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Substituted 5,7-Diphenyl-pyrrolo[2,3*d*]pyrimidines: Potent Inhibitors of the Tyrosine Kinase c-Src

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Abstract—5,7-Diphenyl-pyrrolo[2,3*d*]pyrimidines represent a new class of highly potent inhibitors of the tyrosine kinase c-Src (IC_{50} < 50 nM) with specificity against a panel of different tyrosine kinases. The substitution pattern on the two phenyl rings determines potency and specificity and provides a means to modulate cellular activity.

Introduction

Compelling evidence indicates that the protein tyrosine kinase c-Src plays a unique and crucial role in osteoclastic bone resorption.[1–4] Mutant mice with a disrupted Src gene have functionally inactive osteoclasts in the absence of any other overt pathological signs. c-Src may be responsible for the phosphorylation of cytoskeletal and/or docking proteins involved in the translocation and exocytosis of vesicles during the resorptive process. Inhibitors of c-Src may be useful for the treatment of diseases characterized by excessive bone resorption such as osteoporosis and tumor-induced hypercalcemia. In addition, c-Src inhibitors could also be beneficial in the treatment of c-Src overexpressing tumors, for example colon carcinoma.

We started our investigation of substituted 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines as potential c-Src inhibitors based on the finding that the closely related pyrrazolo-pyrimidine CGP 191 inhibited c-Src phosphorylation with an IC_{50} of 0.5 μM. The pyrrolopyrimidine analogue CGP 62464 proved to be five times more potent (IC_{50} = 0.1 μM).[5] We therefore concentrated our efforts on optimising the 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidine class with the aim to identify potent and specific compounds with good cellular activity.[6]

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Chemistry and methods

The synthesis of a few 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines had been described earlier,[7] but without any data on kinase inhibition. Our substituted 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines have been prepared as outlined by a generic example[8] in Scheme 1.

The first two steps to form the 2-amino-3-cyano-pyrrol usually gave yields between 50 and 80%. The subsequent 3-step cyclization to the pyrrolo[2,3*d*]pyrimidine resulted in purer products than the alternative one pot reaction with formamide at high temperature and produced good yields of 65–85% over all three steps. 5,7-Diphenyl-pyrrolo[2,3*d*]pyrimidines with basic side chains were obtained by conversion of the hydroxymethyl intermediate into the corresponding chloride, followed by treatment with various amines (neat or in a polar solvent). The proposed binding mode of CGP 62464 (compared to that of ATP) based on the published X-ray structure of human Src[9] is shown in Figure 2. According to this model, the 5,7-diphenyl-pyrrolo-[2,3*d*]pyrimidines bind at the ATP binding site in an orientation similar to that of ATP, making use of the same two hydrogen-bonds to and from the backbone of the enzyme (Met 341 and Glu 339). The 5-phenyl locks into a rather small pocket, which is not used by ATP itself. Similar interactions, as well as the existence of a hydrophobic pocket, have been proposed for the binding mode of EGF-receptor inhibitors.[10]

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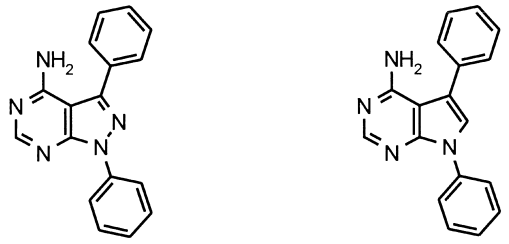


