Novel Anti-Angiogenic Compounds for Application in Tumor Therapy – COP9 Signalosome-Associated Kinases as Possible Targets

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Abstract: Preclinical studies revealed that curcumin, the yellow curry pigment, emodin, a compound derived from grapes, and taurolidine, derived from a biogenic amino acid, and some of their structural homologs possess anti-angiogenic and cancer chemopreventive properties. Whereas curcumin and emodin can act *via* inhibition of COP9 signalosome-associated kinases, taurolidine blocks protein biosynthesis.

Key Words: Angiogenesis, tumor, COP9 signalosome, curcumin, emodin, taurolidine.

INTRODUCTION

For a tumor to develop a highly malignant and deadly phenotype, it must first recruit and sustain its own blood supply, a process called tumor angiogenesis [1-3]. A tumor that is unable in recruiting its own vascular maintenance is called a non-angiogenic tumor and is microscopic in size remaining limited to less than 1 mm diameter, because of its dependency on oxygen and nutrients supply [3]. Angiogenesis allows tumor growth and facilitates local invasion and metastasis. Therefore, inhibition of tumor angiogenesis is a major strategy in current tumor therapy.

Angiogenesis is a dynamic process induced by genetic changes or by local alterations such as hypoxia, glucose deprivation and oxidative or mechanical stress [4, 5]. Physiological angiogenesis is tightly regulated by endothelial growth factors and occurs in a sequence of complex and interrelated steps. Pro-angiogenic proteins include the vascular endothelial growth factor (VEGF), which binds to VEGF receptors on endothelial cells. VEGF, also known as VEGF A, has a number of isoforms, e.g. VEGF-121 and VEGF-165. VEGF also is classified by related factors such as VEGF B, C and D. VEGF, the basic fibroblast growth factor (bFGF) and the platelet-derived growth factor (PDGF), two additional pro-angiogenic proteins, function as mitogens and chemoattractants to recruit endothelial cells. They activate tissue endothelial cells (ECs), circulating ECs and endothelial progenitor cells, which enter the circulation and generate new blood vessels [6]. Activated ECs destroy the extracellular matrix by secreting matrix metalloproteases, which allows them to migrate and to invade the surrounding tissue. The migration of cells is also supported by the extracellular plasminogen activator inhibitor 1 (PAI-1) as well as by specific integrins expressed in the cell membrane of ECs [7, 8].

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Non-angiogenic tumors stay microscopic in size and might remain asymptomatic and non-detectable for the life of a person. Why and when a non-angiogenic tumor switches to the angiogenic phenotype is not well understood at the moment. This so called angiogenic switch is thought to be the result of a disrupted balance between pro- and anti-angiogenic regulators [3, 9]. It is widely accepted that genetic instability promotes the angiogenic switch. This can be explained by compromised checkpoint genes in tumor cells causing their higher mutation rates. For example, experiments with transgene mouse models show that overexpression of the oncogene RAS led to elevated VEGF A levels and the switch into an angiogenic tumor [10].

Hypoxia is a common feature of solid tumors and a negative prognosis factor for the survival of cancer patients. Essential for the induction of angiogenesis under hypoxic conditions is the heterodimeric hypoxia-inducible factor (HIF) complex consisting of α and β subunit proteins and belonging to helix-loop-helix transcriptional activators [11, 12]. Under normoxic conditions the subunit HIF-1α is continually expressed but quickly degraded by the ubiquitin (Ub) proteasome system (UPS) [13]. It is targeted to ubiquitination by hydroxylation *via* oxygen-iron-2-oxoglutarate-dependent oxygenase in the cytoplasma of mammalian cells [14]. Hydroxylation of two proline residues on HIF-1α is necessary for binding to a cullin-RING Ub ligase (CRL) that modifies HIF-1α with Ub chains prior to fast degradation by the 26S proteasome. The specific CRL complex contains cullin 2 (Cul-2) as a scaffold and the von Hippel-Lindau protein (pVHL) as the substrate binding component. The high instability of HIF-1α is reversed during hypoxia. Under this condition the protein is no longer hydroxylated and ubiquitinated. Stable HIF-1α forms the heterodimeric transcription factor, enters the nucleus and induces the production of VEGF and PDGF [15].

Besides HIF-1α another important pro-angiogenic transcription factor is c-Jun. It was first identified as a viral oncoprotein and is frequently overexpressed in human cancers [16]. It forms heterodimers called activating protein 1 (AP-

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1). There is a direct correlation between c-Jun levels, tumor growth and angiogenesis [17]. Reduction of c-Jun blocks solid tumor growth and VEGF-induced neovascularization in rodents [18]. C-Jun is activated/stabilized by the jun kinase family [19]. In addition, growth factors, UV irradiation, hypoxia as well as transforming oncoproteins stimulate c-Jun and activate AP-1-dependent transcription [20]. Moreover, the transcription factor is phosphorylated by COP9 signalosome (CSN)-associated kinases [21], a process that stabilizes c-Jun towards degradation by the UPS [22].

1. THE COP9 SIGNALOSOME AND ITS POSSIBLE ROLE IN ANGIOGENESIS

The CSN is a conserved protein complex in eukaryotic cells, which consists of eight subunits (CSN1 to CSN8) [23]. It has been first identified in plants as a negative regulator of constitutive photomorphogenesis (COP) [24, 25]. In human cells it is associated with kinase activity [21]. The CSN has a similar architecture as compared to its paralog complex, the 26S proteasome lid [21, 26]. Moreover, it seems that the CSN can act as an alternative lid as it has been shown recently [27, 28]. However, the exact mechanism of the CSN-lid exchange and its regulation is unknown.

Significant progress has been made towards understanding the CSN structure and function by analyzing different organisms. Thus, the complex participates in processes such as DNA repair [29, 30], cell cycle regulation [30], signal transduction [31], MAPK signaling [32], and is involved in myriads of developmental events encompassing embryogenesis, cytokine response, light response in plants, yeast morphogenesis and pheromone response [33]. Currently, many of these pleiotropic effects can be explained by its regulatory impact on the UPS *via* deneddylation of cullins. An intrinsic metalloprotease activity of the CSN, mapped to the MPN^{+}/JAMM domain of CSN subunit 5 (CSN5) [34], removes the Ub-like protein NEDD8 from cullins and perhaps from other targets. Cullins 1 to 7 are scaffolding proteins of CRLs consisting of a RING domain Ub ligase and substrate binding proteins determining the specificity of the Ub system [35]. In mammalian cells hundreds of CRL complexes exist that ubiquitinate transcription factors, cell cycle regulators and other important proteins of cells. Cycles of cullin neddylation and CSN-mediated deneddylation regulate the activity of CRLs. Moreover, the CSN is associated with a cystein protease that removes Ub from target proteins. It is an Ub specific protease called USP15, which cleaves polyUb chains and is responsible for the protection of the CRLs from auto-ubiquitination [36, 37].

