Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 18, pp. 2415-2420, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00445-9

DISCOVERY AND STRUCTURE–ACTIVITY STUDIES OF A NOVEL SERIES OF PYRIDO[2,3-*d*]PYRIMIDINE TYROSINE KINASE INHIBITORS

Cleo J. C. Connolly,[a] James M. Hamby,[a] Mel C. Schroeder,[a] Mark Barvian,[a] Gina H. Lu,[b] Robert L. Panek,[b] Aneesa Amar,[c] Cindy Shen,[c] Alan J. Kraker,[c] David W. Fry,[c] Wayne D. Klohs,[c] and Annette M. Doherty[a]

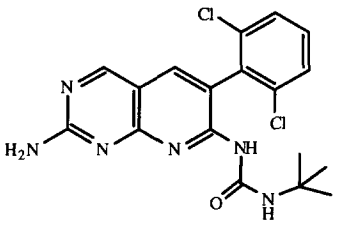
Departments of [a]Chemistry, [b]Vascular and Cardiac Diseases, and [c]Cancer Research, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105.

Abstract: The inhibition of tyrosine kinase–mediated signal transduction pathways represents a therapeutic approach to the intervention of proliferative diseases such as cancer, atherosclerosis, and restenosis. A novel series of pyrido[2,3-*d*]pyrimidine inhibitors of the PDGFr, bFGFr, and c-Src tyrosine kinases was developed from compound library screening and lead optimization.[1] In addition, highly selective inhibitors of the FGFr tyrosine kinase were also discovered and developed from this novel series of pyrido[2,3-*d*]pyrimidines. The syntheses, biological evaluation, and structure–activity relationships of this series are reported.

Introduction: Protein tyrosine kinases (PTKs) catalyze the selective transfer of a phosphate group from ATP to a tyrosine hydroxyl residue of a substrate protein. Tyrosine phosphorylation is a critical event in growth factor mediated signal transduction and PTKs are key components of this process.[2] The aberrant overexpression of receptor PTKs or their cognate ligands has been implicated in the pathogenesis of proliferative diseases. For example, strong evidence exists for the role of the platelet-derived growth factor (PDGF) and the basic fibroblast growth factor (bFGF) in such diseases as restenosis,[3] atherosclerosis,[4] and cancer.[5] FGF is also reputed to be a potent angiogenic factor for which tumor cells are dependent upon to grow and metastasize.[6] In addition, elevated levels of $pp60^{c\text{-}Src}$(c-Src), a nonreceptor cytoplasmic PTK, has been associated with a variety of human malignancies.[7,8] Thus, given the critical role of these PTKs in the propagation of abnormal mitogenic growth signaling, inhibitors of the PDGF receptor (PDGFr), FGF receptor (FGFr) and c-Src tyrosine kinases represent a potential therapeutic strategy for controlling a variety of proliferative disorders.

Results and Discussion: Screening of our compound library for inhibitors of the PDGF and FGF receptor tyrosine kinases led to the discovery of a novel pyrido[2,3-*d*]pyrimidine tyrosine kinase inhibitor, **5**, PD 089828 (see Figure 1). Compound **5** inhibited PDGFr and FGFr tyrosine kinase activity with IC_{50} values of 1.25 and 0.14 μM, respectively. Upon further evaluation, **5** was also found to inhibit the cytosolic c-Src tyrosine kinase activity with an IC_{50} value for inhibition in the submicromolar range (see Table 1). A structure–activity relationship (SAR) study focusing on phenyl substitution in the 6-position was initiated to determine structural requirements for optimizing potency of these three kinases and enzyme selectivity at this position (see Table 1).[9]

FIGURE 1.



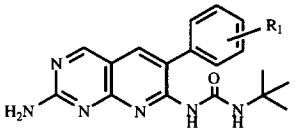
5 (PD 089828)

The SAR study generally revealed that either 2- or 2,6-disubstituted phenyl moieties in the 6-position possessing either methyl or halogen substituents resulted in an increase in PDGFr, FGFr, and c-Src tyrosine kinase inhibitory activity relative to the unsubstituted parent compound, **4**. The greatest increases in activities were realized with the 2,6-disubstituted compounds (**8**, **9**, and **15**) which were a log order more potent against PDGFr and FGFr tyrosine kinases and several orders of magnitude more potent against the c-Src tyrosine kinase compared to **4**. Tyrosine kinase activity was least affected by a 2,6-difluoro substitution (compound **10**) in the phenyl ring producing only a modest increase in FGFr-TK and c-Src kinase activity. Larger groups in the 2-position of the phenyl ring such as ethyl (compound **22**), or methoxy (compound **24**), resulted in a decrease in activity against PDGFr-TK and FGFr-TK with little or no effect on c-Src activity. Substitutions in the 4-position of the phenyl ring resulted in a loss or decrease in activity against all three kinases as exemplified by **14**, **26** and **27**. However, the 2,4,6-trimethyl analog, **18**, retained similar tyrosine kinase activity relative to the 2,6-diMe analog, **15**, even though it contained a methyl substituent in the 4-position.

