Selective Inhibition of Src Protein Tyrosine Kinase by Analogues of 5-*S*-Glutathionyl-*β*-alanyl-L-dopa

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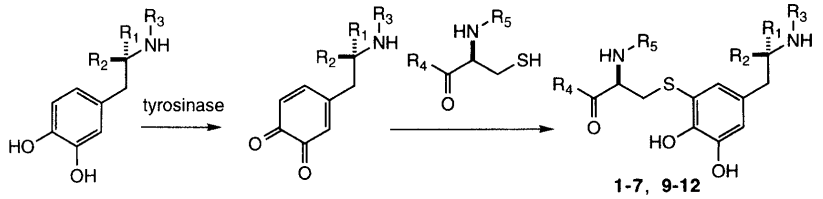
Twelve analogues of the antibacterial phenolic peptide 5-*S*-glutathionyl-*β*-alanyl-L-dopa (5-*S*-GA-L-D: 1) were synthesized *via* orthoquinones using tyrosinase. Several synthesized compounds inhibited the v-Src autophosphorylation tyrosine kinase reaction with an IC_{50} value comparable to that of herbimycin. The inhibition of c-Src substrate phosphorylation was much less active than v-Src autophosphorylation inhibition. The analogues showed no effects on substrate phosphorylation by epidermal growth factor receptor (EGFR), and this selectivity is the most characteristic feature of the analogues (1—12).

Key words protein tyrosine kinase; c-Src; v-Src; EGFR; Raytide

A search for selective protein tyrosine kinase (PTK) inhibitors from natural sources has been conducted to find potential antitumour compounds or useful probes to clarify the role of PTKs in intracellular signal transduction.[1] The phenolic compounds genistein, quercetin, erbstatin, and desmal, and the quinonoid compound herbimycin were identified as PTK inhibitors using different PTK assay systems.[1,2] In the course of a study on insect defense mechanisms, a low molecular-weight antibacterial compound, 5-*S*-glutathionyl-*β*-alanyl-L-dopa (5-*S*-GA-L-D: **1**), was isolated from *E. coli*-challenged imagoes of the flesh fly *Sarcophaga peregrina*.[3] The peptide structure containing tyrosine derivative dopa in (**1**), together with the finding that 5-*S*-cysteinyl-L-dopa (**11**),[4] a structural analogue of (**1**), showed *in vitro* and *in vivo* antitumour activity against several tumour cell lines,[5] led us to investigate the inhibitory effects of (**1**) and its analogues against PTKs. An earlier study demonstrated that (**1**) and (**11**) inhibited v-Src autophosphorylation but not serine/threonine protein kinases.[6]

To clarify the structural requirements of inhibitors of PTKs and possibly to find selective PTK inhibitors, twelve 5-*S*-GA-L-D analogues (**1**—**12**) (Table 1) were synthesized *via* dopa-orthoquinone using mushroom tyrosinase,[4] and their inhibitory activities were evaluated in three different PTK assay systems: autophosphorylation of v-Src[7] and substrate phosphorylation by human c-Src[8] and EGFR.[9] As shown in Table 2, the IC_{50} values of 5-*S*-GA-L-D (**1**), 5-*S*-GADA (**4**), 5-*S*-G-L-D (**5**), and 5-*S*-G-D-D (**6**) which showed relatively high inhibitory activity in v-Src autophosphorylation were in the range of 17 to 28 μM. As in the case with 5-*S*-GA-L-D (**1**),[6] synthetic analogues (**2**—**10**, **12**) showed no significant inhibition of serine/threonene protein kinases when assayed using *v-src*-transformed NIH 3T3 cells. The IC_{50} of the most active 5-*S*-GADA (**4**) was 17 μM, which is comparable to the reported IC_{50} value of herbimycin A (12 μM) against v-Src autophosphorylation.[10]