In addition, the CSN is associated with kinases such as CK2, PKD [38] and inositol 1,3,4-trisphosphate 5/6 kinase [39] (see Fig. (**1**)). The kinases modify substrates of the UPS and determine their stability [40]. For example, the tumor suppressor p53 [41] and the inhibitor of cyclin-dependent kinases $p27^{Kip}$ [42] are phosphorylated by the CSN-associated CK2, which targets the proteins to degradation by the 26S proteasome. In contrast, phosphorylation of c-Jun stabilizes the transcription factor towards the UPS (Fig. (**1**)) [38, 43]. Therefore, kinase inhibitors such as curcumin or emodin elevate the amount of the tumor suppressor p53, which causes apoptosis in tumor cells [44]. On the other hand, as

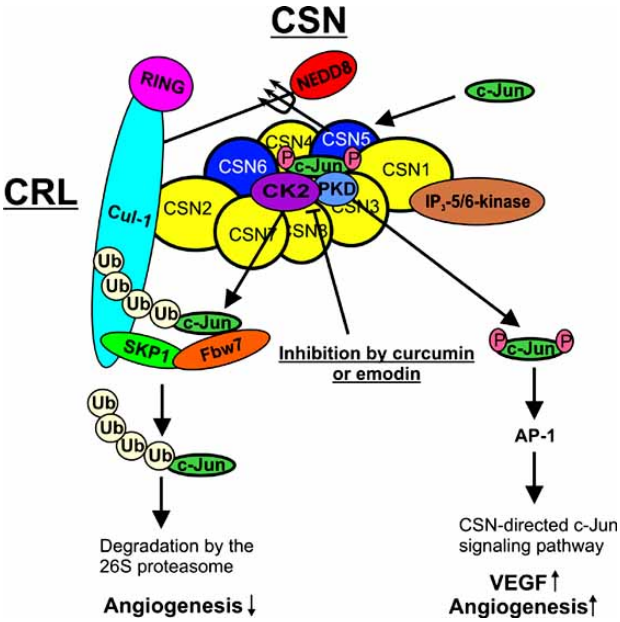

Fig. (1). The CSN-associated kinases decide whether c-Jun is ubiquitinated by a CRL or stabilized to stimulate angiogenesis. The CSN-associated kinases phosphorylate c-Jun and activate the CSN-directed c-Jun signaling pathway, which results in an increased production of VEGF by tumor cells [22], an important prerequisite for tumor-angiogensis. If CSN-associated kinases are blocked by chemopreventive agents such as curcumin or emodin, c-Jun binds to an F-box protein, presumably Fbw7, of a CRL, which ubiquitinates the transcription factor prior to degradation by the 26S proteasome.

shown in Fig. (**1**), by inhibiting phosphorylation these compounds target c-Jun to rapid degradation by the UPS [38, 44], which most likely retards tumor angiogenesis.

Thus, the CSN regulates the UPS by deneddylation of cullins as well as by CSN-mediated phosphorylation of UPS substrates influencing the specific degradation of pro-angiogenic factors.

1.1. Anti-Angiogenic Drugs Acting as Inhibitors of CSN-Associated Kinases

Inhibitors of CSN-associated kinases most likely have at least two effects that are very beneficial for tumor therapy. First, they reduce CSN-mediated phosphorylation of p53 [41] and of p27 [42] causing stabilization of the two cell cycle regulators and inducing apoptosis in tumor cells. Second, diminished CSN-mediated phosphorylation of c-Jun targets the pro-angiogenic transcription factor to degradation by the UPS [38, 43]. Recently inhibitors of the proteasome that completely block degradation by the UPS have been tested in international phase III clinical trials and it appears that proteasome inhibition is efficient in a few cases. However, it certainly cannot be considered for long-term treatment, because of the housekeeping function of the proteasome and the resulting negative side effects (for review see [45]). Therefore, differential influence on the specificity of the UPS by inhibitors of CSN-mediated phosphorylation might be more useful for long-term application as compared to proteasome inhibitors.

1.1.1. Chemistry, Mechanism and Biological Effects of Curcumin and Curcumin-Like Compounds

Curcumin has been identified as a very potent inhibitor of CSN-associated kinases and of CSN-directed c-Jun signaling [22, 38, 39, 46]. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; diferuloylmethane], the yellow pigment in curry, the active component of tumeric, is derived from the rhizomes of *Curcuma longa Linn*. It is a crystalline compound that has been traditionally used in medicine and cooking in India [47]. Curcumin has anti-oxidative, anti-inflammatory and anti-septic properties and is an *in vivo* inhibitor of angiogenesis [48]. It is a chemopreventive agent that blocks initiation steps of carcinogenesis as well as malignant proliferation during tumor growth (for review see [49-53]).

Curcumin is a kinase inhibitor and most of its pleiotropic effects can be explained by this feature. Presumably it fits into the ATP-binding pocket just as it has been shown for emodin [44]. It is not kinase-specific but has higher affinities for PKD as compared to CK2. For example, it also inhibits phosphorylation of IκBα, which stabilizes the inhibitor of NF-κB towards degradation by the UPS (for review see [51, 54, 55]). The activated NF-κB signaling pathway plays a major role in tumorigenesis, since NF-κB induces a number of pro-angiogenic factors including cyclooxygenase 2 (COX-2) and matrix metalloproteases [56]. The expression of these proteins is significantly reduced by the natural polyphenole curcumin [51, 57]. In addition, it has been suggested that curcumin has a direct effect on Ub isopeptidases [58].

We have found efficient inhibition of the CSN-associated kinases, CK2 and PKD, by curcumin [38] and suggest that most of our curcumin effects can be due to this kinase inhibition. Treatment of HeLa cells leads to an increase of p53 as well as to an UPS-dependent disappearance of c-Jun (for review see [40]). In addition, curcumin blocks invasiveness and tubulogenesis of human umbilical vein endothelial cells (HUVEC) as visualized by us in a Matrigel assay [59] and as demonstrated elsewhere [52]. Curcumin-induced increase of p53 causes apoptosis in B8 mouse fibroblasts and HeLa [44] as well as in other tumor cells [60]. Degradation of c-Jun in the presence of curcumin results in reduced VEGF production by tumor cells [22], a major reason for its anti-angiogenic properties. In summary, most of the curcumin effects are due to kinase inhibition and make this compound to a potent anti-angiogenic as well as anti-tumor drug.