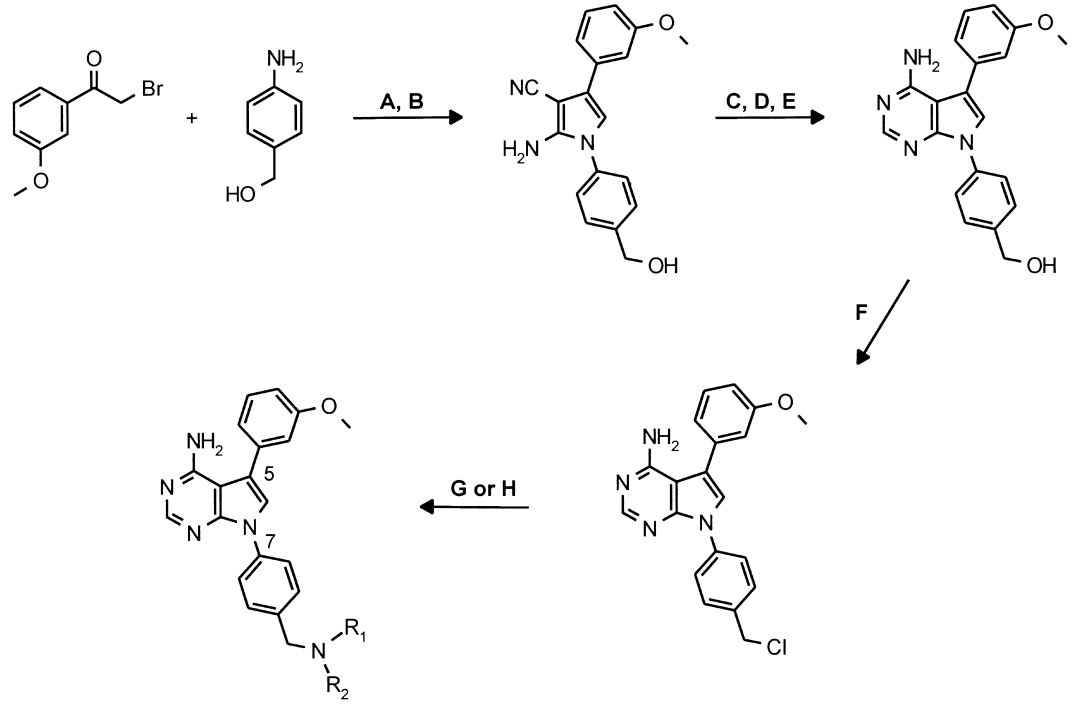
CGP 191: IC_{50} = 0.5μM **CGP 62464: IC_{50} = 0.1μM**

Figure 1.

Results and Discussion

All the 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines synthesised were tested for c-Src inhibition in a liquid phase tyrosine phosphorylation assay with chicken c-Src and the synthetic substrate poly-Glu-Tyr (4:1).[11] Inhibitors with an IC_{50} below 0.5 μM were tested further for specificity in a panel of different in vitro kinase enzyme assays including receptor (EGF-R, VEGF-receptor Kdr) and non-receptor tyrosine kinases (v-Abl), as well as a serine/threonine kinase implicated in cell cycling (Cdc2).

The SAR developed with 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines (Table 1) can be summarized as follows: The two hydrogen bonds indicated in Figure 2 are important for activity. Replacement of the amino-substituent in the 4-position of the pyrimidine ring by hydroxy (which leads to a compound mainly present in its carbonyl tautomeric form) or chlorine as well as *N*-methylation, which prevents this interaction, resulted in a dramatic loss of potency (compounds **1b**–**1d**, Table 1). Furthermore, the substitution pattern on the two phenyl rings proved to be important, for potency as well as specificity. Only limited variations were tolerated at the phenyl ring in the 5 position (compounds **1e**–**1i**). A large substituent in 4′ position (**1i**) or substitution in 2′ position (**1h**) lead to decreased activity, which can be rationalised based on the size of the hydrophobic pocket. At the phenyl ring in position 7 even large substituents in the 3′- or 4′-position (but not in the 2′-position; compounds **1k** and **1o**) lead to potent c-Src inhibitors. This finding can again be explained by the proposed binding mode since substituents in 3′- and 4′-position reach the surface of the enzyme, whereas substituents in 2′-position would disturb the enzyme structure. By adding the aminoethyloxy



Scheme 1. Synthesis of 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines.[8] (A) $NaHCO_3$, EtOH, 50–80 °C; (B) malonodinitrile, NaOEt, EtOH, 50–80 °C, (A + B: 50–80%); (C) triethylorthoformiate, 80–120 °C, acidic work up; (D) NH_3, MeOH, rt; (E) cat. NaOEt, EtOH, reflux; then 2 N NaOH/THF overnight (C–E: 65–85%); (F): chlorenamine, THF, rt; (G) R_1R_2NH, EtOH, 100 °C, sealed tube (75–95%); (H) R_1R_2NH (excess, no solvent), 70–120 °C (80–95%).

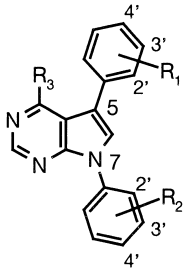
side chain both specificity and solubility (data not shown) could be improved while retaining high potency (compounds **1p**–**1r**; **1t**).

