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POTENT AND SELECTIVE INHIBITORS OF THE ABL-KINASE: PHENYLAMINO-PYRIMIDINE (PAP) DERIVATIVES

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Abstract: Due to its relatively clear etiology, Chronic myelogenous leukemia (CML) represents an ideal disease target for a therapy using a selective inhibitor of the Bcr-Abl tyrosine protein kinase. Extensive optimization of the class of phenylamino-pyrimidines yielded highly potent and selective Bcr-Abl kinase inhibitors. Compound **1** shows high potency (IC_{50} = 38 nM) and selectivity for the Abl tyrosine protein kinase at the *in vitro* level. © 1997, Elsevier Science Ltd. All rights reserved.

INTRODUCTION. Chronic myelogenous leukemia (CML) is a hematological stem cell disorder associated with a specific chromosomal translocation known as the Philadelphia (Ph) chromosome which is detected in 95% of patients with CML and 20% with acute lymphocytic leukemia (ALL)[1]. The molecular consequence of the translocation is the fusion of the *abl* protooncogene to the *bcr* gene resulting in the production of an activated form of the Abl tyrosine protein kinase[2]. The Bcr-Abl protein is capable of inducing leukemia's in mice[3], implicating the protein as the cause of these diseases. As the tyrosine kinase activity of the Bcr-Abl protein is essential to its transforming ability, a specific, well tolerated inhibitor of the Abl tyrosine protein kinase would be a highly attractive therapy for these disorders. This approach could yield a selective anti cancer agent that would be useful for *in vitro* bone marrow purging in preparation for autologous bone marrow transplantation of Bcr-Abl positive leukemia's and for the *in vivo* therapy of these leukemia's. The concept is supported by the fact that inactivation of the *bcr-abl* oncogen by an antisense approach selectively kills leukemia cells harboring this gene[4]. Inhibition of the Abl kinase activity has been reported by low molecular weight compounds such as arylvinylamide, 2-oxindole[5], homophtalimides, benzoisothiazoline-2,2-dioxides, catechols, (for a rewiew of these classes see [6]), tyrphostins[7] and benzopyranones[8]. However, most compounds showed either limited selectivity or low potency. In this communication we enclose our preliminary findings on a series of new phenylamino-pyrimidine (PAP)-derivatives capable of inhibiting phosphorylation by the v-Abl kinase at nanomolar concentrations.

CHEMISTRY. Synthesis of the phenylamino-pyrimidines was started from acetyl-substituted heterocycles or aryl-derivatives **A** which were converted to the enaminones **B** using N,N-dimethl-formamide dimethylacetal. The heterocyclic ring system **C** (**10**, **20**, **22**, **25**, **26**, **29**, **31**, **34-37**) was constructed by the reaction of the substituted phenylguanidines with the enaminones B. The optional functional group transformation of substituents attached at the phenyl ring included hydrogenation (H_2, Pd/C) of the nitro group to give the anilines **16**, **17**, and **24**. Acylation with the required acid chlorides yielded the amides **1-8**, **11-15**, **18**, **19**, **21**, **23**, **27**, **28** (phthalic anhydride was used as the acylation reagent), **30** and **32**. Alkaline hydrolysis of the ester **31**

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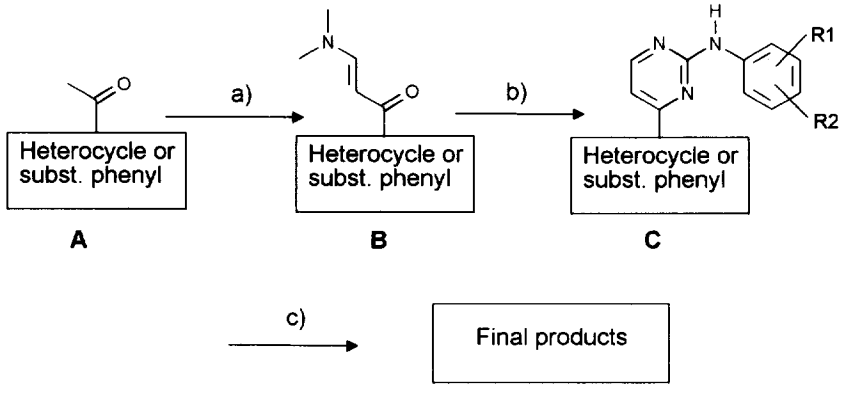
give the carboxylic acid **33**. Synthesis of the required guanidines for the preparation of **20** and **29** are described elsewhere[9]. Synthesis of **9** is depicted in *scheme 2*.