Recently by *in silico* screenings we have identified several curcumin-like substances with similar two- and three-dimensional structures (see Fig. (**2**)) as well as biological effects [44]. Resveratrol and piceatannol are also natural phenols and inhibit the CSN-associated kinases [38, 44]. Piceatannol possesses highest affinities towards the kinases as compared with other inhibitors. All curcumin-like compounds stabilize p53 and induce apoptosis in tumor cells. However, none of these compounds seems to be as effective as curcumin [44]. Just like curcumin, resveratrol (3,4',5-trihydroxy stilbene), a natural product derived from grapes, inhibits COX-2 expression [61] and is a cancer chemopreventive agent [62]. It also blocks tube formation of HUVEC [63]. Piceatannol (3,3',4,5'-tetrahydroxy stilbene), which is derived from resveratrol and also occurs in grapes, inhibits the VEGF production in smooth muscle cells [64] and prevents tubulogenesis of HUVEC [65]. Thus, these curcumin-like substances including BTB00363 (2-Pyridinecarboxylic acid, [(3,5-dichloro-2-hydroxyphenyl)methylene]hydrazide) are potential anti-angiogenic agents and should be tested for clinical application in future tumor therapy.

1.1.2. Chemistry, Mechanism and Biological Effects of Emodin and Emodin-Like Compounds

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) shown in Fig. (**2**) is a naturally occurring anthraquinone present in the roots and barks of numerous plants and an active ingredient of Chinese herbs (for review see [66, 67]). The mechanism of emodin action is very similar to that of curcumin and curcumin-like compounds. It is also known as a inhibitor of NF-κB, which stabilizes IκBα towards degradation by the UPS [68]. Most likely emodin blocks the kinases of the IKK complex responsible for IκBα phosphorylation, which targets the NF-κB inhibitor to ubiquitination and subsequent degradation. As a consequence pro-angiogenic regulators such as COX-2 and matrix metalloproteases are not induced. Moreover, emodin reduced bFGF-induced proliferation and migration of HUVEC and VEGF A-induced tubulogenesis of dermal microvascular endothelial cells [69].

Emodin has been shown to specifically cause protein kinase CK2 inhibition during retinal neovascularization in a mouse model, which regulates angiogenesis (for review see [66]). This is confirmed by the fact that it fits into the ATP-binding pocket of CK2 as revealed by the crystal structure of the kinase and emodin [70] and by competition with ATP-binding to CK2 [71]. Our data demonstrate that emodin is a kinase inhibitor with higher affinity for CK2 as compared to PKD. Interestingly, the IC50 value is 5-times higher with isolated CSN as with recombinant CK2 [38]. Emodin stabilizes p53 in fibroblasts towards degradation by the UPS and induces p53-dependent apoptosis [44, 72]. Its effects on CSN-associated kinases and on c-Jun encourage us to suggest that it also might reduce VEGF production in tumor cells just like curcumin [22] and acts as a suppressor of tumor angiogenesis. Additional agents of the emodin-like group (see Fig. (**2**)), BTB14431 (9,10-dihydroxy-1,4-dihydroanthracene-1,4-dione), JFD02836 (3-methoxy-10-methyl-9, 10-dihydro-9-acridinone), SEW04213 (6-fluoro-3,4-dihydro-2H-pyrano[2,3-b]quinolin-5-amine) and JFD03665 (10-(hydroxymethylene)phenanthren-9(10H)-one), again, were obtained by *in silico* screening using emodin as the lead structure. They all are kinase inhibitors with higher preference for CK2 than for PKD [44]. Based on our data we assume that their biological effects are very similar as compared to emodin and that these synthesized compounds obtained from common data banks are potential anti-angiogenic drugs for possible use in tumor therapy.

1.2. Clinical Application of Curcumin- and Emodin-Like Substances

Preclinical studies have revealed the chemopreventive potentials of curcumin and emodin as well as of related compounds in several animal tumor bioassay systems (see above). Because curcumin has no dose-limiting toxicity,

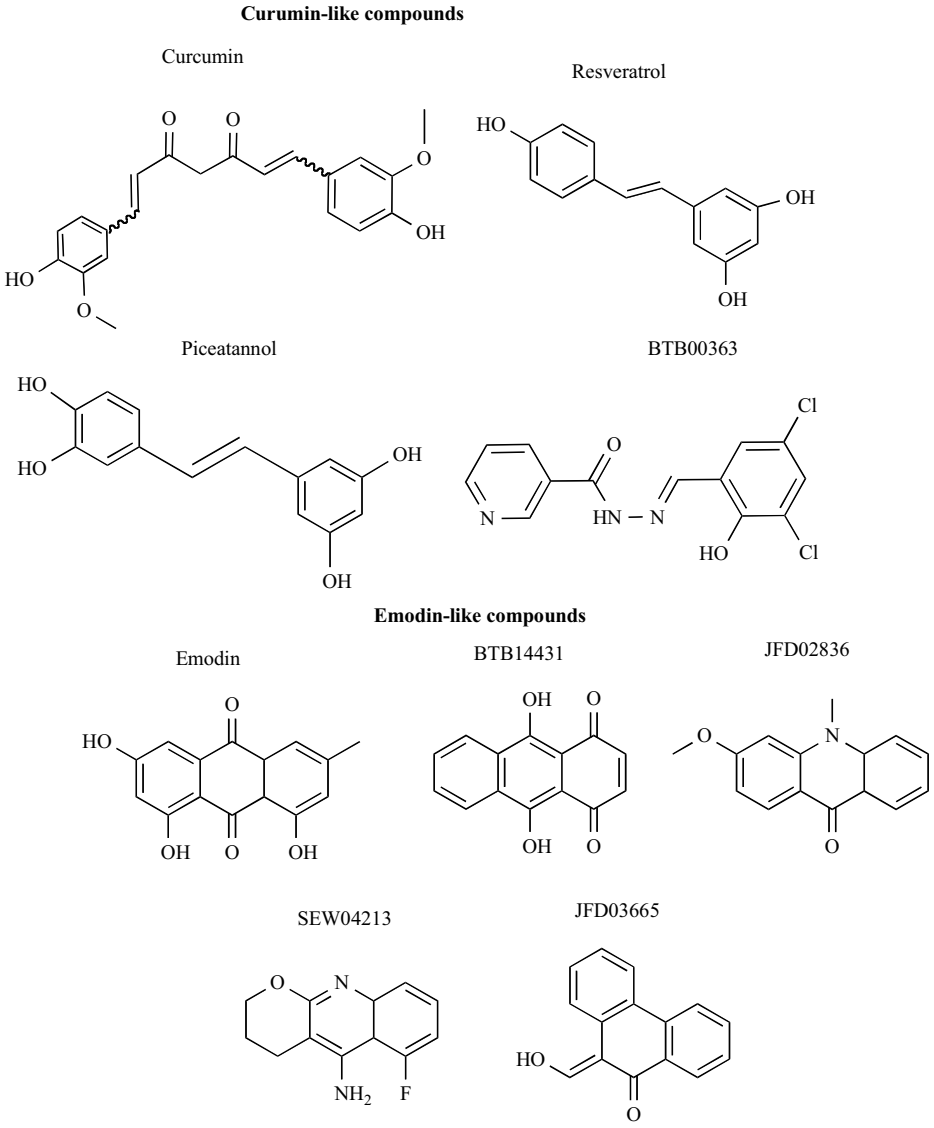

Fig. (2). Structures of curcumin and curcumin-like as well as emodin and emodin-like compounds that inhibit CSN-associated kinases (for details see text).

