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Synthesis and Biological Evaluation of Aeroplysinin Analogues: a New Class of Receptor Tyrosine Kinase Inhibitors

Klaus Hinterding,[a] Axel Knebel,[b] Peter Herrlich[b] and Herbert Waldmann[a,*]

[a]*Universität Karlsruhe, Institut für Organische Chemie, Richard-Willstätter-Allee 2, D-76128 Karlsruhe, Germany*
[b]*Forschungszentrum Karlsruhe GmbH, Institut für Genetik, D-76344 Eggenstein-Leopoldshafen, Germany*

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Abstract—Receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor receptor (PDGFR), are critically involved in the transduction of mitogenic signals across the plasma membrane and therefore in the regulation of cell growth and proliferation. Enhanced RTK activity is associated with proliferative diseases such as cancer, psoriasis and atherosclerosis, while decreased function may be associated for instance with diabetes. EGFR and PDGFR are selectively inhibited by analogues of the marine natural product aeroplysinin. The synthetic inhibitors display IC_{50} values in the low micromolar range and in contrast to the natural product show pronounced inhibitory activity in cultured cells in vivo. The mechanism of inhibition is likely based on a covalent modification of the target enzymes by reaction of epoxy ketone **8** with various nucleophiles.

Introduction

The proliferation and growth of cells are regulated by growth factors such as the insulin-like growth factor (IGF), the nerve growth factor (NGF), the epidermal growth factor (EGF), and the platelet-derived growth factor (PDGF). These growth factors are ligands for specific receptors with tyrosine kinase activity (RTKs). The receptors transmit growth-promoting signals across the cell membrane. After binding of the ligand to the extracellular domain of the receptors, they interphosphorylate tyrosine residues present in their intracellular domains and subsequently trigger various intracellular signalling cascades by a variety of specific protein-protein interactions (Fig. 1).[1] For instance, as a result of several protein–protein interactions the *Ras*-protein, a small GTP-binding protein, is activated. *Ras* then passes the signal on to a kinase cascade that eventually activates transcription factors, which then influence gene transcription in the nucleus. Dysregulation of these well balanced pathways may contribute to the generation of various diseases. For instance, enhanced activity of receptor tyrosine kinases has been implicated in carcinogenesis and in the development of proliferative diseases such as atherosclerosis and psoriasis,[2] whereas decreased function of the insulin RTK is associated with various types of diabetes.[3] Therefore, receptor tyrosine kinases are considered promising targets for the development of new drugs. Such RTK inhibitors are, in addition, likely useful in the dissection of signalling pathways.[2,4] To be used in biological studies, they should preferably be active both in vitro and in vivo.

Natural products like lavendustin A **1**,[5] quercetin **2**,[6] and erbstatin **3**[7] (Fig. 2), which can be applied advantageously in biological investigations, have proven to be RTK blockers. Based on these structures, synthetic inhibitors, the so called tyrphostins **4**,[2a,b,10] were developed. Whereas most compounds introduced so far display IC_{50} values in the low micromolar range,[2,4,8–10] more efficient and specific inhibitors such as **5** and **6** (Fig. 2) have recently been reported.[2a,9] Since numerous

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*Author for correspondence. Tel: +49-721-6082091; Fax: +49-721-6084825;
E-mail: waldmann@ochhades.chemie.uni-karlsruhe.de

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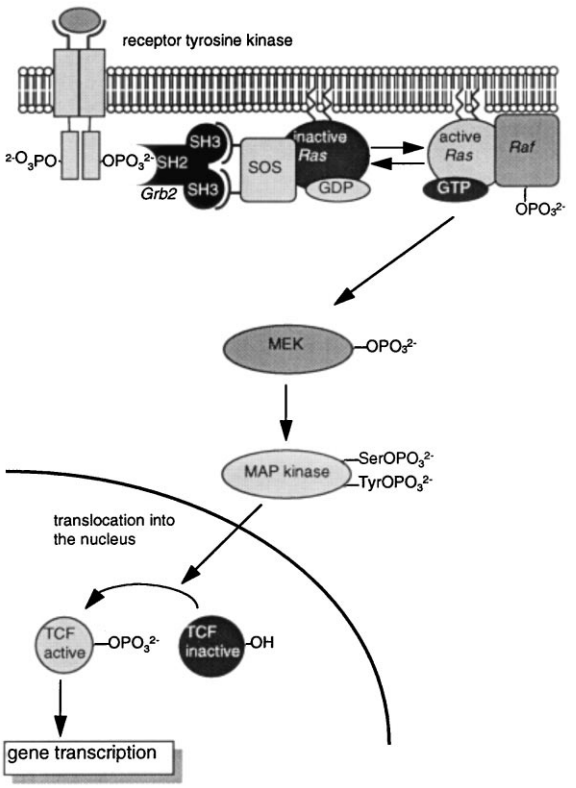


Figure 1. The *Ras*/Map-kinase signal transduction cascade.

tyrosine kinases are involved in various biological phenomena, the identification of new classes of subtype-selective receptor tyrosine kinase inhibitors is still of great importance to bioorganic and medicinal chemistry.

Aeroplysinin **7** (Scheme 1), a highly functionalized marine natural product, is cytotoxic against human breast cancer cells and inhibits the EGFR tyrosine kinase in an in vitro test system.[11] Its structure differs significantly from the structures of the RTK inhibitors reported so far (see Figure 2), which are almost exclusively aromatic compounds. Aeroplysinin might therefore serve as a promising lead for the development of a new class of these biologically active substances. However, compounds with structural similarity to **7** have yet not been investigated and data on the efficiency of **7** in vivo are lacking. We now report on the synthesis and biological evaluation of aeroplysinin analogues that display pronounced RTK inhibitory activity in vivo and receptor subtype selectivity.[12]

Results and Discussion

To investigate the activity of aeroplysinin[13] in a whole cell assay system (vide infra), its inhibitory activity