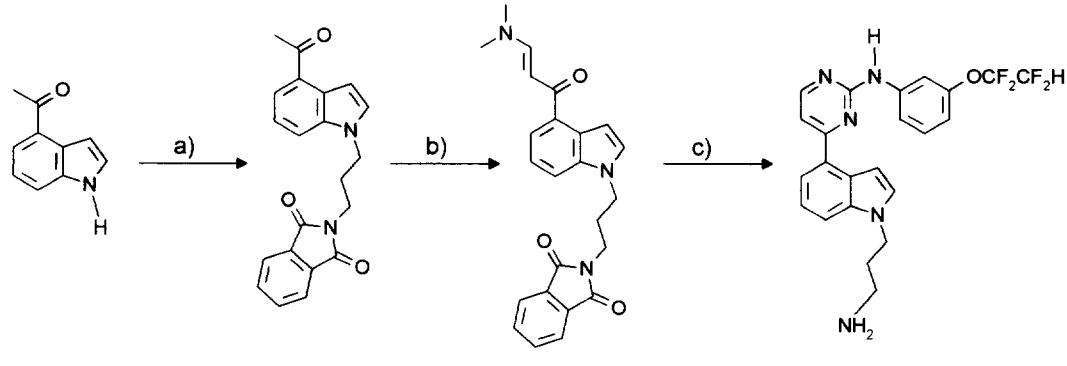
The physicochemical properties of the generally poorly soluble pyrimidine-derivatives can be improved by attachment of hydrophilic substituents on the phenyl ring. For example compound **1**[10] is a polyvalent base (pKa,1 : 8.07; pKa,2 : 3.73; pKa,3 : 2.56, pKa,4 : 1.52), the corresponding methane sulfonate shows good solubility in water (> 100 g/l), yielding an acidic solution (pH: 4.2). The solubility at pH 7.4 however is much lower (49 mg/l). At pH 7.4, the methane sulfonate of **1** is rather lipophilic with a log P of 1.99.

Scheme 1: **General synthesis of the PAP's**



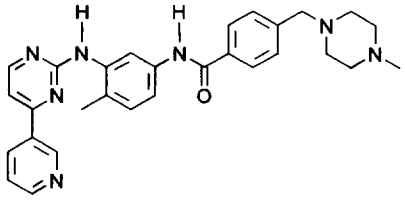
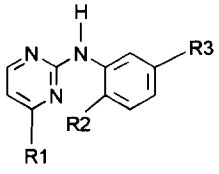
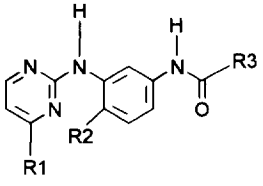
a) Dimethyl-formamide dimethylacetal (neat). b) subst. phenylguanidine, i-propanol, reflux.
c) Optional functional-group transformation of R1/R2.

Scheme 2: **Synthesis of compound 9**



a) N-(3-Bromopropyl)phthalimide, NaH. b) N,N-Dimethylformamide dimethylacetal.
c) i:3-Tetrafluoroethoxyphenylguanidine, i-propanol, rf. ii: Hydrazine hydrate in ethanol

