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

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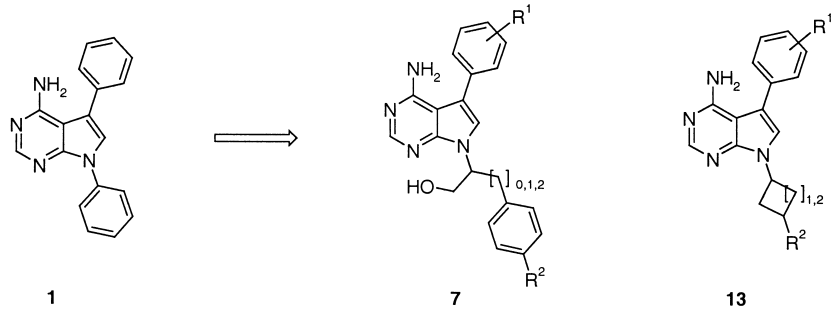
Abstract—7-Substituted-5-aryl-pyrrolo[2,3-*d*]pyrimidines have been prepared starting from α-bromoacetophenones. These compounds represent a novel class of potent inhibitors of the tyrosine kinase $pp60^{c\text{-}Src}$ with good specificity towards other tyrosine kinases (EGF-R, v-Abl). © 2001 Elsevier Science Ltd. All rights reserved.

A number of studies have provided compelling evidence that the non-receptor protein tyrosine kinase $pp60^{c\text{-}Src}$ (c-Src) plays a unique and essential role for osteoclast function.[1–4] As transgenic mice lacking a functional c-Src gene develop normal numbers of osteoclasts, c-Src is probably not involved in osteoclastogenesis. Rather, it appears to be responsible for the phosphorylation of cytoskeletal and/or docking proteins involved in translocation and exocytosis of vesicles during the resorption process. Although c-Src is present in a broad variety of cells, c-Src deficient mice do not show any overt pathological signs other than osteopetrosis, a disease characterized by lack of functionally active osteoclasts.[1] Synthetic c-Src inhibitors reduce osteoclastic bone resorption both in vitro[5] and in vivo[4] and should thus be useful in the treatment of diseases such as osteoporosis and tumor-induced osteolysis.

We report herein on one of our optimization strategies for our lead compound **1** (5,7-diphenyl-pyrrolo[2,3-*d*]pyrimidine, IC_{50} for c-Src inhibition = 0.1 μM), which involves replacement of the N^7-phenyl moiety by different sugar surrogates (Fig. 1).[6]

Based on preliminary data, it was assumed that binding of **1** occurs at the ATP binding site of the enzyme. According to this model, the N^7-phenyl ring is located within the pocket that is usually occupied by the ribose moiety of ATP. On the other hand the 5-phenyl ring extends into a lipophilic pocket which is not exploited by ATP, thus perhaps offering the opportunity to optimize the selectivity profile of analogues of **1** by the introduction of suitable ring substituents. Replacement of the N^7-phenyl substituent by open chain sugar analogues as in **7** or substituted carbacycles as in **13**[7] was expected to improve potency and solubility of our lead compound. The N^7-substituent in compounds of type **7** can be viewed as an open chain sugar analogue, whereas the substituted carbacycles in structures of type **13** represent more direct mimics of the ribose moiety of ATP.



1 **7** **13**

Figure 1.

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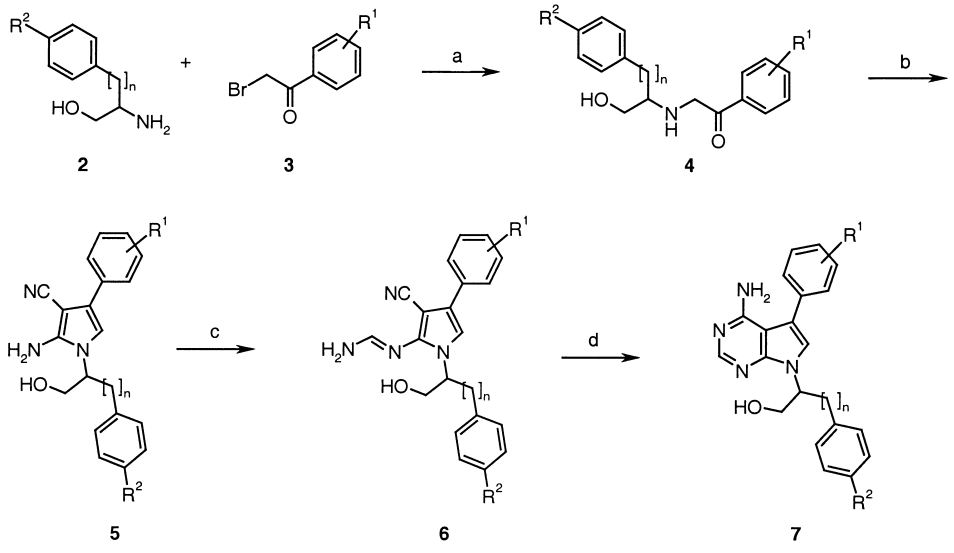
Optimization of the acyclic sugar moiety in analogues **7** was carried out by varying the distance between N^7 and the phenyl ring of the phenyl-alkanol moiety as well as through the introduction of substituents at either or both of the two phenyl rings. The synthesis of these compounds is summarized in Scheme 1.