even when it is administered up to 8 g/day in human clinical trials, it can be used to prevent angiogenesis and to treat cancer [73]. Few years ago curcumin has been tested in a phase I clinical trial in patients with high-risk or premalignant lesions [74]. Curcumin was taken orally for 3 months. This prospective phase-I study evaluated curcumin in patients with one of the following five high-risk conditions: 1) recently resected urinary bladder cancer; 2) arsenic Bowen's disease of the skin; 3) uterine cervical intraepithelial neoplasm; 4) oral leucoplakia; and 5) intestinal metaplasia of the stomach. The authors concluded that curcumin has an effect in the chemoprevention of cancer [74]. Moreover, there exist trials of curcumin in colorectal cancer patients. After a consumption of 3.5 g curcumin daily for 4 months a reduction in inducible PGE2 levels, most likely as a result of a lower COX-2 expression, has been detected in peripheral blood samples [75, 76]. In addition, a reduction in the level of deoxyguanosine adduct, a marker of oxidative DNA damage, has been detected upon curcumin treatment of malignant colorectal tissue [77]. Unfortunately so far information on clinical properties of curcumin is rare and, to our knowledge, resveratrol, piceatannol and BTB00363 have not been studied in the clinic. In the future suitable trials have to be designed. Since curcumin has a poor systemic availability, future studies should focus on the prevention of cancer of the colon, skin or oral cavity (for review see [78]).

In many pre-clinical studies it has been demonstrated that emodin, the most abundant anthraquinone of rhubarb, inhibits cell proliferation, induces apoptosis and prevents angiogenesis as well as metastasis (see above and for review [79]). These capabilities are based on kinase inhibition including CSN-associated kinases [38]. Their impact on c-Jun and p53 levels in tumor cells connected with inhibition of angiogenesis and induction of apoptosis make emodin as well as

emodin-like compounds to potential anti-angiogenic and anti-tumor drugs. Interestingly, emodin also can act as a sensitizer in chemotherapy-based combination regimes [80, 81]. Unfortunately, to date no clinical studies exist, which might help to evaluate the clinical properties of these chemopreventive agents.

2. TAUROLIDINE, AN INHIBITOR OF PROTEIN BIOSYNTHESIS

Taurolidine (TRD, bis-(1,1-dioxoperhydro-1,2,4-thiadiazinyl-4)methane) is a synthetic product derived from the biogenic amino acid taurine and, as far as our analysis revealed, does not act as a kinase inhibitor. It has no direct impact on the CSN-associated kinases or the proteasome [82]. Nevertheless, it possess a broad bactericidal and fungicidal efficacy spectrum as well as anti-tumor effects (for review see [83, 84]), which encouraged us to study its mechanism of action.

2.1. Chemistry, Mechanism and Biological Effects of Taurolidine

TRD consists of two aromatic rings (MW 284), which are linked with a CH_2-group (see Fig. (**3**)) [85, 86]. Hydrolysation divides the molecule into taurultam and methyloltaurultam, two active metabolites. It is further biotransformed to methyloltaurinamide, taurinamide, taurine, and carbon dioxide. After intraperitoneal application the half-live of TRD and taurultam are approximately 2 h and 8 h, respectively, which are significantly shorter after intravenous injection [87, 88].

Originally TRD was described as an antibacterial agent and used in the treatment of peritonitis or in patients with systemic inflammatory response syndrome [89]. It has been hypothesized that the suppression of tumor growth may be explained by intracellular effects causing apoptosis presumably mediated by a mitochondrial cytochrome c-dependent mechanism [90]. Most recently, the agent has been found to exert a direct and selective effect on glial and neuronal brain tumor cells *via* Fas-ligand-mediated cell death [91]. TRD decreases TNFα and IL-1β production of peritoneal macrophages, which might explain the additional antineoplastic effect on local cell growth [92]. TNFα stimulates tumor angiogenesis presumably by activating the production of the major pro-angiogenic factor VEGF in tumor cells. Additionally, TNFα is known to downregulate apoptosis by activating NF-κB, an important pro-angiogenic transcription factor (see above).

Our studies revealed that the pleiotropic effects of TRD can be explained neither by blocking the UPS nor by affecting CSN-associated kinases. We showed that TRD acts as an inhibitor of an early phase in protein biosynthesis [82]. The translation, but not transcription, of all tested proteins such as c-Jun, p53 and IκBα was reduced in the presence of 16 mM TRD (0.5%). Different phases of translation, such as formation of the initiation complex, of the functional 80S ribosome and of polysomes, were separated by density gradient centrifugation and visualized using ^{32}P-mRNA. In these experiments we demonstrated that TRD as well as taurultam affect translation at a very early stage. It seemed that

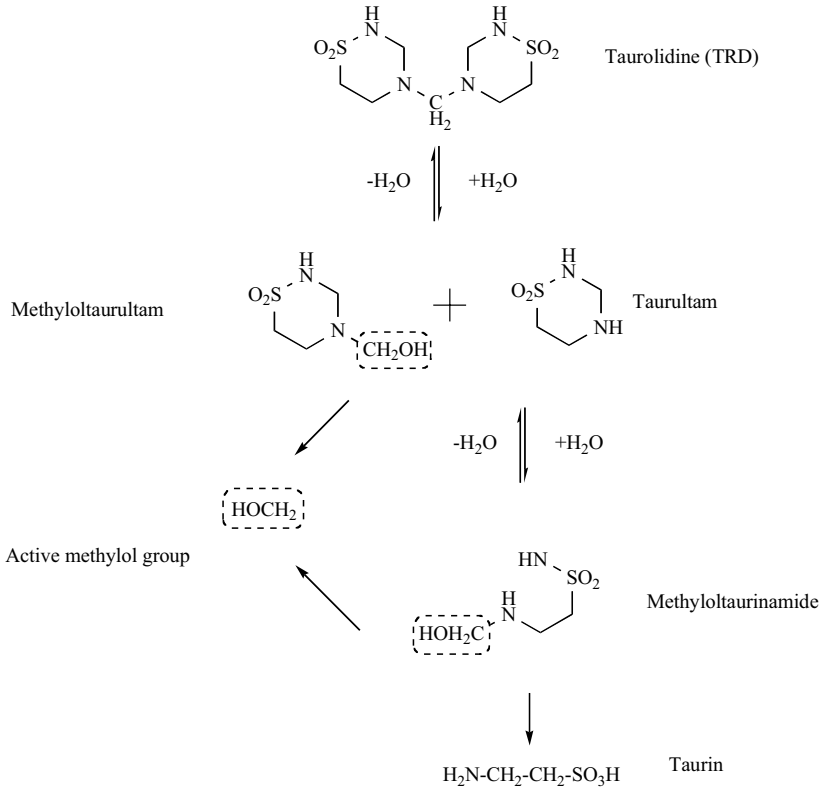

Fig. (3). Hydrolysis of TRD and its products. In the presence of H_2O TRD (half-life ~ 2 h) hydrolyzes to taurultam (half-life ~ 8 h) and to methyloltaurultam. After two more steps taurultam decays to the biogenic amino acid taurine.

