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Oncogene signal transduction inhibitors from Chinese medicinal plants*

Ching-jer Chang,† Curtis L. Ashendel, Thomas C. K. Chan, Robert L. Geahlen, Jerry McLaughlin and David J. Waters

Department of Medicinal Chemistry and Molecular Pharmacology Department of Veterinary Pharmacology and Physiology Department of Veterinary Clinical Sciences Purdue University, West Lafayette, Indiana 47907, USA

Abstract: Oncogene modulated signal transduction based on intracellular phosphorylation of protein tyrosine or serine/threonine has been utilized as a target for oncogene-based anti-cancer drug discovery. Inhibition of protein-tyrosine kinase and protein kinase C directed prescreen has identified numerous potential anti-tumor Chinese medicinal plants. Further bioassay-guided fractionation and separation have led to the discovery of novel protein kinase inhibitors, anthraquinones, stilbenes and polythiophenes as potential anti-tumor agents.

Plants have been important sources for providing many anti-tumor agents with novel structures and unique mechanisms for the control and cure of cancer. The key to the success of the plant natural product drug discovery program resides in bioactivity-directed isolation procedures. The systematic screening for anti-tumor agents from natural sources developed by the US National Cancer Institute was initially guided by the activity in the mouse L1210 and P388 leukemia assay. In the last decade, a 'disease oriented' approach has been employed for screening anti-tumor activity [1,2]. Extracts or compounds are tested directly against human tumor cell panels consisting of 60 cell lines of major human tumors (leukemia, lung, colon, central nerve system, skin, ovary, kidney, prostate and breast cancers). The end point for all these bioassays is cytotoxic effect toward tumor cells. However, the *in vitro* cytotoxic potency is often not a good indicator for the *in vivo* anti-tumor efficacy. Therefore, an alternative approach must be envisioned for discovery of novel anti-tumor agents with unique mechanisms.

It has now been well established that the differentiation and growth of cancer cells are tightly controlled by oncogenic protein induced signaling processes [3,4]. These oncogene-modulated signal transduction pathways therefore offer attractive targets for oncogene-based anti-tumor drug discovery. Our natural product drug discovery group has designed two signal transduction-based bioassays to inhibit the intracellular phosphorylation of the tyrosine or serine/threonine unit of signaling proteins for the bioactivity-directed isolation of anti-tumor agents from Chinese medicinal plants, which have displayed only marginal cytotoxic or noncytotoxic effect against human tumor cell lines.

PROTEIN-TYROSINE KINASE (PTK) INHIBITORS [5–8]

We have focused our research effort on the search for the inhibitors of Src-family kinases because of their involvement in many src-oncogene modulated signal transduction pathways. Lck ($p56^{lck}$) protein-tyrosine kinase is selected as our initial target for the identification of *Src*-family kinase inhibitors. The crude extracts of many Chinese anti-tumor medicinal plants [9,10] *Polygonum cuspidatum (Hu Zhang), Rheum palmaturn (Da Huang), Scutellaria baicalensis (Huang Qin), Polygonum multiforum (He Shou Wu), Ganoderma luidum (Ling Zhi)* and *Cassia occidentalis (Wang Jiang Nan)* were shown to be active in this bioassay.

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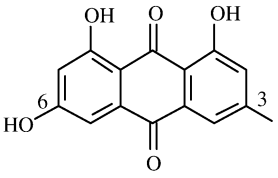
†*Corresponding author.*

Emodin

Extracts from the roots of *Polygonum cuspidatum*, contained inhibitory activity as detected by an *in vitro* peptide phosphorylation assay. Bioassay-directed fractionation of this extract yielded the anthraquinoid, emodin (IC_{50}: 5 μg/mL) [11]. Kinetic analyses indicated that emodin was a competitive inhibitor of Lck with respect to ATP and was noncompetitive with respect to the peptide substrate.



Emodin

Scheme 1

Anti-oncogene activity and selective cytotoxicity

The cytotoxicity profile of emodin as evaluated in the NCI human tumor cell line panels indicated that the compound was only moderately cytotoxic [12]. We sought therefore alternative techniques for the evaluation of noncytotoxic agents such as emodin. One such method is to examine the capacity of agents to selectively alter the growth properties of cells transformed due to the expression of a specific oncogene. Interestingly, we found that emodin was a selective inhibitor of the growth of oncogene-transfected or -overexpressed cells that had been transformed by transfection with an activated oncogene.

Anti-ras

Treatment of transformed bronchial epithelial (TBE) cells with emodin resulted in a dose-dependent inhibition of cell growth at concentrations that had little or no effect on the growth of normal human primary bronchial epithelial (HBE) cells [13]. To explore the mechanism of action of emodin in these cells, we probed TBE and HBE cell lysates with anti-phosphotyrosine antibodies to estimate the relative levels of tyrosine-phosphorylated proteins present in each cell type. We found that TBE cells exhibited elevated levels of phosphotyrosine-containing proteins relative to those found in HBE cells, even though the transforming principal (activated Ras) lacks protein-tyrosine kinase activity. Treatment of intact TBE cells with emodin resulted in a marked decrease in the concentration of cellular protein-tyrosine phosphorylation [13].