Inhibitory effects of (**1**—**12**) on c-Src substrate phosphorylation were determined with human c-Src and a synthetic peptide substrate, Raytide.[8] More than 2-fold higher concentrations of (**1**—**10**) were required to give similar inhibition values to those for v-Src autophosphorylation (see Table 2). A conventional kinetic experiment on the most active 5-*S*-GADA (**4**) with c-Src, Raytide, and ATP revealed that the apparent mode of inhibition was competitive to substrate and noncompetitive to ATP (data not shown). Inhibitory effects against the phosphorylation of Raytide by c-Src were significantly lower in 5-*S*-cysteinyl-dopa (**11** and **12**) than in the other analogues. This suggests that peptide-like structures are required for significant inhibition against v-Src and c-Src. As shown in Table 2, no significant inhibition of the EGFR PTK reaction was observed in 5-*S*-GA-L-D analogues (**1**—**12**) even at the very high concentration of 500 μM. The inhibition of v-Src autophosphorylation by genistein was lower than by the synthetic analogues, although genistein inhibited c-Src substrate phosphorylation more potently. As expected from the mode of inhibition, genistein inhibited EGFR substrate phosphorylation by 98% at a concentration of 500 μM. It may not be appropriate to compare the inhibition percentages with those of genistein, since it is an ATP-competitive PTK inhibitor while 5-*S*-GA-L-D analogues (**1**—**12**) are substrate



R_1, R_2=COOH or H R_3=*β*-alanyl or H
R_4=glycyl or H R_5=*γ*-glutamyl or H.

Chart 1. Synthesis of 5-*S*-GA-L-D Analogues (**1**—**12**)

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Table 1. 5-*S*-GA-L-D and Its Synthetic Analogues

Compound	Abbreviation	Name
1	5-*S*-GA-L-D	5-*S*-Glutathionyl-β-alanyl-L-dopa
2	5-*S*-GA-D-D	5-*S*-Glutathionyl-β-alanyl-D-dopa
3	5-*S*-GAMD	5-*S*-Glutathionyl-β-alanyl-α-methyl-L-dopa
4	5-*S*-GADA	5-*S*-Glutathionyl-β-alanyldopamine
5	5-*S*-G-L-D	5-*S*-Glutathionyl-L-dopa
6	5-*S*-G-D-D	5-*S*-Glutathionyl-D-dopa
7	5-*S*-GDA	5-*S*-Glutathionyldopamine
8	2,5-*S*,*S*-GDA	2,5-*S*,*S*-Bisglutathionyldopamine
9	5-*S*-CysA-L-D	5-*S*-Cysteinyl-β-alanyl-L-dopa
10	5-*S*-CysA-D-D	5-*S*-Cysteinyl-β-alanyl-D-dopa
11	5-*S*-Cys-L-D	5-*S*-Cysteinyl-L-dopa
12	5-*S*-Cys-D-D	5-*S*-Cysteinyl-D-dopa

Table 2. Inhibitory Activities of 5-*S*-GA-L-D Analogues[a)]

Compound	v-Src Autophosphorylation		c-Src Phosphorylation of Raytide	EGFR Phosphorylation of Angiotensin II
	IC_{50} (μM)[b)]	Inhibition (%)[c)]	Inhibition (%)[d)]	Inhibition (%)[e)]
1	25	78.9	52.9	2.2
2	65	69.8	47.5	8.2
3	74	65.8	44.5	5.2
4	17	90.7	43.5	4.9
5	28	79.7	44.1	4.4
6	24	81.4	51.6	≒0
7	45	76.8	32.1	1.0
8	56	67.3	62.4	7.6
9	64	58.1	45.5	2.5
10	42	63.0	36.8	≒0
11	66	59.8	12.2	2.9
12	63	55.3	8.9	≒0
Genistein	>100	36.0	85.7	98.0

a) The three assay methods were modified to measure ^{32}P-labelled phosphorylated proteins or peptides by Fuji Film Bio-image Analyzer BAS 2000. *b*) IC_{50} values are based on three independent determinations. *c*) Inhibition % at a concentration of 100 μM. *d*) Inhibition % at a concentration of 250 μM. *e*) Inhibition % at a concentration of 500 μM.

competitive.

In conclusion, the characteristic feature of 5-*S*-GA-L-D analogues (**1**—**12**) is their specific inhibition of v-Src and c-Src PTK reactions. This is the first example of phenolic natural products that selectively inhibit a nonreceptor-type PTK (Src), but not receptor-type EGFR PTK, although there has been a report on synthetic heterocyclic compounds showing selectivity among different PTKs.[11] Recent findings showed that in almost all breast cancers the level of tyrosine kinase (PTK) activity is elevated up to 25-fold over that normal breast tissue, and 70% of this elevation is attributed to activated c-Src PTK.[12] Furthermore, c-Src acts as a major signal transducer for various receptors including EGF, PDGF, and CSF-1 receptors which play an important driving role in malignant transformations.[12] Therefore (**1**) and its analogues may prove to be useful tools in investigating the roles played by Src PTKs in signal transduction and transformation. Further studies to improve the selectivity and potency of (**1**) and its analogues and to assess their selectivity toward other PTKs are in progress.

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