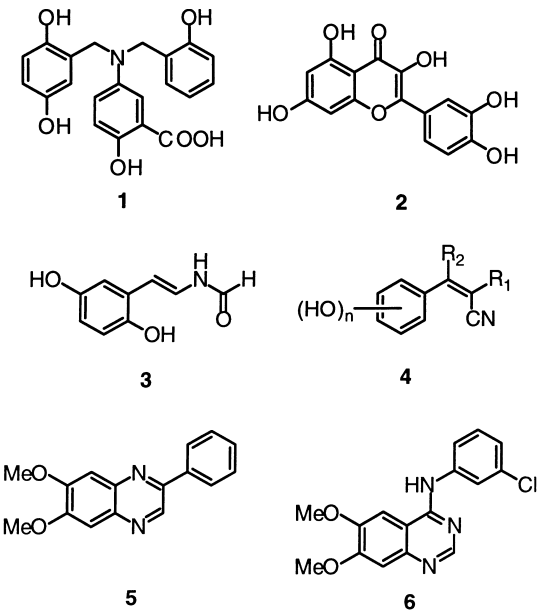
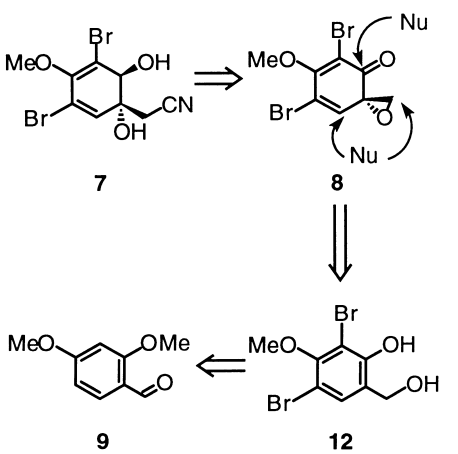


Figure 2. Structures of PTK inhibitors.

against the EGF-receptor was investigated employing immortalized rat-1-HER cells[14] (rat fibroblasts that over-express the human EGF receptor, Fig. 3). Under the assay conditions, however, **7** was not active at all. We reasoned that this lack of inhibition in vivo might be attributed to the inability of **7**, due to the presence of a polar *trans*-diol unit, to pass the plasma membrane and thereby reach the kinase domain of the EGF receptor, which is located in the intracellular domain of the proteins. Therefore, we designed the aeroplysinin analogue **8** as a possible RTK inhibitor for which a higher membrane permeability and, therefore, an enhanced



Scheme 1. Structure of aeroplysinin **7** and the *spiro*-epoxy analogue **8**; retrosynthetic analyses of **7** and **8**.

inhibitory effect could be expected. In **8** the vicinal diol unit and the β-cyano alcohol are replaced by an α-*spiro* epoxy ketone. Thus, **8** should on the one hand be significantly more lipophilic than **7** and on the other hand it should be prone to nucleophilic attack (Scheme 1) at the epoxide and/or the vinylogous ketone function. Thereby an efficient inactivation of the receptor via covalent modification of nucleophiles in the binding site might be brought about. In addition, compound **8** may serve as a promising intermediate for the synthesis of the natural product itself. For this purpose the epoxide in **8** would have to be transformed to the β-cyano alcohol by reaction with cyanide nucleophiles and the keto function would have to be diastereoselectively reduced to the alcohol **7** (Scheme 1). The α-*spiro* epoxy ketone present in **8** can be generated via Becker–Adler[15] oxidation from the *ortho* hydroxy benzylic alcohol **12**, which in turn should be accessible from the commercially available aldehyde **9** (Scheme 1).

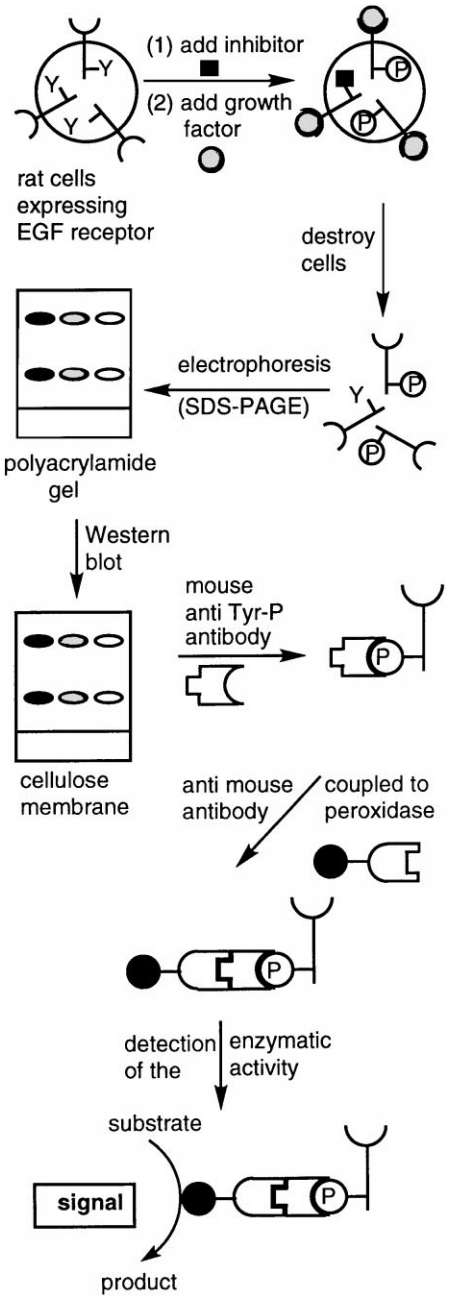
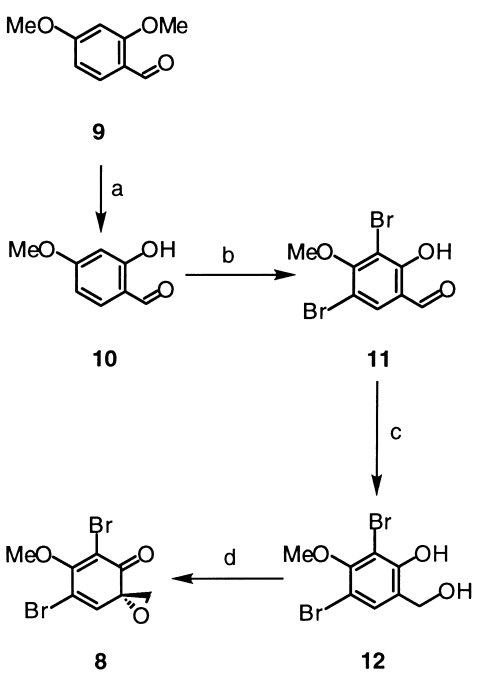


Figure 3. Schematic drawing of the in vivo assay system.