Table 1: Compounds prepared

		
1	**A**	**B**

No.	Form.	R1	R2	R3	mp [°C]
1	1	see above	"	"	207-212
2	B	3-pyridyl	H	identical as in compound 1	198-201
3	B	3-pyridyl	H	3-pyridyl	217-220
4	B	3-pyridyl	CH_3	4-methylphenyl	102-106
5	B	3-pyridyl	H	pentyl	180-184
6	B	3-pyridyl	CH_3	2-naphtyl	97-101
7	B	3-pyridyl	H	4-fluorophenyl	215-216
8	B	3-pyridyl	H	2-thiophenyl	139-141
9	A	see scheme 2	"	tetrafluoroethoxy	amorph.
10	A	3-indolyl	H	tetrafluoroethoxy	140-142
11	B	3-pyridyl	H	phenyl	207-209
12	B	3-pyridyl	CH_3	phenyl	179-180
13	A	3-pyridyl	H	3-aminopropylaminocarbonyl	142-146
14	B	3-pyridyl	H	cyclohexyl	205-206
15	B	3-pyridyl	H	4-pyridyl	224-226
16	A	4-pyridyl	H	amino	200-202
17	A	3-pyridyl	H	amino	58-68
18	B	3-pyridyl	CH_3	2-methoxyphenyl	88-92
19	B	3-pyridyl	CH_3	4-chlorophenyl	216-219
20	A	3-pyridyl	H	2-(1-imidazoly)ethoxy	amorph.
21	B	3-pyridyl	H	4-methylphenyl	214-216
22	A	2-pyridyl	H	nitro	213-219
23	B	3-pyridyl	H	4-cyanophenyl	220-222
24	A	3-pyridyl	CH_3	amino	143-144
25	A	3-pyridyl	H	chloro	146-147
26	A	3-pyridyl	H	hydrogen	143-144
27	B	3-pyridyl	H	2-methoxyphenyl	147-150
28	B	3-pyridyl	H	2-carboxyphenyl	206-209

29	A	3-pyridyl	H	1-imidazolyl	132-134
30	B	3-pyridyl	H	2-pyridyl	187-190
31	A	3-pyridyl	H	methoxycarbonyl	192-195
32	B	3-pyridyl	CH_3	methyl	220-222
33	A	3-pyridyl	H	carboxy	292-294
34	A	3-pyridyl	H	nitro	212-213
35	A	4-chlorophenyl	H	tetrafluoroethoxy	133-135
36	A	4-pyridyl	H	nitro	282-284
37	A	4-pyridyl	H	tetrafluoroethoxy	193-194

RESULTS AND DISCUSSION. The compounds were assayed for the inhibition of four tyrosine kinases (v-Abl, PDGFR-kinase, EGFR-kinase and c-Src) and three serine/threonine kinases (PKCα, PKCδ and PKA)[10], see *table 2*. The optimization aimed at potent inhibition of the phosphorylation by the v-Abl kinase whereas the six other kinases tested served as selectivity criteria. Compounds **1-19** inhibited the v-Abl-kinase *in vitro* with the IC_{50} values of less than 1 μM, the most active compound being tested was derivative **1** (IC_{50} = 38 nM). The most potent compounds on the inhibition of the v-Abl kinase (IC50 < 300 nM) are members of the structural class **B**, e.g. containing an amide function on the phenyl ring. The nature of the acyl-substituent is of minor importance: substituted phenyl (**1**,**4**,**6** and **7**), heterocycles (**3** and **8**) and alkyl (**5**) are tolerated. The 3-pyridyl moiety in 4-position of the pyrimidine can be replaced by an indoyl moiety. In this case, the amide function on the phenyl ring (structure type **B**) is not required any more (**9**, **10**) for efficient inhibitory activity. In this indoyl-series, improvement of the aqueous solubility can be accomplished by attachment of a salt forming group on the indole side chain (**10**). The benzamide moiety in structural type B can be replaced by an 3-aminopropylaminocarbonyl moiety (**13**) and with the corresponding amine although with a slight decrease of potency (**16**, **17**).

The compounds were further screened for selectivity. None of the PAP's showed inhibition (IC_{50} > 1 μM) of the protein kinase A and the EGFR kinase with the exception of compound **9**. The c-Src catalyzed phosphorylation was inhibited by only a few derivatives. The inhibition of the PDGFR-kinase autophosphorylation however was found to be similar to the activity measured for the v-Abl kinase. But also here it is possible to gain selectivity: e.g. the potent v-abl kinase inhibitor **3** was devoid of PDGF activity. The selectivity against PKC-α and -δ was not very difficult to achieve within this series, compound **1** was not inhibiting PKC at all and the v-Abl inactive derivative **37** showed a selective PKC-α inhibition. The methyl-group in 6-position of the phenyl-ring helps to improve on selectivity: compounds **1**, **4** and **6** bearing this "flag-methyl" do not inhibit PKC-α and -δ to a significant extent, whereas compounds lacking this group are dual inhibitors (e.g. **3**).

