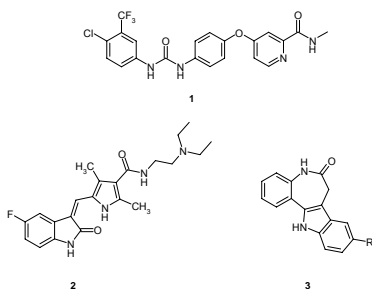


## Introduction

The successful introduction of imatinib (Gleevec) into cancer therapy has proven the suitability of kinase inhibitors as antitumor drugs. [1] However, both the development of resistance and unsatisfactory clinical outcomes, e.g. experienced with gefitinib (Iressa), are major concerns regarding anticancer kinase inhibitors. The development of promiscuous inhibitors that inhibit two or more cancer related protein kinases is a novel concept to overcome these problems. Along these lines, the multiple kinase inhibitors sorafenib (Nexavar; 1) and sunitinib (Sutent; 2) have recently been approved by the FDA as anticancer drugs. In the course of a project aimed at finding novel chemotypes with multiple kinase inhibitor properties we concentrated on the 1-benzazepin-2(1H)-one scaffold as adenino mimetic pharmacophore, because it represents the crucial hinge region binding motif in the paullone class 3 of kinase inhibitors. A small series of 7-alkynyl-substituted 1-benzazepin-2(1H)-one derivatives was generated employing the Sonogashira reaction as key step. The compounds were then tested in a panel of cancer related kinases.



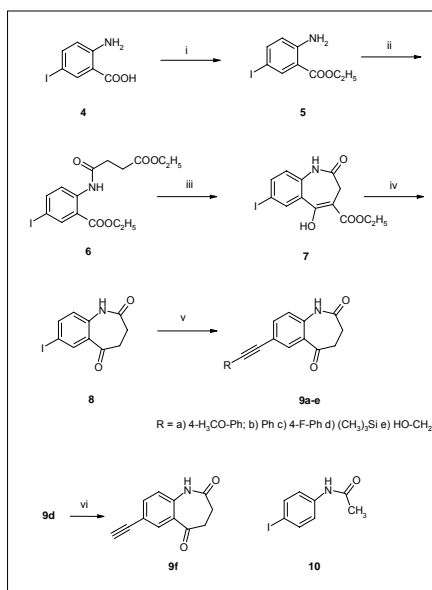
## Chemistry

The synthesis of the 7-ethynyl-1-benzazepine-2,5-diones 9a-f was carried out following a general procedure shown in Scheme 1. First, 2-amino-5-iodobenzoic acid (4) was reacted with iodoethane. The ester 5 was converted to the amide 6 by treatment with ethyl succinyl chloride. Afterwards, 6 underwent a Dieckmann cyclization upon treatment with potassium hydride in DMF/toluene to yield the enol 7. Heating 7 in wet DMSO led to a dealkoxycarbonylation providing 7-iodo-1H-1-benzazepine-2,5(3H,4H)-dione (8). Subsequently, 8 was reacted under Sonogashira reaction conditions furnishing the 7-ethynyl-1-benzazepine-2,5-diones 9a-e.

Test reactions for the set up of suitable reaction conditions of the Sonogashira reaction [2] were carried out starting either with 7-iodo-1-benzazepine-2,5-dione (8) or N-(4-iodophenyl)acetamide (10) as surrogate educt. Several palladium catalysts such as bis-(triphenylphosphine)-palladium(II)-dichloride and tetrakis-(triphenylphosphine)-palladium(0), bases like piperidine, triethylamine and potassium carbonate as well as THF, DMF, piperidine or triethylamine as solvents were tested in different combinations (Table 1). The reactions were performed under conventional or microwave-assisted heating. The standard method employing a conventional heating source (50 °C) and bis-(triphenylphosphine)-palladium(II)-dichloride as catalyst turned out to be favorable reaction conditions. Triethylamine was used as solvent and coevally as base namely because under these conditions the formation of a Glaser-coupling by-product was avoided. [3] Eventually, the synthesis of 7-ethynyl-3,4-dihydro-1H-1-benzazepine-2,5-dione (9f) was accomplished by treating 9d with tetrabutylammoniumfluoride in THF chipping the trimethylsilyl group. Reaction times and yields for the synthesis of 9a-f are shown in Table 2.

