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Synthesis and Evaluation of New Protein-Tyrosine Kinase Inhibitors. Part 2. Phenylhydrazones.

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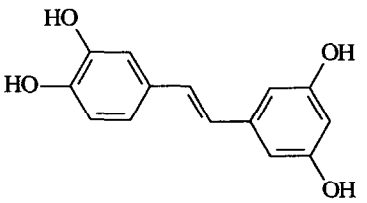
Abstract: A series of 33 phenylhydrazone derivatives of polyhydroxylated benzaldehydes has been prepared and tested for inhibition of the protein-tyrosine kinase p56^{lck}. The most potent of these compounds was a competitive inhibitor of p56^{lck} with respect to a tyrosine-containing peptide substrate and was similar in potency to piceatannol, a naturally occurring protein-tyrosine kinase inhibitor.

Piceatannol (**1**, E-3,4,3',5'-tetrahydroxystilbene) is a plant secondary natural product that was isolated from the seeds of *Euphorbia lagascae* on the basis of its antileukemic activity.[1] Subsequent studies showed that piceatannol could function as a protein-tyrosine kinase inhibitor by competing with a phosphoacceptor peptide.[2] In our program to develop synthetic inhibitors based on this structural lead, we considered the phenylhydrazones of polyhydroxylated benzaldehydes as a potential source of new protein-tyrosine kinase inhibitors. These phenylhydrazones have a close resemblance to piceatannol, are made very easily and are quite stable as compared to polyhydroxylated stilbenes.



Piceatannol (**1**)

For the present study, we prepared an array of phenylhydrazone derivatives using phenylhydrazine, 4-methoxy, 4-bromo and 2,4-dinitro substituted phenylhydrazines and 2-pyridylhydrazine. Hydroxy, dihydroxy, trihydroxy, nitro, and bromo substituted benzaldehydes, as well as 2-, 3-, and 4-pyridinecarboxaldehydes, were also employed as starting meterials. All these compounds were prepared by heating an equimolar mixture of aldehyde and phenylhydrazone in ethanol[3] and the crude products were purified by a single crystallization from ethanol. We prepared 33 phenylhydrazone derivatives that were tested for their inhibitory activity against the phosphoylation of angiotensin I catalyzed by p56^{lck}.[4] The IC_{50} values are summarized in Table I.

Table 1. Physical Characteristics and Protein-Tyrosine Kinase Inhibitory Data of Phenylhydrazones. **(Ar-CH=N-NH-Ar')**

compd	Ar	Ar'	mp (° C)	lit mp (° C)/ M. Formula	PTKI IC_{50} (μM)
2	2,3-dihydroxyphenyl	phenyl	165-166	167[5]	1262
3	3,4-dihydroxyphenyl	phenyl	176-177	175-176[5]	70
4	2,4-dihydroxyphenyl	phenyl	161-162	158[5]	350
5	2,5-dihydroxyphenyl	phenyl	209-210	$C_{13}H_{12}N_2O_2$**	>3500
6	2,3,4-trihydroxyphenyl	phenyl	156-157	$C_{13}H_{12}N_2O_3$**	1572
7	3-hydroxy-4-methoxyphenyl	phenyl	121-122	$C_{14}H_{14}N_2O_2$**	2106
8	4-hydroxy-3-methoxyphenyl	phenyl	102-104	105	2586
9	5-bromo-2-hydroxyphenyl	phenyl	149-150	$C_{13}H_{11}BrN_2O_2$**	>2605
10	3-nitrophenyl	phenyl	124-125	124[5]	>3316
11	4-nitrophenyl	phenyl	158-159	159[5]	>3316
12	2-pyridyl	phenyl	175-176	176[6]	>4056
13	3-pyridyl	phenyl	156-157	158[6]	1318
14	4-pyridyl	phenyl	178	178-179 [7]	>4056
15	2,3-dihydroxyphenyl	4-methoxyphenyl	161-162	$C_{14}H_{14}N_2O_3$**	310
16	3,4-dihydroxyphenyl	4-methoxyphenyl	147-148	$C_{14}H_{14}N_2O_3$**	743
17	2,5-dihydroxyphenyl	4-methoxyphenyl	203-204	$C_{14}H_{14}N_2O_3$**	310
18	2,4-dihydroxyphenyl	4-bromophenyl	163-164	$C_{13}H_{11}BrN_2O_2$**	521
19	3,4-dihydroxyphenyl	4-bromophenyl	172	$C_{13}H_{11}BrN_2O_2$**	599
20	3-hydroxy-4-methoxyphenyl	4-bromophenyl	154-155	$C_{14}H_{13}BrN_2O_2$**	>2491
21	5-bromo-2-hydroxyphenyl	4-bromophenyl	185-186	$C_{13}H_{10}Br_2N_2O$**	>2162
22	2,3,4-trihydroxyphenyl	4-bromophenyl	156-157	$C_{13}H_{11}BrN_2O_3$**	1040
23	2-pyridyl	4-bromophenyl	246-247	$C_{12}H_{10}BrN_3$**	>2897
24	3-pyridyl	4-bromophenyl	248-249	$C_{12}H_{10}BrN_3$**	>2897
25	4-pyridyl	4-bromophenyl	284-285	$C_{12}H_{10}BrN_3$**	>2897
26	2,3-dihydroxyphenyl	2,4-dinitrophenyl	295-296	$C_{13}H_{10}N_4O_6$**	>2514
27	3,4-dihydroxyphenyl	2,4-dinitrophenyl	276-277	275[5]	352
28	2,4-dihydroxyphenyl	2,4-dinitrophenyl	300-301	302-303[3]	1005
29	2,3,4-trihydroxyphenyl	2,4-dinitrophenyl	299-300	$C_{13}H_{10}N_4O_7$**	144
30	2,4-dihydroxyphenyl	2-pyridyl	220-221	$C_{12}H_{11}N_3O_2$**	1012
31	3,4-dihydroxyphenyl	2-pyridyl	216-218	$C_{12}H_{11}N_3O_2$**	227
32	2,3,4-trihydroxyphenyl	2-pyridyl	180-182	$C_{12}H_{11}N_3O_3$**	522
33	3,4,5-trihydroxyphenyl	2-pyridyl	237-238	$C_{12}H_{11}N_3O_3$**	179
34	3-pyridyl	2-pyridyl	158-160	$C_{11}H_{10}N_4$**	400
1	Piceatannol		--	--	66