Anti-Her-2/neu

Emodin was also found to be a selective inhibitor of the growth of breast cancer cells that overexpress the HER-2/*neu* proto-oncogene product [14]. These studies showed that emodin was an effective inhibitor of the tyrosine kinase activity of the immunoprecipitated Her-2/*neu* receptor. Treatment of breast cancer cells (MDA-MB453, AU-565 and BT-483), which overexpress the HER-2/*neu* protein-tyrosine kinase receptor, with emodin inhibited the autophosphorylation of the receptor and, as a consequence, inhibited the intrinsic kinase activity of the receptor (as assayed by the phosphorylation of enolase in anti-Her-2/*neu* immunoprecipitates). Treatment of MDA-MB453 breast cancer cells with 40 μM emodin resulted in a 78% inhibition of cell growth. In contrast, the treatment of MCF-7 and MDA-MB231 cells, which express normal levels of HER-2/*neu* receptor, resulted in only a 37% inhibition of growth. Interestingly, HBL-100 cells, which were derived from normal human breast tissue, were insensitive to the growth inhibitory effects of emodin, even at concentrations as high as 80 μM [14]. Cell cycle analyses indicated that emodin blocked the entry of MDA-MB453 cells into the S phase of the cell cycle. MDA-MB453 cells assume a rounded morphology characteristic of cells transformed by oncogenic PTKs. Treatment with emodin resulted in a change in morphology from a rounded to a flat, more normal phenotype [14] and regained contact inhibition. Thus emodin, or related analogs, may prove to be specific chemotherapeutic agents that exhibit selectivity for breast cancer cells in which the HER-2/*neu* proto-oncogene is overexpressed.

Recently, Zhang & Hung [15] demonstrated that emodin selectively suppresses the proliferation of *Her-2/neu*-overexpressing nonsmall cell lung cancer (NSCLC) cells (NCI-H1435 and *Her-2/neu*-transfected

NCI-H460). Furthermore, the combination of emodin with another anti-cancer drug (doxorubicin, etoposide cisplatin) induced synergistic inhibition of the growth of *Her-2/neu*-overexpressing NSCLC cells.

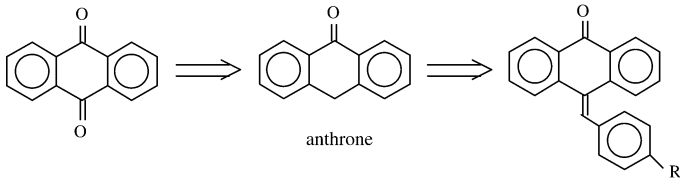
Other anthraquinones

A series of natural and semisynthetic emodin derivatives were recently selected for evaluation of their inhibition of three different kinases (Table 1) [16]. It appears that emodin is the best PTK inhibitor. Deletion or modification of the 6-OH group abolishes all kinase inhibitory activity. Further oxidation of the 3-CH_3 group results in a reduction of activity except for the 3-CHO group. We also examined the requirement for the 10-keto functional group by preparing the anthrone analog of emodin.

Table 1 Inhibition of protein kinases by emodin and its derivatives

			IC_{50} (μg/mL)		
Compound	R_3	R_6	$p56^{lck}$	$p180^{Her\text{-}2/neu}$	PKC
emodin	CH_3	OH	5	6	40
citreorosein	CH_2OH	OH	30	>30	>200
	CH_2Br	OH	30	10	
	CHO	OH	6	30	200
emodic acid	CO_2H	OH	30	>30	200
	$CONH_2$	OH	30	>30	40
physcion	CH_3	OCH_3	>800	>200	
fallacinal	CHO	OCH_3	>800	>200	
chrysophanic acid	CH_3	H	>800	>30	>200
rhein	CO_2H	H	300	>30	>200

This modification results in the loss of kinase inhibitory activitiy and the retention of cytotoxicity. We therefore, explored the carbon analog of the carbonyl group by preparing the quinone methide derivatives, benzylideneanthrone compounds. We have shown recently that 10-(4-acetamidobenzyl-idene)-9-anthrone (R: $NHCOCH_3$) was more effective than emodin in repressing the tyrosine phosphorylation of $p185^{Her\text{-}2/neu}$, selectively inhibiting the proliferation of *Her-2/neu*-transformed NIH3T3 and *Her-2/neu*-overexpressing human breast tumor cells, and suppressing the metastasis-associated secretion of membrane-degrading gelatinase and invasion through a Matrigel basement membrane preparation [17].

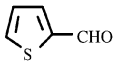
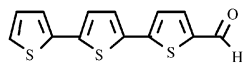
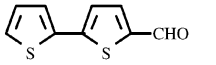
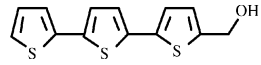
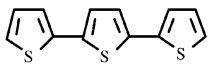
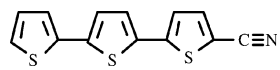


Scheme 2

PROTEIN KINASE C (PKC) INHIBITORS [18–20]

Protein kinase C is a family of serine/threonine protein kinases and is endogenously activated by diacylglycerol, which is produced from mitogen induced hydrolysis of inositol phosopholipids by phosphlipase C. It is a phospholipid-dependent, calcium activated protein kinase. Tumor promoting phorbol esters can also activate this protein in a manner similar to diacylglycerol. The crude extracts of Chinese anti-tumor medicinal plants [9,10], *Eclipta prostrata* (*Li Chang*), *Echinop grisijii* (*Lou Lu*), and *Echinop latifolius* (*Lou Lu*), were shown to contain protein kinase C inhibitory activity. On the basis of this inhibitory activity. we have discovered a series of polythiophenes as novel PKC inhibitors (Table 2)

Table 2 Inhibition of protein kinase C

Compounds	IC_{50} (M)	Compounds	IC_{50} (M)
	$>4\times10^{-3}$		7×10^{-7}
	7×10^{-4}		4×10^{-6}
	1×10^{-5}		2×10^{-6}

[21]. We observed that the PKC inhibitory effect increased as the number of thiophene ring increased in the aldehyde series. 2-Formyl-α-terthiophene is 10 times more potent than the corresponding hydroxymethyl or cyano analogs. Most of the functionalized α-terthiophenes exhibited highly differential cytotoxicity against the renal and ovarian cancer panels in the NCI human tumor cell panels cytotoxocity profiles.

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