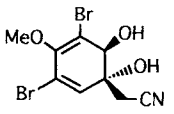
Selective Inhibition of Receptor Tyrosine Kinases by Synthetic Analogues of Aeroplysinin**

Herbert Waldmann,* Klaus Hinterding, Peter Herrlich,* Hans Jobst Rahmsdorf, and Axel Knebel

Various transmembrane receptors—for example the receptors of insulin-like growth factor (IGFR), nerve growth factor (NGFR), epidermal growth factor (EGFR), and platelet-derived growth factor (PDGFR)—phosphorylate themselves on tyrosine residues in response to the binding of extracellular ligands. Thereby these receptor tyrosine kinases (RTKs) gain the ability to trigger various intracellular signaling cascades by which cell growth, proliferation, and other genetic programs are regulated.[1] Dysregulation may contribute to the generation of various diseases. For instance, enhanced activity of RTKs can promote tumor growth and has been implicated in carcinogenesis and as participant in the development of proliferative diseases such as psoriasis.[2] Therefore, these signal-transducting proteins are considered as promising targets for drug development. In addition, such drugs are likely to be useful in dissecting signaling pathways.[2, 3] Essential to the use of a RTK inhibitor in biological studies is its efficacy both in vitro and in vivo.

Whereas most compounds developed so far display IC_{50} values in the low micromolar range[2-6]—the tyrphostins[2a, b, 6] are most often used—more efficient inhibitors were recently reported.[5, 6] As these values leave substantial room for improvement, identification of new and alternative classes of RTK inhibitors is of great importance to bioorganic and medicinal chemistry.

Aeroplysinin **1**, a highly functionalized marine natural product, is cytotoxic against human breast cancer cells and inhibits the EGFR receptor in an in vitro test system.[7] Its structure differs significantly from that of the RTK inhibitors reported so far, which are almost exclusively aromatic compounds; **1** might therefore serve as a promising lead for the development of a new class of these biologically active substances. However,



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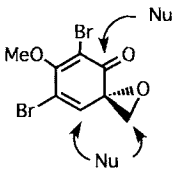

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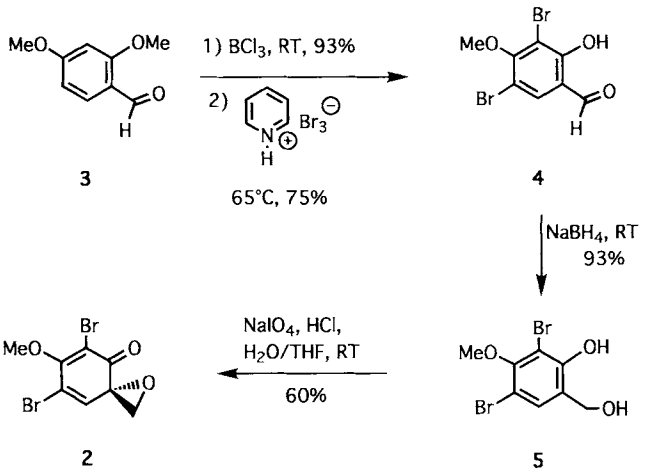
compounds with structural similarity to **1** have not been investigated, and data on the efficiency of **1** in vivo are lacking. To fill this gap we studied the EGFR-inhibiting activity of **1**[8] in a whole-cell assay system (see below) in which it, however, was not at all active. Since this lack of inhibition in vivo most probably has to be attributed to the inability of **1** to pass through the plasma membrane (the kinase domain of RTKs is on the cytosolic face of the membrane) due to the presence of a polar *trans*-diol unit, we designed the aeroplysinin analogue **2** as a possible RTK inhibitor for which higher membrane permeability and an enhanced inhibitory effect could be expected. In **2** the vicinal diol unit and the β-cyano alcohol are replaced by an α-spiroepoxy ketone. Thus **2** should be significantly more lipophilic than **1** and prone to nucleophilic attack at the epoxide and/or the vinylogous ketone functionality. This would lead to efficient inactivation of the receptor by covalent modification of nucleophiles in the binding site.

Ketone **2** was synthesized in a four-step sequence from the readily available aldehyde **3**[9] (Scheme 1). The methoxy group *ortho* to the aldehyde group of **3** was regioselectively cleaved by treatment with BCl_3, and the resulting phenol converted into the dibromo compound **4** with the pyridiniumhydrobromide/bromine complex.[10] Reduction of the aldehyde functionality of **4** generated the benzyl alcohol **5**, which was oxidized to **2** by a Becker–Adler oxidation.[11]



Scheme 1. Synthesis of the spiroepoxy aeroplysinin analogue **2**.

To investigate if **2** inhibits RTKs in vivo the inhibition of the receptor tyrosine kinase of the epidermal growth factor was studied first. In the assay system used, immortalized rat-1-HER cells[12] (rat fibroblasts that overexpress the human EGF receptor) were incubated with varying concentrations of inhibitor for five minutes followed by addition of EGFR to induce phosphorylation of unblocked receptor. The cells were subsequently lysed, and the proteins resolved by electrophoresis (SDS-PAGE). The extent of tyrosine phosphorylation of the EGFR was determined by phosphotyrosine immunoblotting (Figure 1).[13]

The results were very promising: **2** inhibited the kinase activity of the EGF receptor with an IC_{50} value of 10 μM (see Table 1). Preincubation of the cells for just a few minutes was sufficient to induce this effect; under the conditions of our assay inhibition of the RTK with tyrphostins required preincubation of the cells for at least several hours. These findings prove substantial in vivo activity of **2** and its sufficient lipophilicity. In a first attempt to determine whether **2** is receptor-subtype specific, the inhibition of the PDGF receptor was investigated by subjecting NIH-3T3-β-PDGFR cells to the assay conditions described above.[14] Ketone **2** did not inhibit this RTK up to a concentration of 100 μM, indicating pronounced subtype specifity.

