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PHENYLAMINO-PYRIMIDINE (PAP) - DERIVATIVES: A NEW CLASS OF POTENT AND HIGHLY SELECTIVE PDGF-RECEPTOR AUTOPHOSPHORYLATION INHIBITORS

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Abstract: Phenylamino-pyrimidines represent a novel class of inhibitors of the PDGF-receptor autophosphorylation with a high degree of selectivity versus other tyrosine and serine/threonine kinases. Optimum activity of ca 10 nM (IC50) was observed when the phenylamino-group which is attached to the pyrimidine carries a benzamide-moiety with a lipophilic substituent in 4-position.
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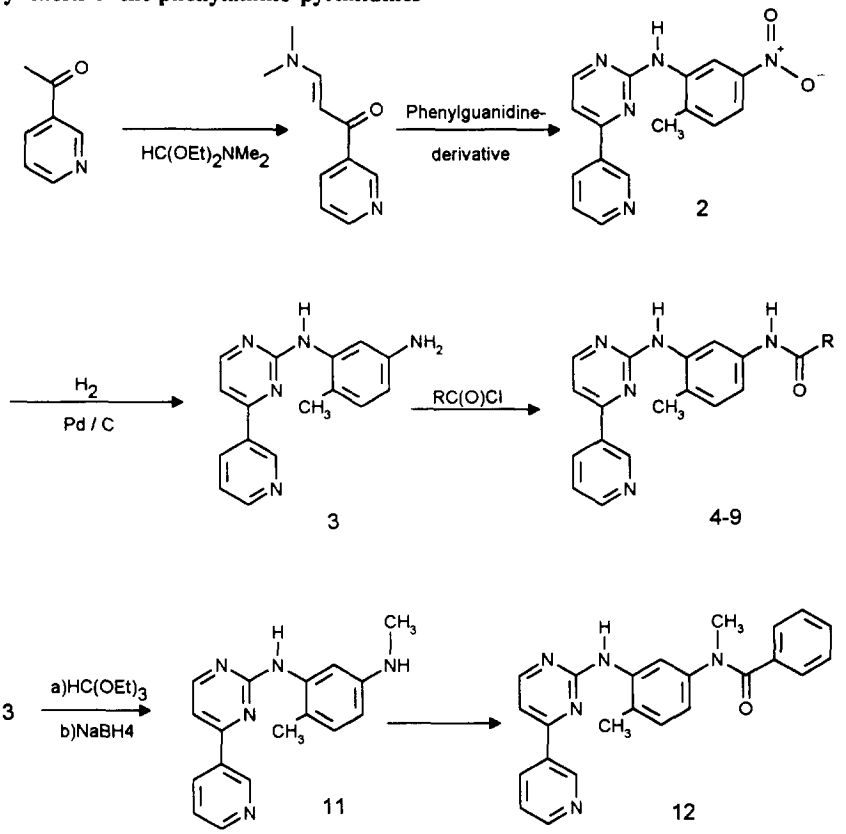
INTRODUCTION. Platelet-derived growth factor (PDGF) is a potent mitogen and chemotactic factor for a variety of cells of mesenchymal origin such as fibroblasts, vascular smooth muscle cells and brain glial cells[1] . There are three isoforms of PDGF[2,3] which bind with different affinities to two related tyrosine kinase receptors (PDGF-R)[4,5]. Binding of PDGF to the extracellular part of either receptor type leads to dimerization of receptor molecules, followed by activation of the receptor tyrosine protein kinase[6] and generation of phosphorylation mediated signals which initiate the biological response[7,8]. Much of the interest in PDGF emanates from the finding that the v-*sis* oncogene of the simian sarcoma virus is a retroviral homolog of the cellular gene encoding the B-chain of PDGF[9,10]. Gene transfer experiments have shown that overexpression of the normal human PDGF B-chain gene (c-*sis* protooncogene) causes the generation of fibrosarcomas[11]. Evidence for the involvement of an autocrine mechanism in the transformation of cultured cells by the *sis* oncogene has emerged from the finding that agents preventing the interaction between PDGF and its receptor such as PDGF antibodies[12] or suramin[13] revert the transformed phenotype. Furthermore, dominant-negative mutants of PDGF have been shown to break the autocrine loop and to revert the transformed phenotype of both, PDGF-transformed BALB/c 3T3 cells and human astrocytoma cell lines[14]. The hypothesis that unscheduled production of PDGF may contribute to the growth of spontaneous tumors is supported by the finding that PDGF is frequently produced by cell lines from human tumors (reviewed in[15]). Furthermore, PDGF has been implicated in the pathogenesis of several non-malignant proliferative diseases including fibrosis, atherosclerosis[16] and restenosis following vascular angioplasty[17,18].