The α-*spiro* epoxy ketone **8** was synthesized in a four-step sequence from aldehyde **9**[16] (Scheme 2). To this end, the methyl ether *ortho* to the aldehyde group of **9** was regioselectively cleaved in 93% yield by treatment with BCl_3. The Lewis acid coordinates to the aldehyde and then regioselectively cleaves the *ortho* methoxy group. The resulting phenol **10** was converted to the dibromo compound **11** (75% yield) through the agency of the pyridinium–hydrobromide–bromine complex.[17] Reduction of the aldehyde function of **11** with sodium borohydride generated compound **12** in 93% yield. This *ortho*-hydroxy-substituted benzyl alcohol was subjected to oxidation with sodium periodate in an acidic medium. Under these conditions the desired α-*spiro*-epoxy ketone **8** was formed in 60% yield. The amount and



Scheme 2. Synthesis of the *spiro*-epoxy aeroplysinin analogue **8**; (a) BCl_3, CH_2Cl_2, rt, 93%; (b) Br_2·HBrpyridine, pyridine, 65 °C, 75%; (c) $NaBH_4$, THF, rt, 93%; (d) $NaIO_4$, HCl, H_2O, THF, rt, 60%.

concentration of hydrochloric acid present in the reaction mixture of this Becker–Adler[15] oxidation had to be carefully controlled, since otherwise the aldehyde **11**—after formation of an intermediary benzylic periodate ester—was formed predominantly.

To investigate whether the α-*spiro*-epoxy ketone **8** and other analogues of **7** inhibit RTKs in vivo, the inhibition of the EGFR tyrosine kinase was first studied. We used the assay system mentioned above. Immortalized rat-1-HER cells[14] (rat fibroblasts that over express human EGF receptor) were incubated with varying concentrations of inhibitor for 5 min. After this short time period, human EGF was added to induce signal transduction and phosphorylation of unblocked receptor. The cells were subsequently lysed, the proteins resolved by electrophoresis (SDS–PAGE) and then transferred onto an Immobilon-PDVF membrane via electroblotting. The extent of tyrosine phosphorylation of the EGFR was determined by phosphotyrosine immunoblotting (see Figure 3).[18] Antibody binding was visualized with an antibody-linked peroxidase that caused an emission of light proportional to the amount of phosphorylated protein. This was used to darken a photo plate. Therefore, the more active the investigated compound inhibited the autophosphorylation of the RTK, the less dark were the corresponding protein patches on the Immobilon-PDVF membrane. Figure 4 demonstrates that compound **8**, but not compound **13**, inhibits the EGF-induced increase of EGF receptor tyrosine phosphorylation in rat-1-HER cells.

The results were very promising: **8** inhibited the kinase activity of the EGF receptor with an IC_{50} value of 10 μM (Table 1). Preincubation of the cells for just a few minutes was sufficient to induce this effect, whereas under the conditions of our assay an inhibition of the receptor tyrosine kinase with tyrphostins required preincubation of the cells for at least several hours. These findings prove substantial in vivo activity of **8** and its sufficient lipophilicity. In a first attempt to determine whether **8** is receptor-subtype specific, the inhibition of the platelet derived growth factor receptor was

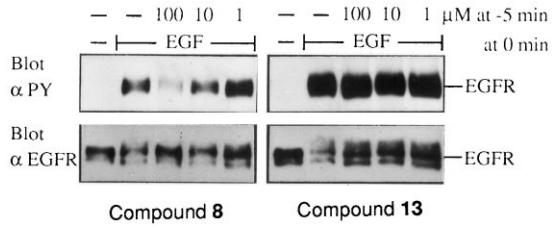
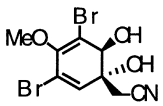
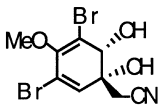
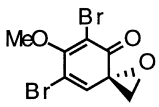
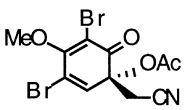
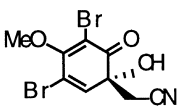
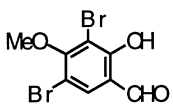
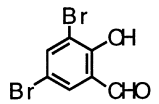
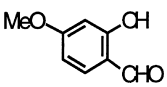
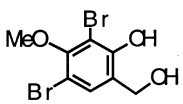
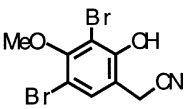
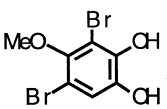
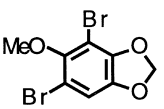
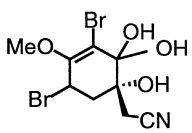


Figure 4. Determination of the IC_{50} values for the inhibition of the EGFR by immunoblotting of phosphotyrosine (αPY) and EGFR) to confirm that equal amounts of proteins were loaded.

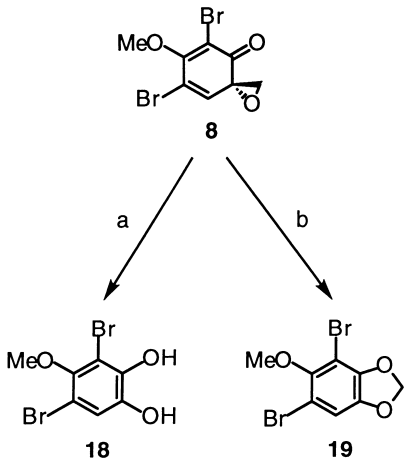
investigated by subjecting NIH-3T3-β-PDGFR cells to the assay conditions described above.[18] Compound **8** inhibited this tyrosine kinase receptor not at all up to a concentration of 100 μM, indicating a pronounced subtype-specifity.