** All new compounds gave satisfactory spectral and microanalytical data.

Results and Discussion

Among the 33 substances prepared and tested, the eight compounds **3**, **4**, **15**, **17**, **29**, **31**, **33** and **34** were the most potent ($IC_{50} \leq 400$ μM). Compound **3** was the most potent among the compounds tested ($IC_{50} = 70$ μM) and is essentially as active as piceatannol ($IC_{50} = 66$ μM). Transfer of the two hydroxy groups to other positions (compounds **2**, **4** and **5**), addition of a hydroxy group (compound **6**), and methylation of these hydroxy groups (compounds **7** and **8**) decreased the inhibitory activity. Similarly, introduction of a nitro group on Ar or substitution of Ar' with pyridyl rings also reduced the potency (Table I). Introduction of a bromo, methoxy or dinitro groups on the phenyl ring of Ar' of the most active gave substances **16**, **19**, and **27** with reduced inhibitory activity. However, the 2,4-dinitrophenylhydrazone derivative of 2,3,4-trihydroxybenzaldehyde (compound **29**) and 2-pyridylhydrazones of 3,4-dihydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde (compounds **31** and **33**) were moderately active ($IC_{50} \leq 227$ μM). The most active of these compounds (**3**) was investigated in greater detail to determine its mechanism of inhibition. As shown in Fig. 1, compound **3** was a competitive inhibitor of $p56^{lck}$ with respect to a tyrosine-containing peptide substrate (angiotensin I) (Fig. 1A) and was a noncompetitive inhibitor with respect to ATP (Fig. 1B). This pattern of inhibition is similar to that described for piceatannol[2] and for erbstatin and its derivatives.[8-11] It is felt that compounds that interact at the peptide-binding site would be potentially more selective for the inhibition of protein-tyrosine kinases than would be compounds that interact at the ATP-binding site, since individual protein-tyrosine kinases all utilize ATP, but have different substrates. Additional efforts are currently underway to prepare and evaluate compounds having hydroxy groups on both the phenyl rings for more potency and selectivity.

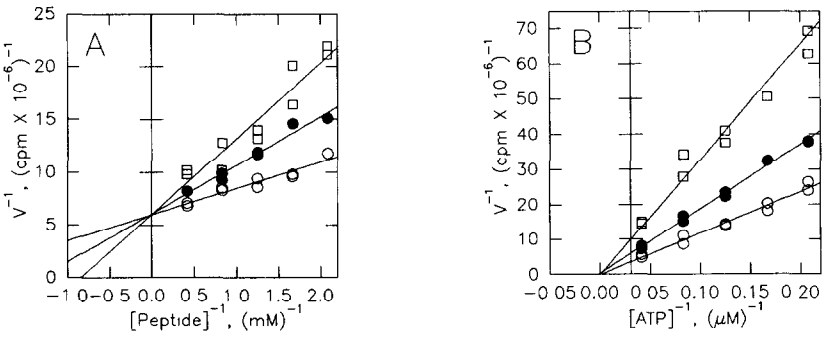


Fig. 1. Lineweaver-Burk plots showing inhibition of $p56^{lck}$ by compound **3**. A, Effect of increasing concentrations of angiotensin I on the inhibition of $p56^{lck}$ by **3** {0 (o), 24 (●) or 40 (⊡) μg/ml}. B, Effect of increasing concentrations of [γ-^{32}P]ATP on the inhibition of $p56^{lck}$ by **3** {0 (o), 24 (●) or 40 (⊡) μg/ml}. Assay conditions are as described.[4]

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