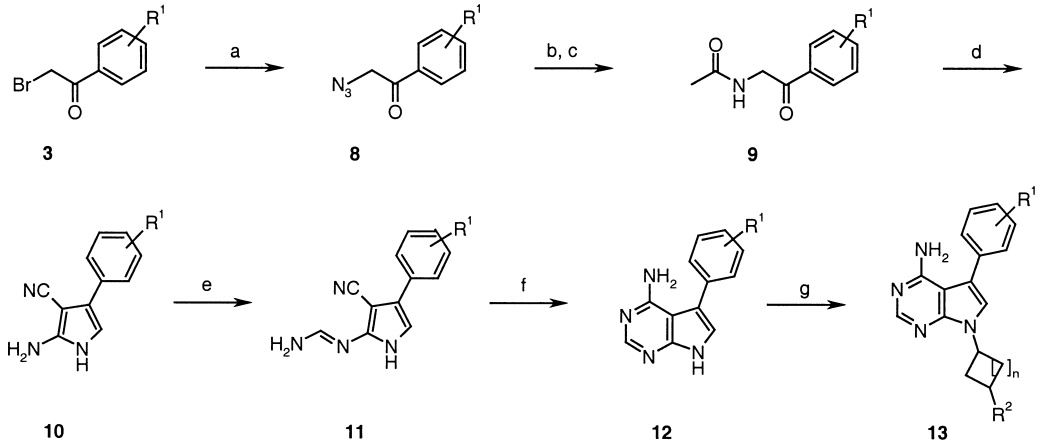
Reaction of amino alcohols **2** with α-bromoacetophenones **3** produced the corresponding substituted amino ketones **4**, which were then converted to 2-amino-3-cyanopyrroles **5** by treatment with malononitrile in the presence of sodium ethoxide. Reaction of **5** with triethyl orthoformate followed by ammonolysis resulted in amidines **6**, which could be transformed into the desired pyrrolo-pyrimidines **7** by sodium ethoxide-induced cyclization and subsequent acidic work-up.

The synthesis of 7-cycloalkyl-pyrrolo-pyrimidines **13** (Scheme 2) in the first part involved the preparation of 4-amino-5-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines **12**,[8] which were obtained by a sequence of reactions similar to those outlined in Scheme 1 for the synthesis of **7**. Treatment of α-bromoacetophenones **3** with sodium azide gave the α-azidoacetophenones **8**, which were subsequently reduced to the corresponding amines. Acetylation of the primary amino group provided substituted acetamides **9**. Condensation of **9** with malononitrile furnished 2-amino-3-cyano-4-phenyl-pyrrole **10**, which was then converted to the amidine **11** by reaction with triethyl orthoformate followed by ammonolysis of the resulting iminoester. Cyclization to the desired 4-amino-5-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidines **12** was achieved by treatment with sodium ethoxide. Attachment of the (substituted) cycloalkyl moieties[9] was achieved via mesylate displacement (**12**→**13a–k**, **13m–p**).

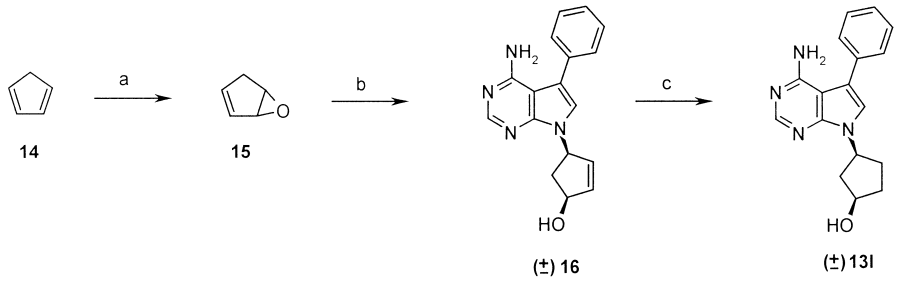
Preparation of the cyclopentanol derivative **13l** (Scheme 3) involved reaction of **12** with the known epoxide **15**[10] to give **16**,[10] followed by catalytic hydrogenation of the olefinic double bond.