Iodoarene	Acetylene (R-C≡CH)	Reaction conditions	Number of by-products*	Literature
10	R = (CH <sub>3</sub> ) <sub>2</sub> Si	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> , piperidine, 70 °C, 10 min., N <sub>2</sub>	-	[4]
8	R = (CH <sub>3</sub> ) <sub>2</sub> Si	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> , piperidine, 70 °C, 10 min., N <sub>2</sub>	4	[4]
10	R = (CH <sub>3</sub> ) <sub>2</sub> Si	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, triethylamine, 50 °C, 30 min., N <sub>2</sub>	-	[5]
8	R = (CH <sub>3</sub> ) <sub>2</sub> Si	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, triethylamine, 50 °C, 25 min., N <sub>2</sub>	-	[5]
10	R = (CH <sub>3</sub> ) <sub>2</sub> Si	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> , piperidine, microwave 80 - 100 °C, 3 - 15 min., (N <sub>2</sub> )	3 - 4	[4]
8	a) R = Ph b) R = HO-CH <sub>2</sub> c) R = 4-F-Ph d) R = 4-H <sub>2</sub> CO-Ph	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, triethylamine, 50 °C, 10 - 40 min., N <sub>2</sub>	-	[5]
8	R = HO-CH <sub>2</sub>	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, DMF / K <sub>2</sub> CO <sub>3</sub> , 50 °C, 80 min., N <sub>2</sub>	2	[6]
8	R = N-(methoxy)-phthalimide <sup>b</sup>	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, triethylamine, 50 °C, 125 min., N <sub>2</sub>	2	[5]
8	R = N-(methoxy)-phthalimide <sup>b</sup>	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, DMF / K <sub>2</sub> CO <sub>3</sub> , 50 °C, 29 h, N <sub>2</sub>	5	[6]
8	R = N-(methoxy)-phthalimide <sup>b</sup>	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub> / Cul, THF / K <sub>2</sub> CO <sub>3</sub> , 50 °C, 3 d 12 h, N <sub>2</sub>	5	[7]
8	R = N-(methoxy)-phthalimide <sup>b</sup>	Pd(PPh <sub>3</sub> ) <sub>4</sub> / Cul, triethylamine / THF, 50 °C, 2 d 12 h, N <sub>2</sub>	5	[8] and [5]

Table 1: Test of different Sonogashira reaction conditions  
\* determined by tlc  
<sup>b</sup> satisfactory results were not obtained with this alkyne



Scheme 1: (i) I<sub>2</sub>H<sub>5</sub>, K<sub>2</sub>CO<sub>3</sub>, DMF, RT, 2 h (ii) ethyl succinyl chloride, pyridine, toluene, 2 h reflux (iii) potassium hydride, DMF, toluene, N<sub>2</sub>, -10 °C → 80 °C, 2 h (iv) DMSO, H<sub>2</sub>O, N<sub>2</sub>, 150 °C, 4 h (v) R-C≡CH, PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, Cul, Et<sub>3</sub>N, N<sub>2</sub>, 50 °C (vi) Bu<sub>4</sub>NF x 3 H<sub>2</sub>O, THF, N<sub>2</sub>, RT, 5 min.

	Reaction time [min]	Yield [%]
9a	10	55.2
9b	10	79.0
9c	20	59.5
9d	10	86.2
9e	40	63.6
9f	5	35.8

Table 2: Reaction times and yields of 7-ethynyl-1-benzazepine-2,5-diones 9a-f

## Biological Evaluation

In an initial screening 9a-f were tested at one concentration (10 μM) for inhibition of a panel of 16 cancer-related protein kinases (ATP = 1 μM). While 9b-f failed to exhibit significant kinase inhibition, results with the 4-methoxy derivative 9a justified further studies. The determination of IC<sub>50</sub> values within the kinase panel revealed that 9a inhibits kinases of the survival (IGF1-R), the angiogenesis (VEGF-R2), and the metastasis (SRC) subpanel in the low micromolar concentration range.

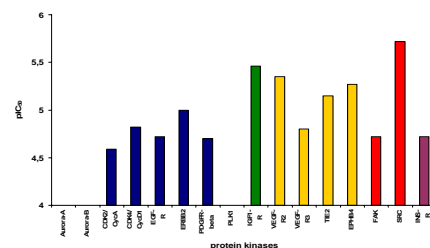


Fig. 1: pIC<sub>50</sub> values of compound 9a tested on 16 cancer-related kinases (c (ATP) = 1 μM). Missing columns indicate that the IC<sub>50</sub> of the distinct kinase is > 10<sup>4</sup> μM. Color code of kinase subpanels: proliferation (blue), survival (green), angiogenesis (yellow), metastasis (red), counter-kinase (purple). Tests were carried out using the <sup>33</sup>PanQinase<sup>®</sup> Activity Assay on a BeckmanCoulter/Sagion robotic system. As a statistic quality parameter, the Z'-factors were determined, which did not drop below 0.47 and were above 0.70 in most cases, indicating a very good to excellent assay quality.

## Conclusion

While the selectivity profile of 9a appears interesting with regard to a horizontal kinase inhibition, further synthetic chemistry studies are required to improve the kinase inhibitory potency. Results of these investigations will be communicated in due course.

## Acknowledgement

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