Most interestingly, substitutions at the 3- and 5-positions of the phenyl ring showed a high degree of selectivity for the FGFr tyrosine kinase relative to that of the other kinases (see Table 2). Initially, the 3,5-dimethyl analog, **17**, was synthesized and displayed selectivity for the FGFr-TK (IC_{50} = 1.13 μM) relative to the PDGFr-TK (IC_{50} = 52.9 μM) and the c-Src tyrosine kinase (IC_{50} > 50 μM). This interesting finding led us to investigate further the effects of 3,5-substitution on FGFr selectivity. The 2,3,5,6-tetramethyl analog, **19**, was selective for FGFr-TK with an IC_{50} value of 0.71 μM and was inactive against PDGFr and c-Src tyrosine kinases. Compared to **19**, the 3,5-dimethoxy analog, **28**, was an order of magnitude more potent against the FGFr-TK with an IC_{50} value of 0.06 μM. Similar to **19**, **28** was inactive against the PDGFr-TK and c-Src tyrosine kinases (Table 3). Increasing the size of the 3,5-dialkoxy substituents from 3,5-diOMe (**28**) to 3,5-diOEt (**30**) retained FGFr-TK selectivity but was accompanied by a decrease in potency for inhibition of the FGFr-TK (IC_{50} = 1.65 μM for **30** vs IC_{50} = 0.06 μM for **28**). The 3,5-diEt (**23**) and 3,5-diNMe_2 (**31**) analogs were also selective but less potent than compound **28**. The 3,5-diF (**11**) analog revealed a decrease in potency and showed no selectivity against FGFr-TK while the 3,5-diCF_3 (**12**) analog was inactive against PDGFr, FGFr, and c-Src tyrosine kinases.

Structure–Activity Relationships:

Table 1. Substituted Phenyl Analogs

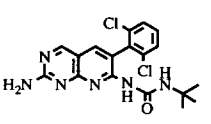
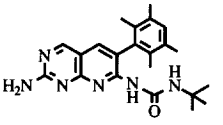
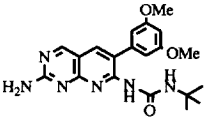


No.	R_1	PDGFr-TK (IC_{50}, μM)[10]	FGFr-TK (IC_{50}, μM)[10]	c-SRC-TK (IC_{50}, μM)[10]
4	H	13.24	8.0	19.33
5	**2,6-diCl**	**1.25**	**0.14**	**0.22**
6	2,3-diCl	2.26	0.16	4.41
7	2,3,6-triCl	2.96	0.11	1.41
8	2,6-diBr	1.42	0.29	0.21
9	2-Br, 6-Cl	0.62	0.18	0.21
10	2,6-diF	1.67	0.11	0.80
11	3,5-diF	8.97	1.10	1.35
12	3,5-diCF_3	>50	>50	>50
13	2-Me	1.05	1.40	0.41
14	4-Me	6.31	1.67	17.50
15	2,6-diMe	0.34	0.40	0.11
16	2,3-diMe	6.05	0.34	4.17
17	3,5-diMe	52.90	1.13	>50
18	2,4,6-triMe	1.47	0.27	0.36
19	**2,3,5,6-tetraMe**	**>50**	**0.71**	**>50**
20	2,3,4,5,6-pentaMe	>50	1.62	>50
21	2,6-diMe, 3-$OCH_2CH_2NEt_2$	11.81	0.89	1.65
22	2-Et	4.48	11.22	10.43
23	3,5-diEt	>50	7.76	>50
24	2-OMe	4.48	11.22	10.43
25	3-OMe	22.93	0.36	36.40
26	4-OMe	2.89	3.97	>50
27	3,4-diOMe	>50	20.25	>50
28	**3,5-diOMe**	**>50**	**0.06**	**>50**
29	3-OEt	23.00	0.67	>50
30	3,5-diOEt	>50	1.65	>50
31	3,5-diNMe_2	>50	16.00	>50