Inhibition of cellular phosphorylation was investigated by measuring the c-Src mediated phosphorylation of the 120 kDa protein FAK in IC8.1 fibroblasts transfected with chicken c-Src (Western blot analysis).[12] For specificity determination the compounds were tested in the same cellular system for the inhibition of the c-Src deactivating kinase Csk. In separate experiments the inhibition of receptor autophosphorylation in intact cells after stimulation with the corresponding growth factor was measured (cellular ELISA; Table 2).[13]

The cellular potency of compounds **1a**, **1e**, **1l** and **1p** was in the micromolar range (Table 2). But for compounds **1q** and **1r** with comparable potency in the liquid phase assay but with polar 4′-substituents at the 7 phenyl ring inhibition of c-Src in intact cells below 1 μM could be achieved. Increasing the length of the side chain by one carbon (**1t**) resulted in a strong c-Src inhibitor in the liquid phase enzyme assay and in intact cells that had a favorable specificity profile against other kinases. For the amide **1s** similar potency in the liquid phase assay but a clear drop in cellular activity was observed, which indicated the importance of a free amino group in the side chain. In intact cells, good specificity was found with these inhibitors against all kinases investigated,

Table 1. Potency and specificity of 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines

1a - 1t



Compound	R1	R2	R3	c-Src (μM)	EGF-R (μM)	Kdr (μM)	v-Abl (μM)	Cdc-2 (μM)	mp (°C)
1a	H	H	NH_2	0.1	0.5	0.5	0.02	54.0	200
1b	H	H	Cl	8.0	nd	nd	nd	nd	155–157
1c	H	H	OH	10.0	nd	nd	nd	nd	255
1d	H	H	$NHCH_3$	>10.0	31.0	nd	>10.0	>10.0	120
1e	3′-OCH_3	H	NH_2	0.05	0.5	>1.0	0.06	43.0	159–160
1f	3′-Cl	H	NH_2	0.2	0.7	1.0	0.6	>10.0	173–175
1g	4′-OCH_3	H	NH_2	0.15	0.35	0.2	0.4	>10.0	187–188
1h	2′-CH_3	H	NH_2	6.0	2.65	nd	nd	90.0	181–182
1i	4′-COOEt	H	NH_2	10.0	1.3	1.1	>10.0	>10.0	158–160
1k	H	2′-CH_2OH	NH_2	0.3	1.1	nd	3.4	>10.0	174–175
1l	H	3′-CH_2OH	NH_2	0.06	0.6	nd	0.2	47.0	165
1m	H	4′-CH_2OH	NH_2	0.02	0.7	0.6	0.07	30.0	207–209
1n	3′-OCH_3	3′-CH_2OH	NH_2	0.06	0.8	>1.0	0.05	33.0	151–152
1o	H	2′-$CH_2NH(CH_2)_2OH$	NH_2	5.0	6.4	nd	nd	>10.0	170
1p	H	3′-$CH_2NH(CH_2)_2OH$	NH_2	0.01	3.4	3.6	nd	>10.0	265–Dec.
1q	H	4′-$CH_2NH(CH_2)_2OH$	NH_2	0.03	1.4	nd	1.0	82.5	120–122
1r	3′-OCH_3	4′-$CH_2NH(CH_2)_2OH$	NH_2	0.03	0.3	>1.0	0.23	>10.0	126–128
1s	3′-OCH_3	4′-CH_2CONH-$(CH_2)_2OH$	NH_2	0.02	0.8	>1.0	0.22	>10.0	148–150
1t	3′-OCH_3	4′-$CH_2CH_2N(CH_3)$-$(CH_2)_2OH$	NH_2	0.03	0.3	>1.0	0.3	>10.0	104–106

Table 2. Cellular potency and specificity of selected 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines:

Compound	R1	R2	c-Src Enz. (μM)]	c-Src Cell[12] (μM)	Csk Cell[12] (μM)	EGF-R Cell[13] (μM)	PDGF-R Cell[13] (μM)
1a	H	H	0.1	3.5	>5.0	3.0	nd
1e	3′-OCH_3	H	0.05	3.3	>5.0	>10.0	2.5
1l	H	3′-CH_2OH	0.06	>5.0	>5.0	12.0	10.0
1p	H	3′-$CH_2NH(CH_2)_2OH$	0.01	4.0	5.0	nd	nd
1q	H	4′-$CH_2NH(CH_2)_2OH$	0.03	0.7	4.3	nd	nd
1r	3′-OCH_3	4′-$CH_2NH(CH_2)_2OH$	0.03	0.5	>5.0	6.4	0.6
1s	3′-OCH_3	4′-CH_2CONH-$(CH_2)_2OH$	0.02	3.9	>5.0	nd	nd
1t	3′-OCH_3	4′-$CH_2CH_2N(CH_3)$-$(CH_2)_2OH$	0.03	0.2	>5.0	10.0	0.3

