



# SYNTHESIS AND KINASE INHIBITORY ACTIVITY OF PHENYLPROPENOYL BENZAZEPINONES



Berger, B.<sup>1</sup>, Kubbutat, M. H. G.<sup>2</sup>, Schächtele, C.<sup>2</sup>, Totzke, F.<sup>2</sup>, Bednarski, P.<sup>3</sup> and Kunick, C.<sup>1</sup>

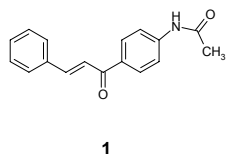
<sup>1</sup> Technische Universität Carolo-Wilhelmina zu Braunschweig, 38106 Braunschweig, Germany  
<sup>2</sup> ProKinase/ KTB Tumorforschungs GmbH, 79106 Freiburg, Germany  
<sup>3</sup> Ernst-Moritz-Armdt-Universität Greifswald, 17487 Greifswald, Germany

## INTRODUCTION

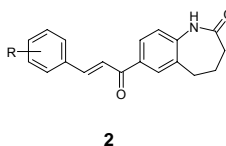
Among the manifold therapeutic targets identified for anticancer drugs, the protein kinase enzyme family has captured the focus of antitumor drug design programmes. Currently a majority of efforts in this field converges upon novel small ATP-competitive inhibitors of oncogenic kinases. [1]

A recently published three-dimensional multicenter database search using a kinase pharmacophore model as template revealed *N*-[4-(3-phenylacryloyl)phenyl]acetamide (**1**) as an inhibitor of the Plasmodium cyclin-dependent kinase homologue Pfmrk. [2] Assuming that **1** might represent a general scaffold suitable to fit into the ATP binding site also of oncogenic kinases, 7-(3-phenylprop-2-enyl)-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-ones **2** were synthesized as rigid analogues of **1**.

Actually, the 4-bromo derivative **2d** (R = 4-Br) turned out to be a multiple inhibitor of cancer-related protein kinases with considerable antiproliferative activity against tumor cells in vitro.



**1**



**2**

## SYNTHESIS

The synthesis of 7-(3-phenylprop-2-enyl)-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-ones **2** comprises three steps.

First,  $\alpha$ -tetralone (**3**) was reacted with sodium azide in a Schmidt rearrangement yielding 1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one (**4**). Upon treatment of **4** with acetyl chloride in carbon disulfide in the presence of  $\text{AlCl}_3$ , 7-acetyl-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one (**5**) was obtained by means of a Friedel-Crafts acylation. Subsequent reaction of **5** with aromatic aldehydes in ethanol/potassium hydroxide (30 minutes at 0 °C, then at room temperature over night) furnished the desired 7-(3-phenylprop-2-enyl)-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-ones **2** in moderate to good yields. (Scheme 1)

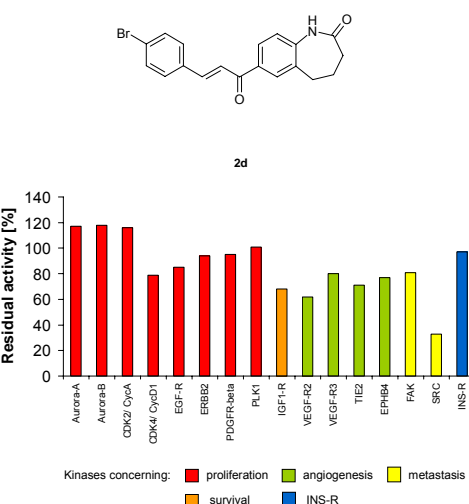


Figure 1: Residual activity of 15 cancer-related kinases and INS-R after incubation with **2d** (10  $\mu\text{M}$ , ATP-concentration 1  $\mu\text{M}$ )

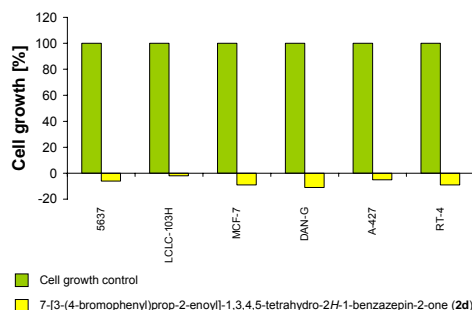


Figure 2: Residual cell growth of 6 cancer cell lines after incubation with **2d** (20  $\mu\text{M}$ )

## BIOLOGICAL EVALUATION

The novel 7-(3-phenylprop-2-enyl)-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-ones **2** were tested in a custom kinase screening assay comprising 15 kinases with relevance for tumor growth, angiogenesis, survival and metastasis. Furthermore, the inhibitory potency of the compounds on the insulin receptor kinase was investigated. Inhibition of the latter kinase is unfavorable because it can lead to unwanted side effects if a kinase inhibitor is used as cancer therapeutic. In this regard, a high selectivity for cancer relevant kinases versus the insulin receptor kinase is an important criterion in the selection process of a compound for further development.