Since compounds **19** and **28** were the most potent and selective inhibitors of the FGFr-TK from this series, they were selected for further profiling against an expanded panel of protein kinases. As seen in Tables 1 and 2, compounds **19** (PD 162628) and **28** (PD 166866) retained their high degree of selectivity for the FGFr-

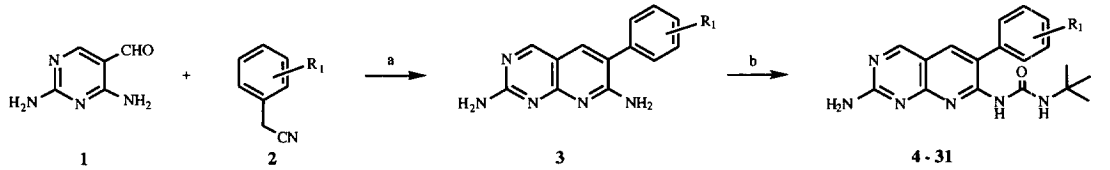
TK relative to this expanded panel of enzymes. In contrast, the initial lead, compound **5**, showed broad activity against this panel of enzymes. The highly FGF-selective compound, **28**, was further evaluated for its ability to inhibit FGF-stimulated human umbilical vein endothelial cells (HUVECs) growth. HUVECs has shown to be dependent upon FGF for growth.[11] In a 4-day growth delay assay,[12] compound **28** inhibited FGF-stimulated HUVEC growth with an IC_{50} of 0.059 μM.

Table 2. FGFr Selective TK Inhibitor Profile

Kinase (IC_{50}, μM)[10]	 **5** PD 089828	 **19** PD 162628	 **28** PD 166866
MAPK	7.1	>50	>50
CDK4	>50	>40	>40
EGFr	0.45	6.68	50
InsR	>50	>50	>50
PKC	50	>50	>50

Synthesis: Compounds **4–31** were prepared according to the general method described in Scheme 1.[13] The previously reported aldehyde **1**[14–16] was condensed with an appropriately substituted phenylacetonitrile (**2**) in refluxing 2-ethoxyethanol under basic conditions (0.4 equiv sodium hydride) to afford the corresponding 2,7-diamino-(6-aryl)-pyrido[2,3-*d*]pyrimidine (**3**) in variable yields (27–97%). Subsequently, a suspension of **3** in DMF at room temperature was treated with 1.1 equiv of NaH, stirred for 1 h, followed by the addition of *tert*-butylisocyanate to afford the desired target compounds **4–31** in yields ranging from 30–70%.

Scheme 1. Pyrido[2,3-*d*]pyrimidine Analogs

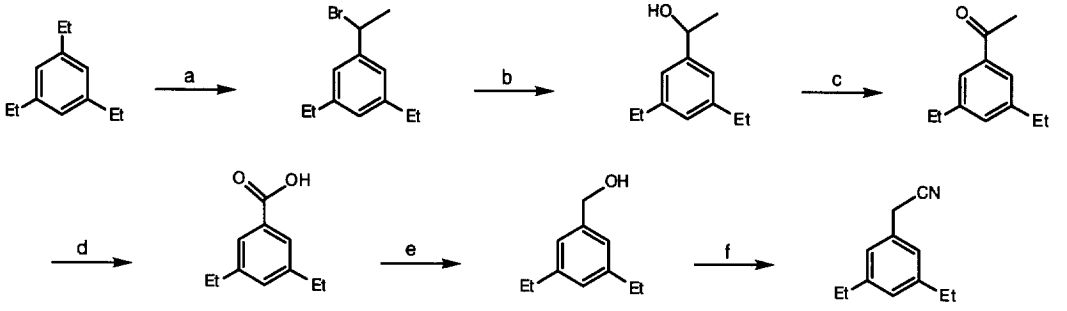


1 2 3 4 - 31

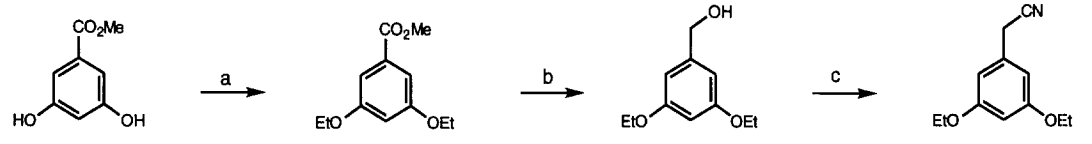
(a) NaH, 2-ethoxyethanol, 27–98% yield; **(b)** i. NaH, DMF; ii. t-BuNCO, 30–70% yield.