The accumulating evidence for the involvement of PDGF in human proliferative disorders has led to a search for specific inhibitors of the PDGF receptor tyrosine protein kinase. Inhibition of the PDGF receptor kinase activity has been reported for low molecular weight compounds from the staurosporine[19-21], tyrphostin[22-24] and quinoline[25] classes of compounds. However, most compounds showed either limited selectivity or limited potency at the cellular level. In this Letter we enclose our preliminary findings on a series of new phenylamino-pyrimidine (PAP)-derivatives which are capable of inhibiting cellular PDGF-receptor autophosphorylation at nanomolar concentration. Potential clinical applications of these inhibitors include their use as anticancer agents, as well as a drug for the treatment of conditions characterized by inappropriate fibroblast and vascular intimal hyperplasia.

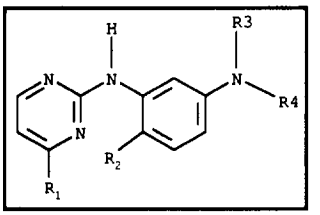
CHEMISTRY. Phenylamino-pyrimidines (PAP) were prepared via the classical route[26] for the synthesis of the pyrimidine-ring system. The acetyl derivatives of different heterocycles were converted into the enaminones using N,N-dimethylformamide-diethylacetal which were then brought in reaction with the corresponding guanidines to give phenylamino-pyrimidines[27]. Catalytic hydrogenation of the nitro group in 2 resulted in the amine 3, which was acylated with the corresponding acid chlorides to give the amides 4-9, vigourous conditions yielded the diamide 10. The methylation of the aniline-group in 3 turned out to be rather difficult. However treatment with ethyl-ortho-formate followed by reduction with sodiumborohydride was finally successful. The methyl-derivative 11 could be isolated in 35% overall yield. The benzoylation with benzoylchloride in pyridine afforded 12. Oxidation of 4 with *m*-chloro perbenzoic acid gave the pyridine-N-oxide 13; mild conditions have to be used to avoid oxidization of the pyrimidine-nitrogens. For the synthesis of 14-16, the same methodology was applied starting either with 3-acetylthiophene or using differently substituted guanidine-derivatives.

Scheme 1: Synthesis of the phenylamino-pyrimidines



In general, the compounds show poor solubility in water (e.g. 4: 1.9 mg/l), but are soluble under acidic conditions (4: HCl 0.1N = 3.3 g/l). The crystalline derivatives are slightly basic (e.g. 4: pK_a = 3.77) and are rather lipophilic (e.g. 4: logP = 3.57).

Table 1. Data for the compounds tested



No	R1	R2	R3	R4	FAB-MS	mp[°C]
1	3-pyridyl	H	H	$C(O)C_6H_5$	368	207-209
2	3-pyridyl	CH_3	O	O	308	195-198
3	3-pyridyl	CH_3	H	H	278	138-140
4	3-pyridyl	CH_3	H	$C(O)C_6H_5$	382	179-180
5	3-pyridyl	CH_3	H	$C(O)$-(o-OCH_3)C_6H_4	412	88-92
6	3-pyridyl	CH_3	H	$C(O)$-(p-CH_3)C_6H_4	396	102-106
7	3-pyridyl	CH_3	H	$C(O)$-(p-Cl)C_6H_4	416	216-219
8	3-pyridyl	CH_3	H	$C(O)CH_3$	320	220-222
9	3-pyridyl	CH_3	H	C(O)-2-naphtyl	432	97-101
10	3-pyridyl	CH_3	$C(O)C_6H_5$	$C(O)C_6H_5$	488	255-258
11	3-pyridyl	CH_3	CH_3	H	292	120-122
12	3-pyridyl	CH_3	CH_3	$C(O)C_6H_5$	396	amorph.
13	3-pyridyl-N-oxide	CH_3	H	$C(O)C_6H_5$	398	238-241
14	2-thiophenyl	CH_3	H	$C(O)C_6H_5$	387	206-207
15	3-pyridyl	Cl	H	$C(O)C_6H_5$	402	221-222
16	3-pyridyl	OCH_3	H	$C(O)C_6H_5$	398	222-223