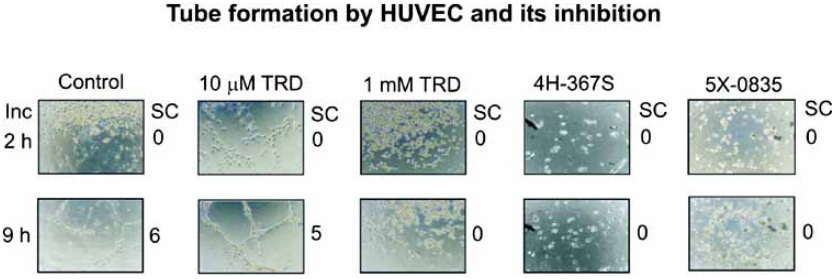

Fig. (4). Inhibition of tubulogenesis of HUVEC by TRD and TRD-like compounds. HUVEC were grown on Matrigel as described recently [59]. Wells were pre-coated with growth-factor-improved Matrigel matrix and incubated (Inc) for 2 h or 9 h as recommended by the manufacturer (Becton Dickinson Labware). The effects of TRD on tubulogenesis were estimated in the presence of 10 μM and 1 mM of the compound. TRD-like substances were used at a concentration of 1 mM. The effects were quantified by counting the number of branch points and a branch point score (SC) is given.

no pre-initiation complex was created anymore. Since protein biosynthesis was blocked not only in mammalian cells but also in bacteria, inhibition of translation might explain most of the effects of TRD including the induction of apoptosis [82].

2.2. Taurolidine Inhibits Tubulogenesis

Inhibition of protein synthesis by TRD and taurultam most likely disturbs the balance of pro- and anti-angiogenic factors and affects tubulogenesis and angiogenesis. Therefore, to study the effect of TRD on tubulogenesis we used the modified Boyden chamber, which is based on a Matrigel matrix consisting of laminin, collagen type IV, entactin and protoheparan sulfate [59]. In this matrix HUVEC proliferate and form cell-junctions, which results in the formation of lumen-like tubes. We tested different concentrations of TRD in this system and quantified the data by counting branching points. With 10 μM of TRD there was only little influence on tubulogenesis whereas 1.0 mM blocked the process almost completely (see Fig. (**4**)). The effect of TRD might be due to diminished translation, which presumably reduced the production of VEGF by HUVEC. Previous studies have already shown that TRD decreases VEGF production of tumor cells [93].

2.3. Identification of Taurolidine-Like Substances

As described previously for curcumin and emodin [44] *in silico* screening was performed to identify TRD-like substances. On the basis of the TRD two- and three-dimensional structures a number of similar compounds was found that exhibit biological effects comparable with TRD. The *in silico* screening revealed the substances 4H-367S, 5X-0835 as well as 5W-0902, which were analyzed in cell experiments (Fullbeck *et al.*, publication in preparation). The results demonstrate that intracellular levels of c-Jun decreased, which presumably reduced VEGF production (our unpublished data). Tubulogenesis was inhibited by 4H-367S as well as 5X-0835 in the Matrigel chamber assay (see Fig. (**4**)).

2.4. Clinical Application of Taurolidine

Anti-septic and anti-tumor features of TRD make the substance very attractive for clinical use. TRD has been applied against infection after elective colorectal surgery [94] and is still successfully used as a disinfectant [95].

There exist numerous studies on the correlation between suppression of tumor growth as well as metastatic spreading and IL-1β response after TRD treatment. Previous studies confirmed a significant association between the IL-1β polymorphisms and increased risk for tumor development in patients with intestinal type or diffuse gastric carcinoma [96]. Comparable conclusions have been drawn for the relationship between IL-1β polymorphisms and *Helicobacter Pylori* infection associated with gastric adenocarcinoma [97, 98]. Moreover, a genetic polymorphism in a pancreatic cancer, which was homozygous for allele 2 of the IL-1β gene had shorter survival rates [99]. A prospective randomized multicenter trial investigated the effects on cytokine release (IL-1β) induced by intraperitoneal 0.25% povidone-iodine versus 0.5% TRD application in 120 patients (our unpublished data, conventional resections of colon cancer, n=57, gastric cancer, n=52 and pancreatic malignancies, n=11). Additionally, the serum- and intraperitoneal levels of IL-6, IL-10 and TNFα as well as the incidence of local recurrences or metastases were analyzed. Randomized patients were intraperitoneally treated with TRD (0.5%) or Ringer´s solution and 0.25% povidone-iodine (control) at the beginning of the operation and after tumor resection. No clinically relevant side effects were observed during administration and after reabsorption of the agent. A reduction of intraperitoneal IL-1β production ($p<0.001$) in the TRD group was noticed. The overall disease-free time and the survival rate of the two groups were not different. The immunological status of the patients was evaluated by the monocyte HLA-DR status. Significantly higher values were detected on the first and second postoperative day in the TRD-group versus povidone-iodine. This result could be interpreted as an increased immune response to TRD stimulation after surgery. Similar immuno-stimulatory effects of TRD have been reported by other authors [100].

Recently, a new study was started in our clinic. The influences of an intravenous therapy (2% TRD, 300 mg/kg body weight per day) on gastric- and pancreatic cancer recurrence or advanced diseases (n=50) were examined. The monthly repeated seven-days treatment sessions were performed using a central vein port catheter. No clinically relevant side effects were seen in 41 patients. In addition, quality of life, response rate and mortality rate were evaluated using a standardized protocol. One patient was lately submitted to his 39th chemotherapy session with clear response and good

quality of life [101]. It seems that the drug is effective on several tumor cells. Two patients with malignant glioblastoma were intravenously treated (2% TRD, 1000 ml = 20 g per day). Although both patients died four months later from acute thromboembolism and pneumonia, a transient improvement in quality of life and a partial tumor remission were observed [100]. A clear response on the TRD treatment was seen in the CT scan. These results highlight the potential anti-tumor effects of TRD through a cytokine modulating effect. The interpretation of the findings in terms of the anti-tumor effects of TRD are currently being investigated in a German multi-center clinical prospective randomized controlled trial (n=2000).

3. PERSPECTIVES

The studies reviewed here are a demonstration of the potencies of curcumin, emodin and taurolidine as well as of their structural homologs to act as inhibitors of angiogenesis and of tumor growth in pre-clinical experiments. All these compounds are potentially attractive alternatives to currently used chemopreventive drugs. The available anti-angiogenic and anti-tumor evidence of most of these compounds supports their advancement into phase III clinical trials. In the near future we will need more well designed clinical studies to better assess their clinical properties and aimed application.