Generally, the substituted phenylacetonitrile intermediates used in this work were obtained from commercial sources or prepared according to literature methods.[1,13,14,17] However, several of the intermediates used in this study were novel, and therefore syntheses were developed to access them. Scheme 2 describes the synthetic routes used to prepare these heretofore unknown substituted phenylacetonitriles, which were used to prepare compounds **23**, **30**, and **31**.

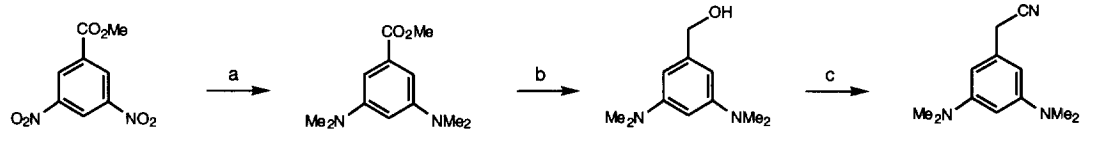
Scheme 2. Arylacetonitrile Intermediates for Compounds 23, 30, and 31



(a) NBS, CCl_4 (88%); (b) $BaCO_3$ dioxane/H_2O (quantitative); (c) 8 N Jones reagent, acetone (53%); (d) Br_2, NaOH/H_2O, dioxane (90%); (e) LAH, THF (92%); (f) i. $SOCl_2$, benzene; ii. KCN, 95% EtOH (45%).



(a) K_2CO_3, EtI, acetone (82%); (b) LAH, THF (82%); (c) PPh_3, DEAD, acetone cyanohydrin (35%).



(a) i. H_2, Ra/Ni, MeOH, ii. 37% HCHO iii. Ra/Ni (77%); (b) LAH, THF (94%); (c) PPh_3, DEAD, acetone cyanohydrin (30%).

Biological Evaluation: The following enzyme assays were run according to previously reported literature procedures: PDGFr-TK/FGFr-TK,[18] c-Src,[19] MAPK,[20] EGFr,[21] InsR,[22] and PKC.[23] The CDK4 assay[24] and HUVEC growth delay assay[12] were performed in our laboratories.

Conclusions: Compound library screening to identify leads with PDGFr and FGFr tyrosine kinase inhibitory activity led to the discovery of a novel series of pyrido[2,3-*d*]pyrimidine compounds with PDGFr, FGFr, and c-Src TK enzyme inhibitory activity. SAR studies focusing on modifications at the 6-phenyl moiety of the initial lead produced a series of broadly active potent TK inhibitors. This study also led to the development of a novel series of 2-amino-6-(3,5-disubstituted-phenyl)-pyrido[2,3-*d*] pyrimidin-7-yl]-3-*tert*-butyl-urea analogs which are highly selective and potent inhibitors of the FGFr tyrosine kinase.

References and Notes:

1. Blankley, C. J.; Doherty, A. M.; Hamby, J. M.; Panek, R. L.; Schroeder, M. C.; Showalter, H. D. H.; Connolly, C. WO PCT Appl. 9615128, 1996; *Chem. Abstr.* **1995**, *125*, 114688.
2. Fry, D. W.; Bridges, A. J. *Curr. Opin. Biotech.* **1995**, *6*, 662.
3. Bornfeldt, K. E. *Trends Cardiovasc. Med.* **1996**, *6*, 143.

