fibroblasts.[5]
[c]Inhibition of epidermal growth factor receptor (EGF-R) tyrosine kinase enzyme activity.
[d]Inhibition of v-Abl tyrosine kinase enzyme activity.
[e]Not determined.

Table 3. Inhibition of c-Src enzyme activity, cellular activity, and selectivity profile of inhibitors **5**



Compd	R^1	R^2	c-Src[a] (enzyme) IC_{50} (μM)	c-Src[b] (cell) IC_{50} (μM)	EGF-R[c] (enzyme) IC_{50} (μM)	v-Abl[d] (enzyme) IC_{50} (μM)
5a	OH	$CH_2CO_2CH_3$	<0.001	0.4	0.8	0.056
5b	OH	CH_2CONH_2	0.001	0.8	0.17	0.21
5c	OH	$CH_2CON(CH_3)_2$	0.003	0.5	0.5	0.12
5d	OH	CH_2CH_2OH	0.001	0.6	2.54	0.098
5e	OMe	$CH_2CO_2CH_3$	0.084	2.8	1.47	1
5f	OMe	CH_2CONH_2	0.022	0.7	0.35	0.55
5g	OMe	$CH_2CON(CH_3)_2$	0.067	>5	0.75	0.51
5h	OMe	CH_2CH_2OH	0.027	1.4	0.9	1.03
5i	OMe	$(CH_2)_2N(CH_3)(CH_2)_2OH$	0.006	0.6	0.295	0.36
5k	OMe	$(CH_2)_2NH(CH_2)_2OCH_3$	0.015	0.29	0.31	0.26
5l	OMe	$(CH_2)_2N(CH_3)(CH_2)_2OCH_3$	0.047	0.5	0.93	0.50

[a]Inhibition of c-Src enzyme activity in the liquid-phase tyrosine phosphorylation assay, c-Src concentration: 830 ng/mL, IC_{50} values are the mean of 2 experiments carried out in duplicate, individual data points in each experiment were within a 3-fold range with each other.
[b]Inhibition of c-Src mediated phosphorylation of Fak in IC8.1 fibroblasts.[5]
[c]Inhibition of epidermal growth factor receptor (EGF-R) tyrosine kinase enzyme activity.
[d]Inhibition of v-Abl tyrosine kinase enzyme activity.

stituents were designed to interact with amino acid side chains that are located in the vicinity of the triphosphate binding pocket. From these derivatives, those bearing a 3-OH substituent on the 5-phenyl ring are nanomolar c-Src inhibitors with a favorable selectivity profile. In the view of the interesting results obtained with the pyrrolidine derivatives it appeared mandatory to investigate the activity of the corresponding piperidinyl-derivatives, particularly in view of the fact that these compounds are achiral.

Table 3 summarizes the c-Src inhibitory activity of the N^7-piperidinyl analogues **5**. Out of this series, **5a**–**d**, bearing a 3-OH substituent on the 5-phenyl ring, are low nanomolar inhibitors of c-Src, which also exhibit good cellular activity, inhibition of cellular phosphorylation of Fak with submicromolar IC_{50}s. In addition, **5a**–**d** possess a remarkable selectivity profile with regard to the inhibition of EGF-R- and v-Abl-kinases.

We therefore investigated the *O*-methylated analogues **5e**–**h.** These 3-methoxy derivatives show a 22- to 84-fold loss of potency for c-Src inhibition compared to the 3-OH analogues **5a**–**5d** and are generally not potent in the cellular assay. This finding is consistent with what we observed for the corresponding pyrrolidine derivatives (**4g**, **h**→**4i**, **k**). Finally attachment of a large substituent incorporating a basic nitrogen at the piperidine nitrogen led to compounds **5i**–**l**, which apart from high potency for c-Src inhibition exhibit cellular activity with submicromolar IC_{50} values in combination with a favorable selectivity profile.[8,9]

The above summarized optimization strategy resulted in novel, extremly potent and remarkably selective inhibitors of the tyrosine kinase c-Src.

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6. The synthesis of the two optically active 2-substituted pyrrolidine mesylates for the preparation of compounds **2** and **3** (Table 1) is described in Baker, G. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* **1961**, *46*, 2954 and in Abraham, D. J.; Mokotoff, M.; Sheh, L.; Simmons, J. E. *J. Med. Chem.* **1983**, *26*, 549.
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8. IC_{50} values for inhibition of lck, another src kinase family member, have been determined for a selected number of compounds and are as follows: **5a** = 0.29 μM; **5b** = 0.20 μM; **5c** = 0.40 μM; **5d** = 0.36 μM; **5i** = 0.34 μM; **5k** = 0.62 μM; **5l** = 0.72 μM.
9. IC_{50} for the inhibition of autophosphorylation of EGF-R in a cellular assay (Elisa) for a number of selected compounds (**4g**, **4h**, **4l**, **4m**, **5a**–**5d**, **5h**, **5k** and **5l**) were >10 μM with one exception (**5i**, IC_{50} = 7.8 μM).