To delineate which structural properties are responsible for the RTK-inhibitory activity of **8**, several analogues of the *spiro*-epoxy ketone were synthesized. Compounds **13**, **14**, **15**, and **17** were synthesized according to a protocol described by Anderson and Faulkner.[19] The γ, δ saturated ketone hydrate **20** was generated from the unsaturated ketone **15** by reduction with $NaBH_4$. In contrast to reference 19 our experiments with this reaction did not form the expected natural product itself. Benzodioxolane **19** was synthesized from epoxy ketone **8** via a 1,3 sigmatropic shift analogous to the rearrangement of vinyl cyclopropanes to cyclopentenes (Scheme 3). Catechol **18**, in principle to be derived from dioxolane **19**, could be instead formed in one step from epoxy ketone **8** by nucleophilic opening of the epoxide (Scheme 3, see Scheme 4 for reaction mechanisms). These compounds were investigated as possible inhibitors of the EGF and PDGF receptors using the in vivo assay systems described above. The data compiled in Table 1 demonstrate that neither aeroplysinin **7**[13] nor its isomer *iso*-aeroplysinin[19] **13** inhibit the EGF or the PDGF RTK in vivo at concentrations up to 100 μM. The analogue **14** in which the epoxide is opened and the resulting tertiary alcohol is masked as an ester also inhibits the EGF receptor although it is less efficient than **8**. Likewise **14** does not block the kinase activity of the PDGF receptor at all. Enhancing the polarity by saponifying the acetate (**14**→**15**) yielded a compound inactive under the assay conditions probably due to its reduced ability to sufficiently pass the plasma membrane of the cells within the time of assay. Interestingly, also the aromatic aldehydes **16** (which does not embody a methoxy group) and **11** both inhibited the EGFR (entries 6 and 7) albeit less efficient than **2**. Furthermore **16** (but not the methoxyaldehyde **11**) inhibited the PDGFR with an IC_{50} value of 30 μM. On the other hand, neither the aldehyde **10** (entry 8), nor the alcohols **12**, **17** and **20** (entries 9, 10, and 13), the catechol **18** (entry 11) or the benzodioxolane **19** (entry 12) are inhibitors. These findings taken together suggest the following requirements for inhibitory activity: (1) the bromine atoms are necessary, probably to effect lipophilicity (Table 1, compare entries 6, 7, and 8); (2) more than one free hydroxy-functions render the compounds inactive under the assay conditions (Table 1, compare entries 6, 9, and 11); (3) an electrophilic group is required (Table 1, compare entries 3, 4, 5, 6, and 12); (4) the nature and position of the electrophilic group may vary (Table 1, compare entries 3, 4, and 6). The IC_{50} values reported in this study are in the low micromolar

Table 1. Inhibition of the EGF and the PDGF receptor tyrosine kinases by means of aeroplysinin analogues in an in vivo assay system (for the description of the assay see the text)

Entry	Compd	Structure	Inhibition of the EGF receptor IC_{50}[a] (μM)	Inhibition of the PDGF receptor IC_{50}[a] (μM)
1	**7**		inactive	inactive
2	**13**		inactive	inactive
3	**8**		10	inactive
4	**14**		55	inactive
5	**15**		inactive	inactive
6	**11**		35	inactive
7	**16**		120	30
8	**10**		inactive	inactive
9	**12**		inactive	inactive
10	**17**		inactive	inactive
11	**18**		inactive	inactive
12	**19**		inactive	inactive
13	**20**		inactive	inactive

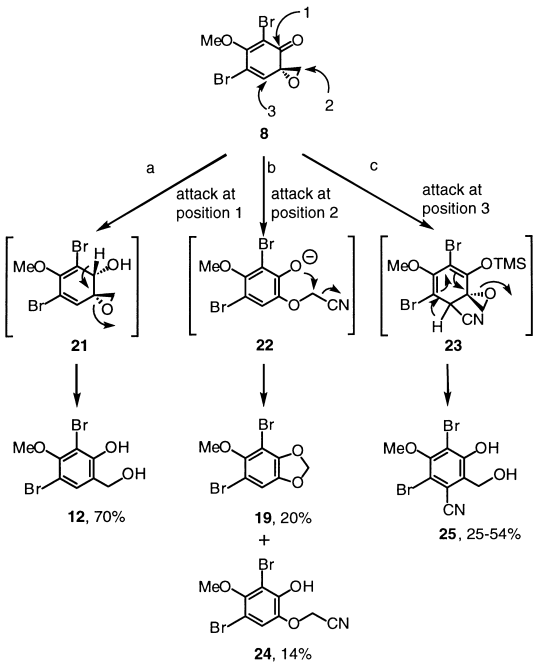
[a]Concentration at which 50% of the enzymatic activity are inhibited.
'inactive' = no inhibition at $< 100\,\mu M$ inhibitor concentration.



Scheme 3. Synthesis of the aeroplysinin analogues **18** and **19**; (a) THF, 1 N HCl, 70 °C, 72%; (b) toluene, 60 °C, 78%.

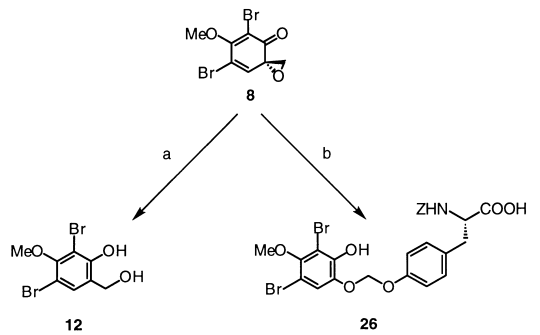
range and therefore do not reach the best values reported so far (vide supra). We stress, however, that the values are determined by employing a whole-cell-based assay system, whereas many of the IC_{50} values reported in the literature were determined by employing isolated kinases. Consequently, in our assay system the inhibitors have to diffuse across the plasma membrane to the interior of the cells. We do not know their intracellular concentration which is sufficient for the observed inhibition. It is, however, prudent to assume that the cytosolic inhibitor concentration is significantly lower than the concentrations reported in Table 1.