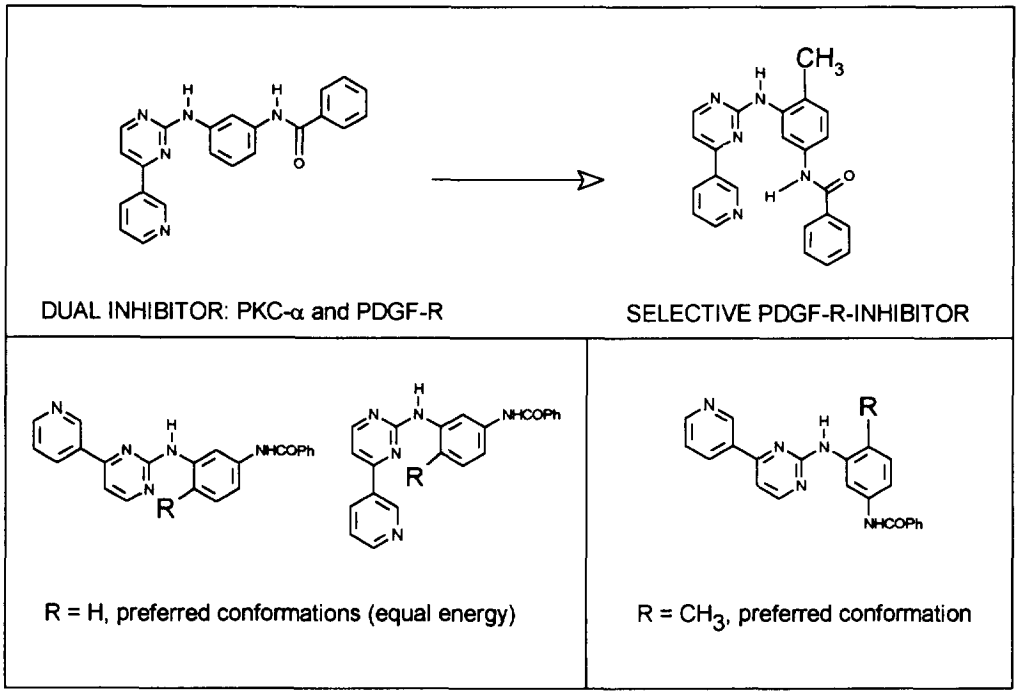
RESULTS AND DISCUSSION. The compounds were assayed for the inhibition of three tyrosine kinases (PDGF-receptor, EGF-receptor and c-Src) and three serine/threonine kinases (PKC-α, PKC-δ and PKA)[28]. The IC50's are the average of at least two independent experiments, the standard deviation is +/- 10%.

At the onset of our work in the PDGF-program the phenylamino-pyrimidine 1 was identified as an attractive lead. However this compound is a dual inhibitor, acting on PKC-α and PDGF-R in the submicromolar range. With the introduction of a methyl group in 6-position of the phenyl-ring, the inhibition of PKC-α was lost, whereas the potency for inhibition of the PDGF-receptor autophosphorylation was enhanced. The dramatic loss of activity on PKC-α can either be explained by the forced change of the preferred conformation upon the introduction of this flag-methyl (scheme 2). Either the required shape of the inhibitor for a tight binding is then not achievable or the flag-methyl group severely clashes with the wall of the ATP-binding pocket within the protein kinase C family[29]. This "flag-methyl" effect works reliably, the nitro- and amino-derivatives without this substituent are potent PKC-α inhibitors (IC50: 1-5 μM; data not shown) but upon introduction of this flag-methyl (2,3), inhibition of PKC is lost. Compounds 2,3,4,6,7 and 9 were also tested on PKC-β1, β2, γ, ε, and η (data not shown), which where not inhibited (IC50 > 100 μM).