4. Reidy, M. A.; Bendeck, M. P. In Coronary Restenosis: From Genetics to Therapeutics; Feuerstein, G., Ed.; Dekker: New York, 1997, Vol. 28, pp 55-67.
5. Fry, D. W. *Expert Opin. Invest. Drugs* **1994**, *3*, 577.
6. Klein, S.; Roghani, M.; Rifkin, D. B. *EXS* **1997**, *79*, 159.
7. Brunton, V. G.; Ozanne, B. W.; Paraskeva, C.; Frame, M. C. *Oncogene* **1997**, *14* , 283.
8. Verbeek, B. S.; Vroom, T. M.; Adriaansen-Slot, S. S.; Ottenhoff-Kalff, A. E.; Geertzema, J. G. N.; Hennipman, A.; Rijksen, G. *J. Pathol.* **1996**, *180* , 383.
9. Connolly, C. J. C.; Hamby, J. M.; Schroeder, M.C.; Lu, G. H.; Panek, R. L.; Amar, A.; Shen, C.; Kraker, A. J.; Doherty, A. M. 211th ACS National Meeting: New Orleans, LA, March 1996, MEDI 193.
10. IC_{50} values reported for kinase inhibition represent the means of at least two separate experiments each done in triplicate with typical variations of less than 30% between replicate values.
11. Garfinkel, S.; Hu, X.; Prudovsky, I. A.; McMahon, G. A.; Kapnik, E. M.; McDowell, S. D.; Maciag, T. *J. Cell Biol.* **1996**, *134*, 783.
12. HUVEC growth delay assay conditions: HUVECs were grown in EGM culture medium supplemented with 10 ng/mL human recombinant epidermal growth factor, 1 mg/mL hydrocortisone, 2% fetal bovine serum, 50 mg/mL gentamicin, 50 ng/mL amphotericin-B, 12 mg/mL bovine brain extract, and 10 mg/mL heparin. Experiments were preformed with HUVECs between passage 3 and 5. HUVECs were seeded in a 96-well cell culture plate at a concentration of 1800 cells/well. Cells were allowed to attach overnight in a 37 °C , 5% CO_2 incubator after which concentrations of compounds were added to wells and cells were incubated with the indicated compounds for 4 days. The effect of the compounds was determined with sulphorhodamine B as previously described in the following reference: Skehan, P.; Stoneng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.; Bokesch, H.; Kenney, S.; Boyd, M. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
13. Bennett, L. R.; Blankley, C. J.; Fleming, R. W.; Smith, R. D.; Tessman, D. K. *J. Med. Chem.* **1981**, *24*, 382.
14. Blankley, C. J.; Boschelli, D. H.; Doherty, A. M.; Hamby, J. M.; Klutchko, S.; Panek, R. L. WO PCT Appl. 9634867, 1996; *Chem. Abstr.* **1996**, *126*, 59965.
15. Gossett, L. S.; Shih, C. J. WO PCT Appl. 9509845, 1995; *Chem. Abstr.* **1995**, *123*, 286711.
16. Stuart, A.; Paterson, T.; Roth, B.; Aig, E. *J. Med. Chem.* **1983**, *26*, 667.
17. Boyer, J.; Bruylants, A. *Bull. Soc. Chim. Fr.* **1958**, 453.
18. (a) PDGFr-TK/FGFr-TK inhibition assay conditions: Escobedo, J. A.; Keating, M. T.; Ives, H. E. *J. Biol. Chem.* **1988**, *263*, 1482. (b) Lu, G. H.; Connors, R.; Panek, R.; FASEB Meeting, Anaheim, CA, Spring 1994, Abstract 253.
19. C-Src tyrosine kinase inhibition assay conditions: Thompson, A. M.; Fry, D. W.; Kraker, A. J.; Denny, W. A.; *J. Med. Chem.* **1994**, *37*, 21.
20. MAPK inhibition assay conditions : Alessi, D. R.; Cuenda, A.; Cohen, P.; Dudley, D. T.; Saltiel, A. R. *J. Biol. Chem.* **1995**, *270*, 27489.
21. EGFr tyrosine kinase inhibition assay conditions: Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093.
22. For insulin receptor (InsR) tyrosine kinase inhibition assay conditions see: Fry, D. W.; Kraker, A. J.; Connors, R. C.; Elliot, W. L.; Nelson, J. M.; Showalter, H. D. H.; Leopold, W. R. *Anti-Cancer Drug Des.* **1994**, *9*, 331.
23. Protein kinase C (PKC) inhibition assay conditions: Martiny-Baron, G.; Kazanietz, M. G.; Mischak, H.; Blumberg, P. M.; Kochs, G.; Hug, H.; Marme, D.; Schachtele, C. *J. Biol. Chem.* **1993**, *268*, 9194.
24. CDK4 inhibition assay conditions: The total volume in 96-well Millipore filter plates was 0.1 mL with a final concentration of 20 mM TRIS (Tris[hydroxmethyl]aminomethane), pH 7.4, 50 mM NaCl, 1 mM dithiothreitol, 10 mM $MgCl_2$, 25 μM ATP (containing 0.25 μCi of [^{32}P]ATP), 20 ng of recombinant CDK4/cyclin D, 1 μg of retinoblastoma and appropriate dilutions of inhibitor. All components except the ATP are added to the wells and the plate is placed on a plate mixer for 2 min. The reaction is started by adding [^{32}P]ATP and the plate is incubated at 25 °C for 15 min. The reaction terminated by addition of 0.1 mL 20% trichloroacetic acid (TCA). The plate is kept at 4 °C for at least 1 h to allow the substrate to precipitate. The wells are then washed 5 times with 0.2 mL of 10% TCA and ^{32}P incorporation determined with a β-plate counter.

(*Received in USA* 30 *June* 1997; *accepted* 25 *August* 1997)