To prove the high susceptibility of **8** towards nucleophilic attack, which may be responsible for the inhibition of the tyrosine kinases the epoxy ketone was treated with different nucleophiles. By variation of the reaction conditions a specific attack at three different positions 1, 2 and 3 (Scheme 4) could be induced. Reducing agents like $NaBH_4$ attacked the ketone (position 1) and yielded the benzyl alcohol **12** after rearrangement of the intermediate **21**. Cyanide nucleophiles NaCN in DMSO or Et_2AlCN attacked the epoxide followed by an unexpected aromatization of the system via phenolate **22**, yielding benzodioxolane **19** and cyanohydrin **24**. A nucleophilic attack at position 3 could be initiated with Bu_4NCN or PPh_3 in TMSCN. Deprotonation of the acidic position of intermediate **23** yielded the hexasubstituted benzene derivative **25**. All attempts to synthesize aeroplysinin from the intermediate **8** therefore failed (Scheme 4). Reduction of the ketone **8** with different reducing agents yielded the benzyl alcohol **12**, whereas the desired alcohol **21** could not be isolated. On the other hand, reaction with cyanide nucleophiles gave the aromatic compounds **19** and **24** after cleavage of the C–C bond instead of the desired alcohol **15**, which



Scheme 4. Reaction of epoxide **8** with nucleophiles at different electophilic positions. (a) $NaBH_4$, $CeCl_3$, MeOH, 70%; (b) NaCN, DMSO or Et_2AlCN, toluene, **19**: 20%, **24**: 14%; (c) TMSCN, Bu_4NCN, CH_2Cl_2, 54% or TMSCN, PPh_3, CH_2Cl_2, 25%.

requires the expected cleavage of the C–O bond. To test whether these electrophilic groups may be able to covalently modify the enzyme by reaction with nucleophiles in the active site, compound **8** was treated with biologically relevant nucleophiles, which may occur in the side chains of amino acids (Scheme 5). In the event reaction with Z-Tyr gave oxoacetal **26** by nucleophilic attack at the epoxide function (position 2). On the other hand epoxy ketone **8** was reduced to benzylic alcohol **12**



Scheme 5. Reaction of epoxy ketone **8** with biological relevant nucleophiles at different electophilic positions. (a) *N*-Ac-Cys, TosOH, THF, 50 °C, 56%; (b) Z-Tyr, TosOH, THF, 50 °C, 27%.

(attack at position 1) upon treatment with *N*-acetyl-Cys. These findings indicate that a covalent modification of the enzyme may be responsible for the inhibitory activity of the compounds described.

Conclusion

In conclusion, we have demonstrated that α-substituted cyclohexadienones like **8** and **14** and structurally related aromatic compounds such as **11** and **16** inhibit receptor tyrosine kinases, possibly by covalent modification of the enzyme in its active site. They display a marked in vivo activity and appreciable receptor-subtype specificity. Analogues of the natural product aeroplysinin are thus interesting lead compounds for the development of new drugs and tools in bioorganic and medicinal chemistry (i.e., for the dissection of signal transduction pathways) and the introduction of new tyrosine kinase inhibitors into medical use.

Experimental

General

Melting points were determined in open capillaries using a Büchi 535 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250, AM 400 or DRX 500 spectrometers at room temperature. IR spectra were recorded on a Bruker IFS 88 spectrometer. Mass spectra and high-resolution mass spectra (HRMS) were measured on a Finnigan MAT MS70 spectrometer. Elemental analyses were performed on a Heraeus CHN-Rapid apparatus.

Materials

Solvents were dried by standard methods and stored over molecular sieves. For column chromatography silica gel (40–60 μm, Baker) was used. Commercial reagents were used without further purification. Pyridinium–hydrobromide–perbromide was prepared according to reference 17. Compounds **13**, **14**, **15**, and **17** were synthesized according to reference 19. Human EGF was purchased from Sigma (Munich), PDGF-BB from Biomol (Hamburg), Immobilon-PDVF membrane from Millipore (Bedford, UK), antibodies from Transduction Laboratories (Exeter, UK) except for peroxidase-linked antibodies, which were from DAKO (Hamburg).

2-Hydroxy-4-methoxy-benzaldehyde (10).[21] To a solution of the aldehyde **9** (11.07 g, 0.067 mol) in dry CH_2Cl_2 (100 mL) was added dropwise a 1 M solution of BCl_3 in CH_2Cl_2 (100 mL, 0.1 mol) at 0 °C. After stirring at room temperature for 16 h the mixture was cooled in an ice-bath and 100 mL of 1 N HCl were added followed by 200 mL of water. The aqueous phase was extracted with CH_2Cl_2 (3×100 mL) and the combined organic layers were washed with brine (2×150 mL). Drying over Na_2SO_4 and removal of the solvent in vacuo yielded an oil, which slowly crystallized. The crude pink product (9.89 g, 0.065 mol) was purified by chromatography on silica gel (ethyl acetate/hexane = 1/3 (v/v), R_f = 0.30). Yield: 9.49 g, 0.062 mol, 93%, white crystals, mp 42 °C (lit. 21; 42 °C); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.87 (s, 3H, OMe), 6.43 (d, J = 1.5 Hz, 1H, CH), 6.54 (dd, J = 8.6 Hz, J = 1.5 Hz, 1H, CH), 7.42 (d, J = 8.6 Hz, 1H, CH), 9.72 (s, 1H, OH), 11.50 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.75 ppm (OMe), 100.68 (CH), 108.43 (CH), 115.21 (C-CHO), 135.28 (CH), 164.57 (C-OH), 166.87 (COMe), 194.43 (CHO).

3,5-Dibromo-2-hydroxy-4-methoxy-benzaldehyde (11). A solution of pyridinium–hydrobromide–perbromide (4.5 g, 14 mmol) in pyridine (12 mL) was added dropwise at 45 °C to a solution of aldehyde **10** (1.0 g, 6.6 mmol) in pyridine (12 mL). After stirring for 120 min at 45 °C the mixture was hydrolysed with ice (100 mL) and water (500 mL) to yield a brownish precipitate, which was filtered and dried in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/2 (v/v), R_f = 0.46) gave white crystals: 1.71 g, 5.5 mmol, 83%. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.99 (s, 3H, OMe), 7.75 (s, 1H, arom. H), 9.78 (s, 1H, OH), 11.75 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 60.94 (OMe), 107.84 (CBr), 107.88 (CBr), 118.67 (CCHO), 136.29 (CH), 159.50 (COH), 161.11 (COMe), 194.10 (CHO); HRMS: calcd for $C_8H_6Br_2O_3$ 309.8664, found: 309.8646; Anal. calcd for $C_8H_6Br_2O_3$: C 31.00% H 1.96%, found: C 31.16% H 2.00%.