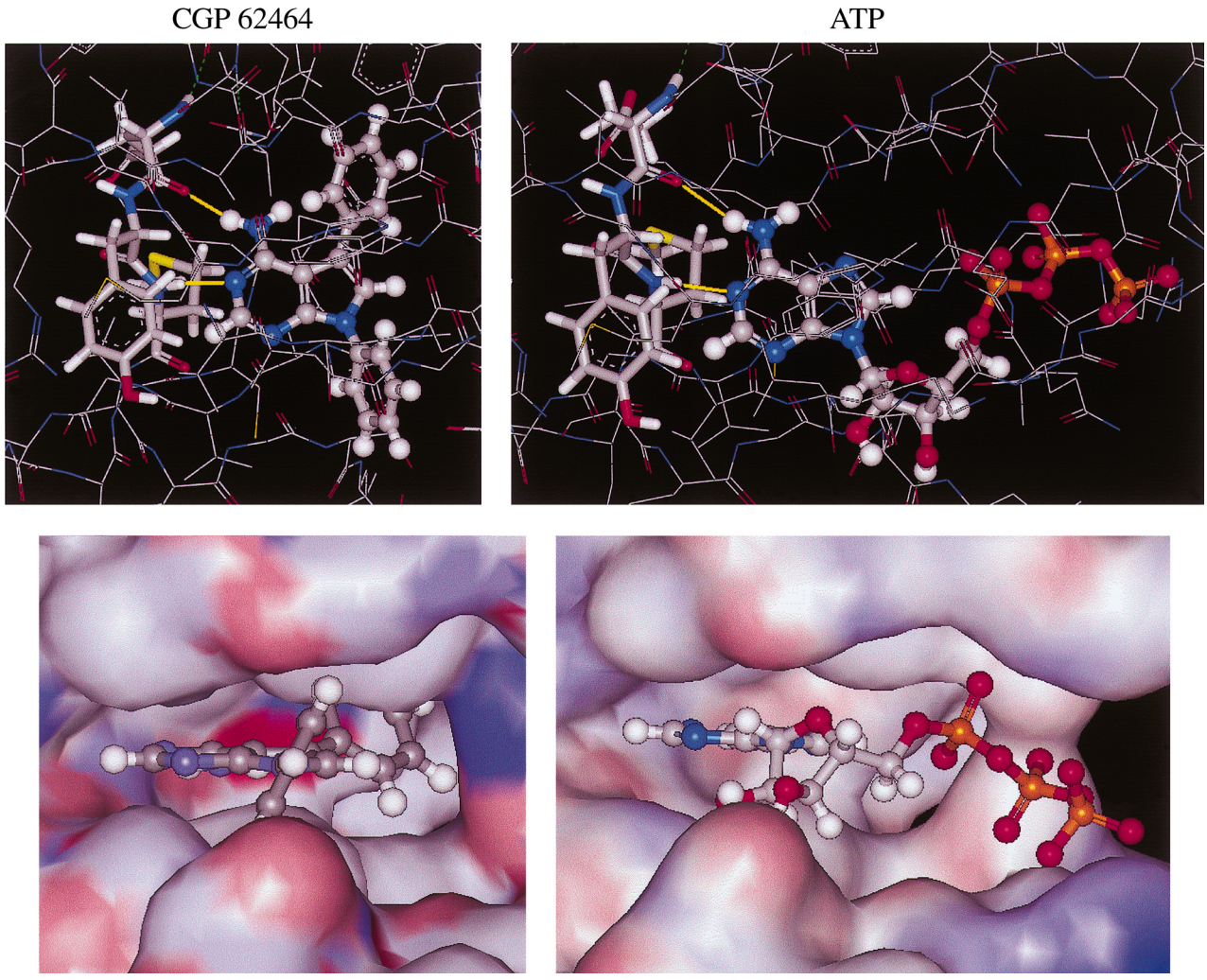


Figure 2.

except for the PDGF-receptor, which was inhibited at concentrations comparable to those necessary for c-Src inhibition.

Conclusions

Our optimization strategy resulted in highly potent and remarkably selective inhibitors of the tyrosine kinase c-Src in vitro. They exhibit potent inhibition of c-Src mediated phosphorylation of intracellular substrates in intact cells. Polar substituents at the phenyl ring in position 7 of 5,7-diphenyl-pyrrolo[2,3*d*]pyrimidines increase the potency of c-Src inhibition in the enzyme assay and the use of substituted ethylamine side chains improves cellular potency.

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