The kinase activity was determined by the measurement of incorporated radioactive phosphate into the substrates in the presence or absence of the inhibitor.

The antiproliferative activity of the 4-bromo derivative **2d** was evaluated in an assay comprising six cancer cell lines including bladder, lung, pancreas and mammary cancer. The percent cell growth following a 96 h treatment with 20  $\mu\text{M}$  substance was determined. Untreated controls showed 100% growth. Cell growth was measured with a microtiter assay based on the staining of cellular material with crystal violet. [3]

## RESULTS AND DISCUSSION

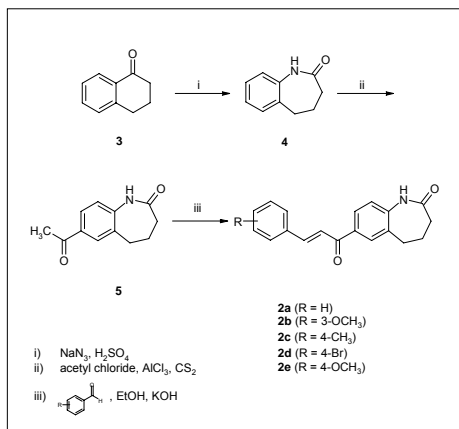
The results of the protein kinase screening assays revealed that the novel analogues **2a** – **2e** exhibit inhibitory activity for the investigated kinases. **2d** proved to be the best compound of the series, displaying selectivity for IGF1-R, SRC and VEGF-R2 with inhibition rates between 33% and 68% at 10  $\mu\text{M}$ . (Figure 1) The determination of  $\text{IC}_{50}$  values for the aforementioned kinases confirmed this observation ( $\text{IC}_{50}$ s : IGF1-R 4.3  $\mu\text{M}$ , SRC 3.1  $\mu\text{M}$ , VEGF-R2 4.5  $\mu\text{M}$ ). Of note, **2d** shows a clear selectivity versus the insulin receptor kinase which is inhibited with an  $\text{IC}_{50}$  of 27  $\mu\text{M}$ .

Furthermore, **2d** showed compelling results in the cancer cell line assay. At a concentration of 20  $\mu\text{M}$ , **2d** completely inhibited the growth of all six investigated cancer cell lines. (Figure 2)

In conclusion, the 7-(3-phenylprop-2-enyl)-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-one scaffold **2** proved to be a useful starting structure for the development of kinase inhibitors. A further structure modification programme directed to find compounds with enhanced kinase inhibitory activity and selectivity appears to be rewarding.

## ACKNOWLEDGEMENT

Funding of the project by the European Commission in the 6th framework programme (Contract No. LSHB-CT-2004-503467, to B. B., M. H. G. K., C. S., F. T. and C. K.) is gratefully acknowledged.



Scheme 1: Synthesis of 7-(3-phenylprop-2-enyl)-1,3,4,5-tetrahydro-2*H*-1-benzazepin-2-ones **2**

## REFERENCES

- [1] Sawyer, T. K.: Novel protein kinase inhibitors for cancer therapy. *Curr. Med. Chem. – Anti-Cancer Agents* **2004**, *4*, 449-455.
- [2] Bhattacharjee, A. K., Geyer, J. A., Woodard, C. L., Kathcart, A. K., Nichols, D. A., Prigge, S. T., Li, Z., Mott, B. T. and Waters, N. C.: A three-dimensional in silico pharmacophore model for inhibition of Plasmodium falciparum cyclin-dependent kinases and discovery of different classes of novel Pfmrk specific inhibitors. *J. Med. Chem.* **2004**, *47*, 5418-5426.
- [3] Saczewski, F., Reszka, P., Gdaniec, M., Grunert, R. and Bednarski, P. J.: Synthesis, X-ray crystal structures, stabilities, and in vitro cytotoxic activities of new heteroarylacrylonitriles. *J. Med. Chem.* **2004**, *47*, 3438-3449.