3,5-Dibromo-2-hydroxy-4-methoxy-benzyl alcohol (12). $NaBH_4$ (0.117 g, 3.1 mmol) was added at room temperature to a stirred solution of aldehyde **11** (0.96 g, 3.1 mmol) in THF (30 mL). After 60 min 1 N HCl (10 mL) was added and the aqueous phase was extracted with ether (3×30 mL). The organic layers were dried with Na_2SO_4 and the solvent was removed in vacuo to yield a yellow oil, which was purified by chromatography on silica gel (ethyl acetate/hexane = 1/1 (v/v), R_f = 0.33;). Yield: 0.9 g, 2.9 mmol, 93%, white crystals. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 2.24 (t, J = 5.7 Hz, 1H, OH), 3.88 (s, 3H, OMe), 4.76 (d, J = 5.7 Hz, 2H, CH_2), 6.75 (s, 1H, OH), 7.37 (s, 1H, arom. H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 60.73 ppm (CH_2), 62.20 (OMe), 107.18 (CBr), 107.40 (CBr), 124.24 (*C*-CH_2OH), 130.77 (CH), 151.89 (COH), 153.88 (COMe); MS (70 eV, EI): *m*/*z* (%): 310/312/314 (16/27/11) [M^+], 292/294/296 (47/100/49) [$M^+ - 18$], 264/266/268 (6/11/6), 249/251/253 (3/7/4), 213/215 (12/12), 91 (6), 63 (7); HRMS: calcd for

$C_8H_8Br_2O_3$ 311.8820, found: 311.8809; Anal. calcd for $C_8H_8Br_2O_3$: C 30.81% H 2.59%, found: C 30.87% H 2.59%.

2,4-Dibromo-3-methoxy-cyclohexa-2,4-diene-1-one-5-spiro-oxirane (8). To a solution of the benzylic alcohol **12** (0.5 g, 1.6 mmol) in a mixture of THF (8 mL) and 1 N HCl (1 mL) was added dropwise a solution of $NaIO_4$ (2.74 g, 12.8 mmol, 8 equiv) in water (15 mL) and 1 N HCl (5 mL). After stirring at room temperature for 90 min the deep-yellow solution was extracted with ether (3×30 mL). The combined organic layers were dried with Na_2SO_4 and the solvent was removed in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/5 (v/v), R_f = 0.27) yielded yellow crystals. Yield: 0.3 g, 1 mmol, 60%. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.26 (d, J = 8.6 Hz, 1H, CH_2), 3.43 (d, J = 8.6 Hz, 1H, CH_2), 4.14 (s, 3H, OMe), 6.54 (s, 1H, CH); ^{13}CNMR ($CDCl_3$, 100 MHz) δ 57.55 (quart. C), 59.19 (CH_2), 61.84 (OMe), 110.80 (CBr), 119.82 (CBr), 138.23 (CH), 164.44 (COMe), 187.06 (C = O); IR (KBr): ν3337, 3064, 2944, 2849, 1762, 1679, 1534, 1460, 1051 cm^{-1}; MS (70 eV, EI): *m/z* (%): 308/310/312 (40/75/35) [M$^+$], 293/295/297 (53/100/50) [M$^+$−15], 265 (5), 235/237/239 (4/10/5), 209 (8), 131 (18), 77 (15); HRMS: calcd for $C_8H_6Br_2O_3$ 309.8664, found: 309.8648; Anal. calcd for $C_8H_6Br_2O_3$: C 31.00% H 1.96%, found: C 31.05% H 1.99%.

Reduction of the ketone 8 with $NaBH_4/CeCl_3$. To a solution of the ketone **8** (0.3 g, 0.97 mmol) and $CeCl_3$ (0.89 g, c = 0.4 M) in MeOH (6 mL) was added at 0 °C under stirring $NaBH_4$ (0.036 g, 0.97 mmol). After 30 min the mixture was hydrolysed with 1 N HCl (3 mL) and extracted with ether (5×20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/2 (v/v), R_f = 0.23) yielded white crystals of alcohol **12**. Yield: 0.21 g, 0.67 mmol, 70%.

3,5-Dibromo-4-methoxy-benzo-1,2-dioxolane (19) and 3,5-dibromo-2-hydroxy-4-methoxy-phenoxyacetonitrile (24). To a solution of the epoxyketone **8** (100 mg, 0.32 mmol) in toluene (2 mL) was added dropwise under nitrogen a 1 M solution of Et_2AlCN (2.24 mL, 2.24 mmol, 7 equiv) at room temperature. After stirring at room temperature for 3 h the reaction mixture was poured into 2 N NaOH (10 mL) and ice (10 mL). The aqueous phase was extracted with CH_2Cl_2 (4×20 mL) and the combined organic layers were dried over Na_2SO_4. After evaporation of the solvent in vacuo, chromatography on silica gel (ethyl acetate/hexane = 1/4 (v/v)) yielded the benzodioxolane **19** (20 mg, 0.07 mmol, 20%, white crystals; R_f = 0.45) and the cyanohydrin **24** (15 mg, 0.04 mmol, 14%, white crystals; R_f = 0.35). **19**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.82 (s, 3H, OMe), 6.05 (s, 2H, CH_2), 6.94 (s, 1H, CH); ^{1}H NMR (DMSO, 250 MHz) δ 3.74 ppm (s, 3H, OMe), 6.16 (s, 2H, CH_2), 7.25 (s, 1H, CH); ^{13}C NMR ($CDCl_3$, 250 MHz) δ 60.92 (OMe), 98.02 (CBr), 102.35 (CH_2), 107.96 (CBr), 111.10 (CH), 144.32 (CO), 146.70 (CO), 148.92 (CO); IR (KBr): ν 3090, 2943, 2908, 1606, 1495, 1466, 1404, 1245, 1201, 954, 838 cm^{-1}; MS (70 eV, EI): *m/z* (%): 308/310/312 (50/100/49) [M$^+$], 293/295/297 (44/88/43) [M$^+$−15], 235/237/239 (5/9/4), 209 (7), 143 (9), 131/133 (14/13), 77 (18), 53 (14); HRMS: calcd for $C_8H_6Br_2O_3$: 309.8664 found: 309.8647; Anal. calcd for $C_8H_6Br_2O_3$: C 31.00% H 1.96%, found: C 31.18% H 2.16%. A more effective protocol for preparing compound **19** is the following: a solution of epoxyketone **8** (50 mg, 0.16 mmol) in toluene (3 mL) is heated for 10 h at 60 °C. After evaporation of the solvent in vacuo the residue is purified by chromatography on silica gel (ethyl acetate/hexane = 1/4 (v/v), R_f = 0.45). Yield: 39 mg, 0.13 mmol, 78%, white crystals, mp: 86 °C.