[*] Prof. Dr. H. Waldmann, Dipl.-Chem. K. Hinterding
Institut für Organische Chemie der Universität
Richard-Willstätter-Allee 2, D-76128 Karlsruhe (Germany)
Fax: Int. code +(721)608-4825
e-mail: waldmann@ochhades.chemie.uni-karlsruhe.de
Prof. Dr. P. Herrlich, Prof. Dr. H. J. Rahmsdorf, Dipl.-Biol. A. Knebel
Institut für Genetik, Forschungszentrum Karlsruhe GmbH
D-76344 Eggenstein-Leopoldshafen (Germany)
Fax: Int. code +(7247)825-070
e-mail: genetic@igen.fzk.de

[**] This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

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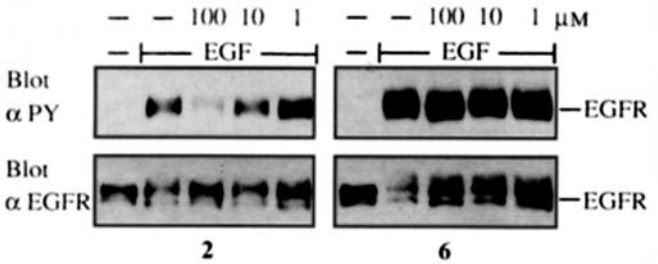
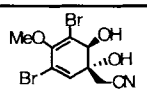
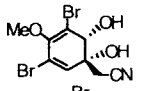
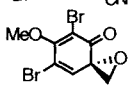
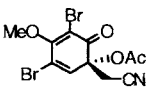
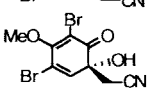
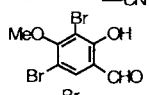
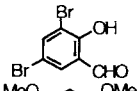
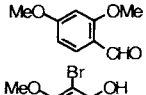
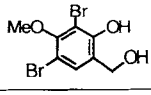


Figure 1. Determination of the IC_{50} values for the inhibition of the EGFR by immunoblotting of phosphotyrosin (αPY) and EGFR (αEGFR) to confirm that equal amounts of proteins were loaded. The aeroplysinin analogue **2**, but not *iso*-aeroplysinin **6**, inhibits the EGF-induced increase of EGF-receptor tyrosine phosphorylation in rat-1-HER cells (representative experiment). The numbers 100, 10, and 1 μM in the first row show the concentration of inhibitor for the corresponding incubation.

To delineate which structural properties are responsible for the RTK-inhibitory activity of **2**, several analogues of the spiroepoxy ketone were synthesized and investigated as possible inhibitors of the EGF and PDGF receptors in the in vivo assay systems described above. Table 1 demonstrate that neither aeroplysinin **1**[8] nor its isomer *iso*-aeroplysinin[7b] **6** inhibit the EGF or the PDGF RTK in vivo at concentrations up to 100 μM. The analogue **7**—in which the epoxide is opened, but the resulting tertiary alcohol is masked as ester—also inhibits the EGF receptor although it is less efficient than **2**. Likewise, **7** does not block the kinase activity of the PDGF receptor at all. Enhancing the polarity by saponifying the acetate (**7** → **8**) yielded a compound that is inactive under the assay conditions. This is probably due to its reduced ability to sufficiently pass through the plasma membrane of the cells within the time of assay. Interestingly the aromatic aldehydes **9**, which does not embody a methoxy group, and **4** also inhibited the EGFR (entries 6 and 7) albeit less efficiently than **2**. Furthermore, **9**, but not the methoxyaldehyde **4**, inhibited the PDGFR with an IC_{50} value of 30 μM. On the other hand neither aldehyde **3** (entry 8) nor alcohol **5** (entry 9) are inhibitors. These findings taken together suggest that the presence of the bromine atoms, probably to effect lipophilicity, and an electrophilic group are required for inhibitory activity. The nature of the electrophilic group may vary.

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

Entry	Compound		Inhibition of the EGF receptor IC_{50} [μM]	Inhibition of the PDGF receptor IC_{50} [μM]
1		**1**	inactive	inactive
2		**6**	inactive	inactive
3		**2**	10	inactive
4		**7**	55	inactive
5		**8**	inactive	inactive
6		**4**	35	inactive
7		**9**	120	30
8		**3**	inactive	inactive
9		**5**	inactive	inactive

[a] Concentration at which 50% of the enzymatic activity are inhibited; "inactive" = no inhibition at <100 μM inhibitor concentration.

In conclusion we have demonstrated that α-substituted cyclohexadienones such as **2** and **7** as well as structurally related compounds inhibit RTKs. They display a marked in vivo activity and appreciable receptor-subtype specificity. Analogues of the natural product **1** are thus interesting lead compounds for the development of new drugs and tools in bioorganic and medicinal chemistry, that is, for the dissection of signal transduction pathways and the introduction of new tyrosine kinase inhibitors into medical use.

Received: January 8, 1997 [Z99751E]
German version: *Angew. Chem.* **1997**, *109*, 1553–1555

Keywords: aeroplysinin · bioorganic chemistry · enzyme inhibitors · kinases

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