ABBREVIATIONS

VEGF	=	Vascular endothelial growth factor
Ub	=	Ubiquitin
UPS	=	Ubiquitin proteasome system
CRL	=	Cullin-RING ligase
CSN	=	COP9 signalosome
CK2	=	Protein kinase CK2
PKD	=	Protein kinase D
IκBα	=	Inhibitor of nuclear transcription factor κB alpha
COX-2	=	Cyclooxygenase-2
HUVEC	=	Human umbilical vein endothelial cells
TRD	=	Taurolidine

REFERENCES

[1] Folkman, J. *N. Engl. J. Med.*, **1971**, *285*, 1182.
[2] Liu, C.C.; Shen, Z.; Kung, H.F.; Lin, M.C. *World J. Gastroenterol.*, **2006**, *12*, 6941.
[3] Naumov, G.N.; Akslen, L.A.; Folkman, J. *Cell Cycle*, **2006**, *5*, 1779.
[4] Gasparini, G.; Longo, R.; Toi, M.; Ferrara, N. *Nat. Clin. Pract. Oncol.*, **2005**, *2*, 562.
[5] Folkman, J.; Hochberg, M. *J. Exp. Med.*, **1973**, *138*, 745.
[6] Longo, R.; Sarmiento, R.; Fanelli, M.; Capaccetti, B.; Gattuso, D.; Gasparini, G. *Angiogenesis*, **2002**, *5*, 237.
[7] St Croix, B.; Rago, C.; Velculescu, V.; Traverso, G.; Romans, K.E.; Montgomery, E.; Lal, A.; Riggins, G.J.; Lengauer, C.; Vogelstein, B.; Kinzler, K.W. *Science*, **2000**, *289*, 1197.
[8] Stupack, D.G.; Cheresh, D.A. *Curr. Top. Dev. Biol.*, **2004**, *64*, 207.
[9] Ribatti, D.; Nico, B.; Crivellato, E.; Roccaro, A.M.; Vacca, A. *Leukemia*, **2006**.
[10] Watnick, R.S.; Cheng, Y.N.; Rangarajan, A.; Ince, T.A.; Weinberg, R.A. *Cancer Cell*, **2003**, *3*, 219.
[11] Brahimi-Horn, C.; Pouyssegur, J. *Bull. Cancer*, **2006**, *93*, E73.
[12] Melillo, G. *Mol. Cancer Res.*, **2006**, *4*, 601.
[13] Brahimi-Horn, C.; Pouyssegur, J. *Crit. Rev. Oncol. Hematol.*, **2005**, *53*, 115.
[14] Masson, N.; Ratcliffe, P.J. *J. Cell Sci.*, **2003**, *116*, 3041.
[15] Rini, B.I.; Small, E.J. *J. Clin. Oncol.*, **2005**, *23*, 1028.
[16] Vogt, P.K. *Nat. Rev. Cancer*, **2002**, *2*, 465.
[17] Vleugel, M.M.; Greijer, A.E.; Bos, R.; van der Wall, E.; van Diest, P.J. *Hum. Pathol.*, **2006**, *37*, 668.
[18] Zhang, G.; Dass, C.R.; Sumithran, E.; Di Girolamo, N.; Sun, L.Q.; Khachigian, L.M. *J. Natl. Cancer Inst.*, **2004**, *96*, 683.
[19] Derijard, B.; Hibi, M.; Wu, I.H.; Barrett, T.; Su, B.; Deng, T.; Karin, M.; Davis, R.J. *Cell*, **1994**, *76*, 1025.
[20] Shaulian, E.; Karin, M. *Nat. Cell Biol.*, **2002**, *4*, E131.
[21] Seeger, M.; Kraft, R.; Ferrell, K.; Bech-Otschir, D.; Dumdey, R.; Schade, R.; Gordon, C.; Naumann, M.; Dubiel, W. *FASEB J.*, **1998**, *12*, 469.
[22] Pollmann, C.; Huang, X.; Mall, J.; Bech-Otschir, D.; Naumann, M.; Dubiel, W. *Cancer Res.*, **2001**, *61*, 8416.
[23] Deng, X.W.; Dubiel, W.; Wei, N.; Hofmann, K.; Mundt, K.; Colicelli, J.; Kato, J.; Naumann, M.; Segal, D.; Seeger, M.; Carr, A.; Glickman, M.; Chamovitz, D.A. *Trends Genet.*, **2000**, *16*, 202.
[24] Wei, N.; Chamovitz, D.A.; Deng, X.W. *Cell*, **1994**, *78*, 117.
[25] Wei, N.; Deng, X.W. *Annu. Rev. Cell Dev. Biol.*, **2003**, *19*, 261.
[26] Glickman, M.H.; Rubin, D.M.; Coux, O.; Wefes, I.; Pfeifer, G.; Cjeka, Z.; Baumeister, W.; Fried, V.A.; Finley, D. *Cell*, **1998**, *94*, 615.
[27] Peng, Z.; Shen, Y.; Feng, S.; Wang, X.; Chitteti, B.N.; Vierstra, R.D.; Deng, X.W. *Curr. Biol.*, **2003**, *13*, R504.
[28] Huang, X.; Hetfeld, B.K.; Seifert, U.; Kahne, T.; Kloetzel, P.M.; Naumann, M.; Bech-Otschir, D.; Dubiel, W. *FEBS J.*, **2005**, *272*, 3909.
[29] Groisman, R.; Polanowska, J.; Kuraoka, I.; Sawada, J.; Saijo, M.; Drapkin, R.; Kisselev, A.F.; Tanaka, K.; Nakatani, Y. *Cell*, **2003**, *113*, 357.
[30] Liu, C.; Powell, K.A.; Mundt, K.; Wu, L.; Carr, A.M.; Caspari, T. *Genes Dev.*, **2003**, *17*, 1130.
[31] Naumann, M.; Bech-Otschir, D.; Huang, X.; Ferrell, K.; Dubiel, W. *J. Biol. Chem.*, **1999**, *274*, 35297.
[32] Maytal-Kivity, V.; Piran, R.; Pick, E.; Hofmann, K.; Glickman, M.H. *EMBO Rep.*, **2002**, *12*, 1215.
[33] Karniol, B.; Malec, P.; Chamovitz, D.A. *Plant Cell*, **1999**, *11*, 839.
[34] Cope, G.A.; Suh, G.S.; Aravind, L.; Schwarz, S.E.; Zipursky, S.L.; Koonin, E.V.; Deshaies, R.J. *Science*, **2002**, *298*, 608.
[35] Petroski, M.D.; Deshaies, R.J. *Nat. Rev. Mol. Cell Biol.*, **2005**, *6*, 9.
[36] Hetfeld, B.K.; Helfrich, A.; Kapelari, B.; Scheel, H.; Hofmann, K.; Guterman, A.; Glickman, M.; Schade, R.; Kloetzel, P.M.; Dubiel, W. *Curr. Biol.*, **2005**, *15*, 1217.
[37] Wee, S.; Geyer, R.K.; Toda, T.; Wolf, D.A. *Nat. Cell Biol.*, **2005**, *7*, 387.
[38] Uhle, S.; Medalia, O.; Waldron, R.; Dumdey, R.; Henklein, P.; Bech-Otschir, D.; Huang, X.; Berse, M.; Sperling, J.; Schade, R.; Dubiel, W. *EMBO J.*, **2003**, *22*, 1302.
[39] Sun, Y.; Wilson, M.P.; Majerus, P.W. *J. Biol. Chem.*, **2002**, *277*, 45759.
[40] Bech-Otschir, D.; Kapelari, B.; Dubiel, W. In *Protein Degradation*; Mayer, R., Ciechanover, A., Rechsteiner, M., Eds.; WILEY-VCH Verlag GmbH & Co. KGaA: Weinheim, **2005**; Vol. 1: Ubiquitin and the Chemistry of Life, p. 348.
[41] Bech-Otschir, D.; Kraft, R.; Huang, X.; Henklein, P.; Kapelari, B.; Pollmann, C.; Dubiel, W. *EMBO J.*, **2001**, *20*, 1630.
[42] Huang, X.; Wagner, E.; Dumdey, R.; Peth, A.; Berse, M.; Dubiel, W.; Berndt, C. *Isr. J. Chem.*, **2006**, *46*, 231.
[43] Naumann, M.; Bech-Otschir, D.; Huang, X.; Ferrell, K.; Dubiel, W. *J. Biol. Chem.*, **1999**, *274*, 35297.
[44] Fullbeck, M.; Huang, X.; Dumdey, R.; Frommel, C.; Dubiel, W.; Preissner, R. *BMC Cancer*, **2005**, *5*, 97.
[45] Reinstein, E.; Ciechanover, A. *Ann. Intern. Med.*, **2006**, *145*, 676.
[46] Henke, W.; Ferrell, K.; Bech-Otschir, D.; Seeger, M.; Schade, R.; Jungblut, P.; Naumann, M.; Dubiel, W. *Mol. Biol. Rep.*, **1999**, *26*, 29.
[47] Ammon, H.P.; Wahl, M.A. *Planta Med.*, **1991**, *57*, 1.