24: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.88 (s, 3H, OMe), 6.18 (s, 2H, CH_2), 6.41 (s, 1H, CH); ^{1}H NMR (DMSO, 250 MHz) δ 3.82 (s, 3H, OMe), 6.29 (s, 2H, CH_2), 6.88 (s, 1H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 57.06 (OMe), 90.82 (CBr), 98.47 (CBr), 103.26 (CH_2), 103.69 (CH), 114.25 (CN), 144.77 (CO), 148.23 (CO), 151.91 (CO); IR (KBr): ν 3115, 3083, 2915, 2235 (CN), 1625, 1612, 1472, 1453, 1438, 1366, 1226, 1100, 935 cm^1; MS (70 eV, EI): *m/z* (%):333/335/337 (0.42/0.80/0.36) [M$^+$−2], 318/320/322 (0.33/0.73/0.33) [M$^+$−17], 308/310/312 (0.10/0.18/0.09) [M$^+$−27], 255/257 (100/98), 240/242 (69/67), 210/212 (12/12), 182/184 (8/9), 131/133 (10/10), 90 (5), 75 (12); HRMS calcd for $C_9H_7Br_2NO_3$ 336.8773, found: 336.8739.

3,5-Dibromo-2-hydroxy-4-methoxy-phenol (18). A solution of the epoxyketone **8** (200 mg, 0.65 mmol) in a mixture of THF (35 mL), 1 N HCl (5 mL) and water (20 mL) was heated under reflux for 5 h. After cooling the mixture was extracted with ether (3×50 mL), the combined organic layers were washed with brine (30 mL) and dried with Na_2SO_4. The solvent was removed in vacuo and the residue was purified by chromatography (ethyl acetate/hexane = 2/3 (v/v), R_f = 0.23). Yield: 140 mg, 0.47 mmol, 72%, yellowish crystals, mp 108 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.83 (s, 3H, OMe), 5.30 (br, 1H, OH), 5.54 (br, 1H, OH), 7.11 (s, 1H, CH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 60.86 (OMe), 105.81 (CBr), 107.59 (CBr), 118.10 (CH), 140.34 (COH), 141.19 (COH), 147.27 (COMe); IR (KBr): ν 3352, 2944, 1583, 1486, 1375, 1271, 1138, 964 cm ± 1; MS (70 eV, EI): *m/z* (%): 296/298/300 (50/100/47) [M +], 281/283/285 (45/89/42) [M$^+$−15], 253/255/257 (19/40/18), 175 (8), 131 (8), 77 (13), 53 (12); HRMS (70 eV): calcd for $C_7H_6Br_2O_3$: 297.8664, found: 297.8640; Anal. calcd: C 28.22% H 2.03%, found: C 28.32% H 2.18%.

3,5-Dibromo-2-hydroxybenzaldehyde (16).[22] To a solution of 2-hydroxy benzaldehyde (2.00 g, 16.4 mmol) in pyridine (20 mL) was added dropwise at 45 °C a solution of pyridinium–hydrobromide–perbromide (11.25 g, 35 mmol) in pyridine (35 mL). After stirring for 2 h at 45 °C the mixture was poured onto ice (200 mL). The precipitated solid was filtered off, dried and recrystallized from ethyl acetate/hexane (2/1 (v/v), 20 mL). Yield: 3.90 g, 13.94 mmol, 85%, yellow crystals, mp 81 °C (lit.[22]: 81–84 °C); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.94 (s, 3H, OCH_3), 7.20 (d, J = 8 Hz, 1H, arom. CH), 7.94 (dd, J = 8 Hz, J = 1 Hz, 1H, arom. CH), 8.07 (d, J = 1 Hz, 1H, arom. CH), 12.94 (br, 1H, COOH).

2,6-Dibromo-4-hydroxy-4′-cyanomethyl-1-methoxy-cyclohexene-3-one-hydrate (20). $NaBH_4$ (8 mg, 0.21 mmol) was added at 0 °C under nitrogen to a solution of ketone **15** (35 mg, 0.10 mmol) in dry EtOH (10 mL). After 10 min at 0 °C the reaction was stopped by adding a saturated solution of NH_4Cl in water (5 mL). Extraction with CH_2Cl_2 (3×20 mL) was followed by drying the combined organic layers over $MgSO_4$ and evaporation of the solvent in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/1 (v/v), R_f = 0.15) yielded a colourless solid. Yield: 12 mg (0.03 mmol), 34%. ^{1}H NMR (CD_3OD, 250 MHz) δ 2.50 (t, J = 14 Hz, 1H, CH_2), 2.78 (d, J = 12 Hz, 1H, CH_2CN), 2.85 (d, J = 12 Hz, 1H, CH_2CN), 3.12 (dd, J = 14 Hz, J = 6 Hz, 1H, CH_2), 3.70 (s, 3H, OCH_3), 5.02 (dd, J = 14 Hz, J = 6 Hz, 1H, CHBr); ^{1}H NMR (DMSO-d_6, 250 MHz) δ 2.41 (t, J = 14 Hz, 1H, CH_2), 2.65 (d, J = 15 Hz, 1H, CH_2CN), 2.96 (d, J = 15 Hz, 1H, CH_2CN), 3.04 (dd, J = 14 Hz, J = 6 Hz, 1H, CH_2), 3.63 (s, 3H, OCH_3), 5.15 (dd, J = 14 Hz, J = 6 Hz, 1H, CHBr), 6.25 (br, 1H, OH), 7.11 (br, 1H, OH), 7.58 (br, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 41.18 (CH_2), 44.31 (CH_2), 46.14 (CH), 52.90 (OCH_3), 71.93 (CCH_2CN), 93.60 (CN), 163.45 (quart. C), 172.03 (quart. C), 180.23 (quart. C); IR (KBr): ν 3389, 3267, 3206, 2947, 2250 (CN), 1730, 1621, 1547, 1442, 1343, 1260, 1232, 1000, 771, 634 cm^{-1}; MS (70 eV, EI): m/z (%): 356/358/360 (15/29/13) [M^+ + 1], 355/357/359 (50/100/45) [M^+], 325 (10) [M^+-OCH_3-H], 312 (9), 284 (16) [M^+-2OH-CH_2CN], 250/252 (28/26) [M^+-Br-CN], 249/251 (90/89) [M^+-HBr-CN], 217/219/221 (24/42/20), 189/191 (44/49), 170 (100), 138 (41), 55 (49); HRMS (70 eV): calcd for $C_9H_{11}Br_2NO_4$: 356.9035, found: 356.9058.