Table 2: Enzymatic Profile, IC50 [μM]

No.	PDGF-R	EGF-R	c-Src	PKC-α	PKC-δ	PKA
1	5	>50	15.7	1.2	23	>500
2	>100	>100	>100	>500	>500	>500
3	>10	>100	>100	>100	>500	>500
4	0.1	>100	>100	72	>500	>500
5	0.3	>100	>100	>100	>500	>500
6	**0.01**	**>100**	**>100**	**>100**	**>500**	**>100**
7	**0.01**	**>100**	**>100**	**>100**	**>500**	**>100**
8	50	>100	>100	>100	>500	>500
9	0.05	>100	>100	>100	>500	>100
10	2.5	75	n.d.	>100	>100	>500
11	>10	>100	n.d.	71	>100	>500
12	>100	49	n.d.	85	>100	>500
13	>10	>100	n.d.	>100	>100	>500
14	>10	>100	>100	>100	>100	>500
15	0.15	>100	n.d.	>100	>100	>500
16	>10	>100	n.d.	10	>100	>500

Scheme 2: Increase of selectivity and analysis of the conformation[30]



The pharmacophore of this class for inhibition of PDGF-receptor can be defined as follows: phenylamino-pyrimidine with a pyridine attached in 4-position of the pyrimidine ring system. The substitution pattern of the phenyl group has to be as follows: a methyl- or chloro-substituent in 6-position ($R_2 = CH_3$ or Cl) and a substituted benzamide (R4 = C(O)-phenyl) with a free NH-group (R_3 = H) in 3-position. The benzamide is preferentially substituted again with a small lipophilic substituent (chlorine or methyl) in 4-position of the benzamide moiety (comp. 6 and 7).

Despite the sequence homology in the ATP-binding region among serine/threonine and tyrosine kinases,[31] these results show that ATP-competitive compounds have the potential to be potent and selective inhibitors of individual protein kinases. A possible explanation for the observed high selectivity lies in the fact, that ATP is only weakly bound, so minor changes of amino acids in the ATP-binding pocket do not change the already low affinity for ATP but do alter the affinity of inhibitors. In summary, PAP-derivatives represent a novel class of inhibitors of the PDGF-R family of protein kinases with high selectivity and potency at the enzymatic and cellular level. Furthermore, they are efficiently taken up by the cells. Experimental data show that this high selectivity can be translated into a very specific PDGF-dependent tumor-growth inhibition *in vivo*[32].

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27. A typical synthesis is done as follows: To a solution of 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one[33] (119.8 g, 0.68 mol) in isopropanol (700 ml) was added 3-nitro-phenyl-guanidine nitrate (165.4 g, 0.68 mol), suspended in isopropanol (300 ml). Following the addition of sodium hydroxide (30.0 g, 0.75 mol), the reaction mixture is stirred for 8 hours at reflux. After cooling to room temperature, the suspension is filtrated and the product washed with isopropanol (200 ml). The residue is suspended in water (300 ml), stirred at rt for 30 min, filtrated and washed with ethanol and ether to give 82.4 g (41.3 %) of N-(3-nitrophenyl)-4-(3-pyridyl)-2-pyrimidinamine as a solid. A suspension thereof (17.0 g, 0.058 mol) and palladium (5% on charcoal, 1.7 g) in tetrahydrofurane (1700 ml) was stirred under a hydrogen athmosphere (1 bar) for 21 h at rt. Filtration, evaporation and crystallisation (methylenchloride) gave N-(3-amino-phenyl)-4-(3-pyridyl)-2-pyrimidinamine (9.3 g, 60.9 %). A solution of this amine (9.3 g, 35.3 mMol) and benzoylchloride (7.57 g, 53.9 mmol) in pyridine (300 ml) was stirred at rt for 23 h. Evaporation and crystallisation (dimethylformamide, water) gave 2.37 g (18.3 %) of 1 as a yellowish solid.
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