[48] Arbiser, J.L.; Klauber, N.; Rohan, R.; van Leeuwen, R.; Huang, M.T.; Fisher, C.; Flynn, E.; Byers, H.R. *Mol. Med.*, **1998**, *4*, 376.
[49] Duvoix, A.; Blasius, R.; Delhalle, S.; Schnekenburger, M.; Morceau, F.; Henry, E.; Dicato, M.; Diederich, M. *Cancer Lett.*, **2005**, *223*, 181.
[50] Duvoix, A.; Delhalle, S.; Blasius, R.; Schnekenburger, M.; Morceau, F.; Fougere, M.; Henry, E.; Galteau, M.M.; Dicato, M.; Diederich, M. *Biochem. Pharmacol.*, **2004**, *68*, 1101.
[51] Thangapazham, R.L.; Sharma, A.; Maheshwari, R.K. *AAPS J.*, **2006**, *8*, E443.
[52] Singh, S.; Khar, A. *Anticancer Agents Med. Chem.*, **2006**, *6*, 259.
[53] Campbell, F.C.; Collett, G.P. *Future Oncol.*, **2005**, *1*, 405.
[54] Lin, Y.G.; Kunnumakkara, A.B.; Nair, A.; Merritt, W.M.; Han, L.Y.; Armaiz-Pena, G.N.; Kamat, A.A.; Spannuth, W.A.; Gershenson, D.M.; Lutgendorf, S.K.; Aggarwal, B.B.; Sood, A.K. *Clin. Cancer Res.*, **2007**, *13*, 3423.
[55] Kamat, A.M.; Sethi, G.; Aggarwal, B.B. *Mol. Cancer Ther.*, **2007**, *6*, 1022.
[56] Kim, J.H.; Lee, K.W.; Lee, M.W.; Lee, H.J.; Kim, S.H.; Surh, Y.J. *FEBS Lett.*, **2006**, *580*, 385.
[57] Tunstall, R.G.; Sharma, R.A.; Perkins, S.; Sale, S.; Singh, R.; Farmer, P.B.; Steward, W.P.; Gescher, A.J. *Eur. J. Cancer*, **2006**, *42*, 415.
[58] Mullally, J.E.; Fitzpatrick, F.A. *Mol. Pharmacol.*, **2002**, *62*, 351.
[59] Ismail, M.; Henklein, P.; Huang, X.; Braumann, C.; Ruckert, R.I.; Dubiel, W. *Eur. J. Haematol.*, **2006**, *77*, 157.
[60] Choudhuri, T.; Pal, S.; Agwarwal, M.L.; Das, T.; Sa, G. *FEBS Lett.*, **2002**, *512*, 334.
[61] Subbaramaiah, K.; Chung, W.J.; Michaluart, P.; Telang, N.; Tanabe, T.; Inoue, H.; Jang, M.; Pezzuto, J.M.; Dannenberg, A.J. *J. Biol. Chem.*, **1998**, *273*, 21875.
[62] Jang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W.; Fong, H.H.; Farnsworth, N.R.; Kinghorn, A.D.; Mehta, R.G.; Moon, R.C.; Pezzuto, J.M. *Science*, **1997**, *275*, 218.
[63] Kimura, Y.; Okuda, H. *J. Nutr.*, **2001**, *131*, 1844.
[64] Faffe, D.S.; Flynt, L.; Mellema, M.; Whitehead, T.R.; Bourgeois, K.; Panettieri, R.A., Jr.; Silverman, E.S.; Shore, S.A. *Am. J. Physiol. Lung Cell Mol. Physiol*, **2005**, *288*, L1040.
[65] Kimura, Y.; Baba, K.; Okuda, H. *Anticancer Res.*, **2000**, *20*, 2923.
[66] Srinivas, G.; Babykutty, S.; Sathiadevan, P.P.; Srinivas, P. *Med. Res. Rev.*, **2006**.
[67] Garg, A.K.; Buchholz, T.A.; Aggarwal, B.B. *Antioxid. Redox. Signal*, **2005**, *7*, 1630.
[68] Kumar, A.; Dhawan, S.; Aggarwal, B.B. *Oncogene*, **1998**, *17*, 913.
[69] Kwak, H.J.; Park, M.J.; Park, C.M.; Moon, S.I.; Yoo, D.H.; Lee, H.C.; Lee, S.H.; Kim, M.S.; Lee, H.W.; Shin, W.S.; Park, I.C.; Rhee, C.H.; Hong, S.I. *Int. J. Cancer*, **2006**, *118*, 2711.
[70] Sarno, S.; Moro, S.; Meggio, F.; Zagotto, G.; Dal Ben, D.; Ghiselllini, P.; Battistutta, R.; Zanotti, G.; Pinna, L.A. *Pharmacol. Ther.*, **2002**, *93*, 159.
[71] Yim, H.; Lee, Y.H.; Lee, C.H.; Lee, S.K. *Planta Med.*, **1999**, *65*, 9.
[72] Lin, J.G.; Chen, G.W.; Li, T.M.; Chouh, S.T.; Tan, T.W.; Chung, J.G. *J. Urol.*, **2006**, *175*, 343.
[73] Aggarwal, B.B.; Kumar, A.; Bharti, A.C. *Anticancer Res.*, **2003**, *23*, 363.
[74] Cheng, A.L.; Hsu, C.H.; Lin, J.K.; Hsu, M.M.; Ho, Y.F.; Shen, T.S.; Ko, J.Y.; Lin, J.T.; Lin, B.R.; Ming-Shiang, W.; Yu, H.S.; Jee, S.H.; Chen, G.S.; Chen, T.M.; Chen, C.A.; Lai, M.K.; Pu, Y.S.; Pan, M.H.; Wang, Y.J.; Tsai, C.C.; Hsieh, C.Y. *Anticancer Res.*, **2001**, *21*, 2895.