3,5-Dibromo-6-cyano-2-hydroxy-4-methoxybenzyl alcohol (25). A solution of epoxy ketone **8** (190 mg, 0.61 mmol) in dry CH_2Cl_2 (1.5 mL) and TMSCN (1.5 mL, 1.12 g, 11.25 mmol) was stirred under nitrogen at room temperature for 24 h. The mixture was cooled to 0 °C and Bu_4NCN (165 mg, 0.61 mmol) was added. After 45 min a saturated solution of $NaHCO_3$ in water (5 mL) was added. Extraction with ether (4×30 mL) and drying of the combined organic phases with Na_2SO_4 was followed by evaporation of the solvent in vacuo. Chromatography on silica gel (ethyl acetate/hexane = 1/2 (v/v), R_f = 0.20) gave yellow crystals. Yield: 110 mg, 0.33 mmol, 54%; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.90 ppm (s, 3H, OMe), 5.10 (s,2H,CH_2); ^{1}H NMR (DMSO-d_6, 250 MHz) δ 3.80 ppm (s, 3H, OMe), 4.70 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 58.00 ppm (CH_2), 60.45 ppm (OMe), 110.13 (CCN), 114.46 (CBr), 114.94 (CBr), 115.79 (CN), 130.49 (CCH_2OH), 153.94 (COMe), 154.55 (COH); IR (KBr): ν 3439, 3107, 2947, 2240, 1557, 1400, 1173, 1021 cm^{-1}; MS (70 eV, EI): m/z (%): 335/337/339 (23/30/20) [M^+], 317/319/321 (76/100/74) [M^+−18], 289/291/293 (9/21/13), 246/248/250 (4/9/4), 238/240 (16/17), 223/225 (8/8), 180/182 (3/4), 116 (7), 88 (8), 77 (3); HRMS (70 eV): calcd for $C_9H_7Br_2NO_3$: 336.9663, found: 336.9655.

Reaction of epoxy ketone 8 with Z-Tyr (oxoacetal 26). A solution of epoxide **8** (10 mg, 0.03 mmol), Z-Tyr (20 mg, 0.07 mmol) and *p*-TosOH (0.5 mg, mmol) in dry THF (2 mL) was heated in the presence of molecular sieves (4 A) for 5 h. Ethyl acetate (30 mL) and NEt_3 (0.1 mL) was added and the mixture was washed with water (2×20 mL). After evaporation of the solvent in vacuo, the residue was purified by chromatography (ethyl acetate/hexane = 1/2, R_f = 0.15). Yield: 5 mg, 0.008 mmol, 27%, colourless oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 3.01 (d, J = 7 Hz, 2H, Tyr β-CH_2), 3.88 (s, 3H, OCH_3), 4.60 (m, 1H, α-CH), 5.10 (s, 2H, Ph-CH_2), 5.68 (d, J = 6 Hz, 1H, O-CH_2-O), 5.78 (d, J = 6 Hz, 1H, O-CH_2-O), 6.21 (s, 1H, NH), 6.67 (d, J = 8 Hz, 2H, Tyr arom. CH), 6.90 (d, J = 8 Hz, 2H, Tyr arom. CH), 7.35 (br, 5H, arom. CH); MS (70 eV, EI): m/z (%): 623/625/627 (0.15/0.40/0.16) [$M^+ + H_2O$], 606/608/610 (0.03/0.07/0.04) [M^+], 528 (0.08) [M^+−Br], 386/388/390 (0.14/0.41/0.26), 308/310/312 (12/25/12) [M^+−Tyr], 298 (48), 283 (45), 107 (83), 91 (100); HRMS (70 eV): calcd for $C_{25}H_{23}Br_2NO_7$: 606.9841, found: 606.9858.

Inhibition of RTK autophosphorylation. Immortalized rat-1/HER cells[14] (in the case of EGF-R autophosphorylation) or NIH 3T3-β-PDGFR cells[20] (in the case of PDGF-R autophosphorylation) were grown at 37 °C and 6% CO_2 in Dulbecco's modified Eagle's Medium (DMEM) containing 10% calf serum (FCS) and antibiotics (100 units/mL penicillin, 100 μg/mL streptomycin). Three hours before the experiments the medium was exchanged for DMEM without phenol red and without FCS. The cells were incubated for 5 min with DMSO-solutions of the inhibitors (final concentrations: 1 μM, 10 μM and 100 μM) and then treated for 5 min. with human EGF in the case of EGF-R autophosphorylation (2 ng/mL final concentration in the medium) or PDGF-BB in the case of PDGF-R autophosphorylation (10 ng/mL final concentration in the

medium). After removal of the medium the cells were washed twice with PBS (2×5 mL; buffered isotonic solution: 137 mM NaCl, 2.7 mM KCl, 0.7 mM $CaCl_2$, 0.6 mM $MgCl_2$, 6.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4) and subsequently lysed with sample buffer (0.5 mL; 0.16 M Tris–HCl, 4% SDS, 20% glycerol, 4% β-mercaptoethanol, 0.05% bromophenol blue). The lysate was sonicated to shear the DNA and aliquots were boiled for 5 min. The proteins were resolved by SDS–PAGE (10% acrylamide). After transferring the proteins onto Immobilon-PDVF-membrane by electroblotting immunostaining was performed using the antibody dilutions recommended by the manufacturer (anti-phosphotyrosine PY20-, monoclonal α-EGFR- and monoclonal α-PDGFR-antibodies). Counterstaining was performed with horseradish peroxidase-linked antibodies, which were visualized using ECL detection reagent. In all cases, unblotted parts of the SDS-gels were Coomassie stained to confirm that equal amounts of proteins were loaded.

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