Scheme 1. (a) DIEA, EtOH, 55 °C, 25–39%; (b) $CH_2(CN)_2$, NaOEt, EtOH, 55 °C, 89–97%; (c) (i) $HC(OEt)_3$, 80 °C; (ii) NH_3/MeOH, rt, 25–54%; (d) (i) NaOEt, EtOH, 80 °C; (ii) HCl/MeOH, 26–80%.



Scheme 2. (a) NaN_3, $CH_3N[(CH_2)_7CH_3]_3Cl$ (Aliquat™ 366), toluene, 55 °C, 41–87%; (b) H_2, Pt/C, MeOH, HCl, 86–94%; (c) $(Ac)_2O$, Et_3N, THF, rt, 81–89%; (d) $CH_2(CN)_2$, NaOEt, EtOH, 55 °C, 61–94%; (e) (i) $HC(OEt)_3$, 80 °C; (ii) NH_3/MeOH, rt, 68–82%; (f) NaOEt, EtOH, 80 °C, 88–97%; (g) R^2-$C_{4(5)}H_{6(8)}$-OMes, K_2CO_3, 18-crown-6, DMF, 55 °C, 24–96%.



Scheme 3. (a) (i) CH_3COOOH, Na_2CO_3, CH_2Cl_2; (ii) Na_2SO_3, 11%; (b) (i) $Pd[PPh_3]_4$, DMSO/THF (1:1), rt; (ii) **12**, 0 °→25 °C (exclude light), 21%; (c) H_2, Pd/C (10%), MeOH, 98%.

All 7-substituted-5-aryl-pyrrolo[2,3-*d*]pyrimidines were tested for c-Src inhibition in a liquid phase tyrosine phosphorylation assay using chicken c-Src[11] and the synthetic substrate poly-Glu-Tyr (4:1).[4] Inhibitors with an IC_{50} below 0.5 μM were further tested for specificity against EGF-R- and v-Abl-kinase and in a cellular assay measuring the c-Src mediated phosphorylation of Fak in IC8.1 fibroblasts transfected with chicken c-Src (Western blot analysis).[4] The results are summarized in Tables 1 (analogues **7**) and 2 (analogues **13**).

As illustrated by the data summarized in Table 1, compounds **7** with unsubstituted phenyl rings show submicromolar c-Src activity only for $n=0$ (in the case of the *R*-enantiomer) and $n=1$ (**7b**, **c**). Introduction of substituents at the 4-position of the phenyl ring in the phenyl-alkanol moiety of **7c** (→**7e**), led to reduced inhibitory potency. However, a dramatic increase in potency could be achieved by introducing a 3-OH substitutent on the 5-phenyl ring of **7e** (→**7g**), which resulted in an IC_{50} value for c-Src inhibiton of 42 nM. Most notably, **7g** also exhibits a very favorable selectivity profile and possesses submicromolar cellular activity.[12] Methylation of the 3-OH group on the 5-phenyl ring as in compound **7h** proved to be detrimental for c-Src inhibition, resulting in a ca. 25-fold loss in potency in comparison with **7g**. Table 2 summarizes the c-Src inhibitory activity of analogues of structure **13**. It is immediately obvious that the presence of an unsubstituted cyclopentane or cyclobutane ring at the 7-position of the 5-phenyl-pyrrolo[2,3-*d*]pyrimidine moiety generally produces low nanomolar inhibitors of c-Src. Compounds **13c**–**e** and **13k**, **i** also exhibit potent cellular activity, inhibiting cellular phosphorylation of Fak with submicromolar IC_{50} values. Out of these latter analogues **13d** possesses the most favorable overall profile, as it inhibits the tyrosine kinase activity of the EGF receptor and v-Abl ca. 100-fold less potently than that of c-Src. In contrast, **13a**–**c**, **e**, **f**, **h**, and **i** are significantly less selective, in particular with respect to the inhibition of v-Abl kinase. Attachment of a hydroxyl substituent to the cyclopentane or cyclobutane moiety in the case of an unsubstituted 5-phenyl ring (**13l**, **m**) slightly improved activity against c-Src in the enzymatic assay (3- and 5-fold, respectively) but resulted in a loss of cellular potency for **13m**. Compounds bearing substituents larger than hydroxy on the cyclobutane ring were investigated in combination with a *meta*-methoxyphenyl substituent at the 5-position of the pyrrolo-pyrimidine ring system (**13n**–**p**). Out of this series, compounds **13d** and **13p** emerged as the most attractive analogues investigated, as they combine potent cellular activity with a remarkable selectivity profile versus EGF-R and v-Abl.[12,13] In addition, **13p** possesses significantly improved aqueous solubility compared to most other analogues investigated in this study.