[75] Sharma, R.A.; Euden, S.A.; Platton, S.L.; Cooke, D.N.; Shafayat, A.; Hewitt, H.R.; Marczylo, T.H.; Morgan, B.; Hemingway, D.; Plummer, S.M.; Pirmohamed, M.; Gescher, A.J.; Steward, W.P. *Clin. Cancer Res.*, **2004**, *10*, 6847.
[76] Plummer, S.M.; Hill, K.A.; Festing, M.F.; Steward, W.P.; Gescher, A.J.; Sharma, R.A. *Cancer Epidemiol. Biomarkers Prev.*, **2001**, *10*, 1295.
[77] Garcea, G.; Berry, D.P.; Jones, D.J.; Singh, R.; Dennison, A.R.; Farmer, P.B.; Sharma, R.A.; Steward, W.P.; Gescher, A.J. *Cancer Epidemiol. Biomarkers Prev.*, **2005**, *14*, 120.
[78] Thomasset, S.C.; Berry, D.P.; Garcea, G.; Marczylo, T.; Steward, W.P.; Gescher, A.J. *Int. J. Cancer*, **2007**, *120*, 451.
[79] Huang, Q.; Lu, G.; Shen, H.M.; Chung, M.C.; Ong, C.N. *Med. Res. Rev.*, **2006**.
[80] Zhang, L.; Hung, M.C. *Oncogene*, **1996**, *12*, 571.
[81] Wang, S.C.; Zhang, L.; Hortobagyi, G.N.; Hung, M.C. *Semin. Oncol.*, **2001**, *28*, 21.
[82] Braumann, C.; Henke, W.; Jacobi, C.A.; Dubiel, W. *Int. J. Cancer*, **2004**, *112*, 225.
[83] Simon, A.; Bode, U.; Beutel, K. *Clin. Microbiol. Infect.*, **2006**, *12*, 606.
[84] Jacobi, C.A.; Menenakos, C.; Braumann, C. *Anticancer Drugs*, **2005**, *16*, 917.
[85] Calabresi, P.; Goulette, F.A.; Darnowski, J.W. *Cancer Res.*, **2001**, *61*, 6816.
[86] Darnowski, J.W.; Goulette, F.A.; Cousens, L.P.; Chatterjee, D.; Calabresi, P. *Cancer Chemother. Pharmacol.*, **2004**, *54*, 249.
[87] Knight, B.I.; Skellern, G.G.; Browne, M.K.; Pfirrmann, R.W. *Br. J. Clin. Pharmacol.*, **1981**, *12*, 695.
[88] Knight, B.I.; Skellern, G.G.; Browne, M.K.; Pfirrmann, R.W. *Br. J. Clin. Pharmacol.*, **1981**, *12*, 439.
[89] Billing, A.; Frohlich, D.; Ruckdeschel, G. *Langenbecks Arch. Chir.*, **1992**, *377*, 180.
[90] Han, Z.; Ribbizi, I.; Pantazis, P.; Wyche, J.; Darnowski, J.; Calabresi, P. *Anticancer Res.*, **2002**, *22*, 1959.
[91] Stendel, R.; Scheurer, L.; Stoltenburg-Didinger, G.; Brock, M.; Mohler, H. *Anticancer Res.*, **2003**, *23*, 2309.
[92] Torisu, H.; Ono, M.; Kiryu, H.; Furue, M.; Ohmoto, Y.; Nakayama, J.; Nishioka, Y.; Sone, S.; Kuwano, M. *Int. J. Cancer*, **2000**, *85*, 182.
[93] Braumann, C.; Stuhldreier, B.; Bobrich, E.; Menenakos, C.; Rogalla, S.; Jacobi, C.A. *J. Surg. Res.*, **2005**, *129*, 129.
[94] Bohm, B.; Schwenk, W.; Muller, J.M. *Chirurg*, **1999**, *70*, 453.
[95] Perry, J.D.; Riley, G.; Johnston, S.; Dark, J.H.; Gould, F.K. *J. Heart Lung Transplant.*, **2002**, *21*, 1230.
[96] Machado, J.C.; Pharoah, P.; Sousa, S.; Carvalho, R.; Oliveira, C.; Figueiredo, C.; Amorim, A.; Seruca, R.; Caldas, C.; Carneiro, F.; Sobrinho-Simoes, M. *Gastroenterology*, **2001**, *121*, 823.
[97] Gatti, L.L.; Burbano, R.R.; de Assumpcao, P.P.; Smith Mde, A.; Payao, S.L. *Clin. Exp. Med.*, **2004**, *4*, 93.
[98] Lu, W.; Pan, K.; Zhang, L.; Lin, D.; Miao, X.; You, W. *Carcinogenesis*, **2005**, *26*, 631.
[99] Barber, M.D.; Powell, J.J.; Lynch, S.F.; Fearon, K.C.; Ross, J.A. *Br. J. Cancer*, **2000**, *83*, 1443.
[100] Da Costa, M.L.; Redmond, H.P.; Bouchier-Hayes, D.J. *J. Surg. Res.*, **2001**, *101*, 111.
[101] Braumann, C.; Winkler, G.; Rogalla, P.; Menenakos, C.; Jacobi, C.A. *World J. Surg. Oncol.*, **2006**, *4*, 34.

Received: 22 December, 2006 **Revised: 9 October, 2007** **Accepted: 9 October, 2007**