Remarkable is compound **9**, it showed absolutely no selectivity at all against the seven kinases tested. It is interesting to note that the class of the PAP's can also serve as leads for other kinases (PKC, PKA, c-src, EGF-R-K, PDGF-R-K): compound **9** inhibited the EGF-R autophosphorylation and c-Src with an IC_{50} of 0.53 μM and 0.5 respectively. The very potent (IC_{50}: 0.01 μM) PDGF-R inhibitors **4** and **19** and the PKC-α inhibitors **9**, **10**, **23**, **27**, **34**, **35** and **37** could serve as starting points for the respective projects whereas for the PKC-δ and specially PKA projects none of the tested members of this class could serve as a promising lead.

Table 2: Enzymatic profile, IC_{50} [μM]

No	v-Abl-K	EGFR	c-Src	PDGFR	PKA	PKCα	PKCδ
1	0.038	>100	>100	0.05	>500	>100	>100
2	0.1	>100	7.8	0.2	475	n.d.	n.d.
3	0.15	>100	9	50	>500	1.7	17
4	0.2	>100	>100	0.01	>100	>100	>500
5	0.2	>100	3.7	0.6	n.d.	2.0	19
6	0.2	>100	>100	0.05	>100	>100	>500
7	0.2	>50	>100	3	>500	n.d.	n.d.
8	0.3	>50	3	20	>500	n.d.	n.d.
9	0.35	0.53	0.5	5	2	0.18	4.4
10	0.37	1.8	2.2	>10	73	0.29	1.4
11	0.4	>50	15.7	5	>500	1.2	23
12	0.4	65	>100	0.1	>500	72	>500
13	0.4	45	9.8	>10	n.d.	n.d.	n.d.
14	0.45	>100	46	0.8	>500	n.d.	n.d.
15	0.5	>100	78	>100	>500	1.3	24
16	0.6	n.d.	16	>10	n.d.	n.d.	n.d.
17	0.7	50	14	>100	6.0	210	>500
18	0.8	>100	>100	0.3	>500	80	>100
19	0.8	>100	>100	0.01	>100	>100	>100
20	1.0	7.1	4.9	>10	340	1.5	34
21	1.0	>100	>100	0.8	>500	n.d.	n.d.
22	1.2	>100	>100	>100	>500	2.5	>500
23	1.3	>100	>100	1.5	>500	0.35	>500
24	1.5	>100	>100	>10	>500	240	>500
25	1.5	>100	>100	>10	>500	1.6	23
26	1.8	100	>100	>10	>500	10.5	39
27	1.9	100	>100	1.0	>500	0.32	>500
28	2.0	n.d.	44	>100	>500	n.d.	n.d.
29	3.3	>100	1.6	18	>500	1.0	21
30	3.8	n.d.	90	>100	>500	n.d.	n.d.
31	5.1	n.d.	90	>10	>500	2.5	39
32	5.8	>100	>100	50	>500	500	>500
33	8.0	n.d.	126	>10	n.d.	45	>100
34	34	100	93	>100	>500	0.79	>500
35	>50	>100	>100	>10	>500	0.85	>100
36	61	>100	>100	>100	>500	2.6	>500
37	>100	>100	>100	>10	>500	0.3	>100

The selectivity of compound **1** on the enzymatic assays for Abl- and PDGFR-kinase could be translated to the cellular and *in vivo* situation: *v-abl-* and *v-sis* transformed BALB/c 3T3 cells were inhibited *in vitro* and *in vivo*[11]. The reported findings with compound **1** suggest that it may be a development candidate for use in the treatment of Philadelphia chromosome-positive leukemia's[12]. Additional potential applications for compound **1** may include proliferative diseases that involve abnormal PDGF receptor activation.

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