In conclusion we have prepared a series of novel and potent c-Src inhibitors. Some of these compounds exhibit an excellent selectivity profile against EGF-R and v-Abl tyrosine kinases.

Table 1. Inhibition of c-Src enzyme activity, cellular activity, and selectivity profile of inhibitors **7**

Compd	R^1	R^2	*n*	c-Src (enzyme)[a] IC_{50} (μM)	c-Src (cell)[b] IC_{50} (μM)	EGF-R[c] IC_{50} (μM)	v-Abl[d] IC_{50} (μM)
7a[e]	H	H	0	4.9	n.d.[h]	n.d.[h]	n.d.[h]
7b[f]	H	H	0	0.48	>5	n.d.[h]	n.d.[h]
7c[g]	H	H	1	0.6	n.d.[h]	0.44	10.5
7d[g]	H	H	2	4	n.d.[h]	n.d.[h]	n.d.[h]
7e[g]	H	OMe	1	>1	>5	14.4	>10
7f[g]	H	OH	1	1.9	n.d.[h]	13.2	2.2
7g[g]	3-OH	OMe	1	0.042	0.4	3.4	0.34
7h[g]	3-OMe	OMe	1	1	n.d.[h]	9.7	0.68

[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.
[c]Inhibition of epidermal growth factor receptor (EGF-R) tyrosine kinase enzyme activity.
[d]Inhibition of v-Abl tyrosine kinase enzyme activity.
[e]*S*-Enantiomer.
[f]*R*-Enantiomer.
[g]Racemate.
[h]Not determined.

Table 2. Inhibition of c-Src enzyme activity, cellular activity, and selectivity profile of inhibitors **13**

Compd	R^1	R^2	*n*	c-Src (enzyme)[a] IC_{50} (μM)	c-Src (cell)[b] IC_{50} (μM)	EGF-R[c] IC_{50} (μM)	v-Abl[d] IC_{50} (μM)
13a	H	H	2	0.14	1.8	0.50	0.14
13b	4-OMe	H	2	0.08	1.2	0.57	0.26
13c	4-OH	H	2	0.02	0.3	0.25	0.11
13d	3-OMe	H	2	0.002	0.17	0.46	0.17
13e	3-OH	H	2	0.001	0.2	0.08	0.01
13f	H	H	1	0.11	1	0.73	0.09
13g	4-OMe	H	1	0.20	3	n.d.[h]	n.d.[h]
13h	4-OH	H	1	0.01	1.5	0.6	0.085
13i	3-OMe	H	1	0.04	0.6	0.45	0.05
13k	3-OH	H	1	0.004	0.1	0.3	0.005
13l[e]	H	OH	2	0.05	0.2	0.79	0.04
13m[f]	H	OH	1	0.02	4	2.82	0.39
13n[g]	3-OMe	CH_2OH	1	0.03	2	0.42	0.04
13o[g]	3-OMe	$CONH(CH_2)_2OH$	1	0.02	4	0.33	0.23
13p[g]	3-OMe	$CH_2NH(CH_2)_2OH$	1	0.02	0.3	0.49	0.42

[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.
[c]Inhibition of epidermal growth factor receptor (EGF-R) tyrosine kinase enzyme activity.
[d]Inhibition of v-Abl tyrosine kinase enzyme activity.
[e]*cis*-Racemate.
[f]*trans*-Racemate.
[g]Diastereomeric mixtures.
[h]Not determined.

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12. IC_{50} values for inhibition of lck, a Src kinase family member have been determined for selected compounds: **7g**: 2.85 μM; **13d**: 0.23 μM; **13p**: 0.94 μM.
13. As pointed out by one of the referees, the apparent lack of correlation between enzyme inhibition and cellular activity might arise from inhibition of (unknown) additional or alternative cellular targets, which have not been addressed by our work. Alternatively, the observed discrepancies may be caused by differencies in cellular penetration for the individual compounds. However, it should be noted that the IC_{50} determined for the inhibition of autophosphorylation of EGF-R in a cellular assay (Elisa) for a number of compounds (**7g**, **13c**, **13e**, **13i**) were ≥ 8 μM, with one exception (**13d**, $IC_{50} = 2.4$ μM).