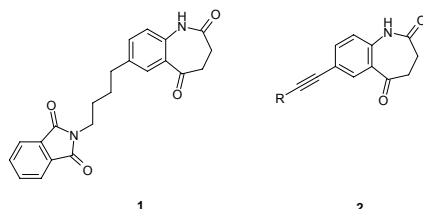


Introduction

A high-throughput screening directed to find novel kinase inhibitory chemotypes disclosed a 7-alkyl substituted 3,4-dihydro-1*H*-1-benzazepine-2,5-dione (**1**) as an inhibitor of several protein tyrosine kinases. [1] In the course of a structure optimization programme 7-ethynyl analogues **2** were prepared by Sonogashira reactions employing 7-iodo-1*H*-1-benzazepine-2,5-(3*H*,4*H*)-dione (**7**) as starting material. Among the resulting title compounds, 7-[(4-methoxyphenyl)ethynyl]-3,4-dihydro-1*H*-1-benzazepine-2,5-dione (**2a**) proved to inhibit several kinases relevant for proliferation, survival, angiogenesis and metastasis of tumor tissues.



Chemistry

The synthesis of the 7-ethynyl-1-benzazepine-2,5-diones **2a-f** was carried out following a general procedure shown in Scheme 1. First, 2-amino-5-iodobenzoic acid (**3**) was reacted with iodoethane. The ester **4** was converted to the amide **5** by treatment with ethyl succinyl chloride. Afterwards, **5** underwent a Dieckmann cyclization upon treatment with potassium hydride in DMF and toluene to yield the enol **6**. Heating **6** in wet DMSO led to a dealkoxycarbonylation providing 7-iodo-1*H*-1-benzazepine-2,5-(3*H*,4*H*)-dione (**7**). Subsequently, **7** was reacted under Sonogashira reaction conditions furnishing the 7-ethynyl-1-benzazepine-2,5-diones **2a-e**. [2] 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. The reactions were carried out either under conventional or microwave-assisted heating. The standard method under conventional heating to 50 °C using bis-(triphenylphosphine)-palladium(II)-dichloride and triethylamine serving as solvent and coevally as base turned out to be favorable for the synthesis of **2a-e**.

	Reaction time [min]	Yield [%]
2a	10	55.2
2b	10	79.0
2c	20	59.5
2d	10	86.2
2e	40	63.6
2f	5	35.8

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

The other catalyst, bases, solvents and microwave conditions provided no advantages for the reaction times and yields. The synthesis of 7-ethynyl-3,4-dihydro-1*H*-1-benzazepine-2,5-dione (**2f**) was accomplished by treating **2d** with tetrabutylammoniumfluoride in THF chipping the trimethylsilyl group. Reaction times and yields of **2a-f** are shown in Table 1.

Biological evaluation

In a first screening the inhibitory profiles of the six new synthesized compounds **2a-f** were determined using 13 cancer-related protein kinases. The compounds were tested at one concentration (10 μM) in singlicate. The 4-methoxy derivative **2a** which showed a kinase inhibition profile resembling the lead structure **1** was further characterized by IC₅₀ determinations with the 13 kinases. IC₅₀ values were measured by testing 10 concentrations (1 x 10⁻⁴ M to 3 x 10⁻⁹ M) of **2a** in singlicate. The comparison of the kinase inhibition profiles of the lead structure **1** and **2a** expressed by their pIC₅₀ values is shown in Figure 1. With the exception of the receptor protein tyrosine kinase TIE2, **2a** proved to be a superior inhibitor for all tested kinases compared to the lead structure **1**.

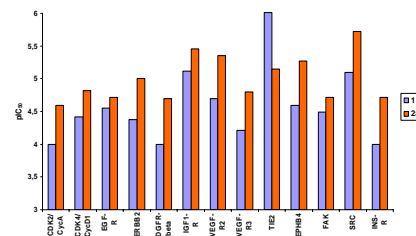


Fig. 1: Comparison of the pIC₅₀ values of compound **1** and **2a** tested on 13 cancer-related kinases

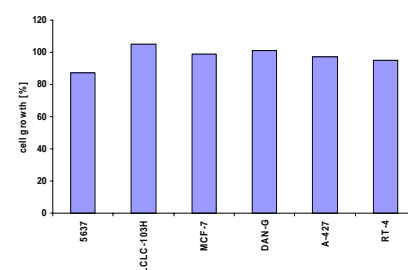
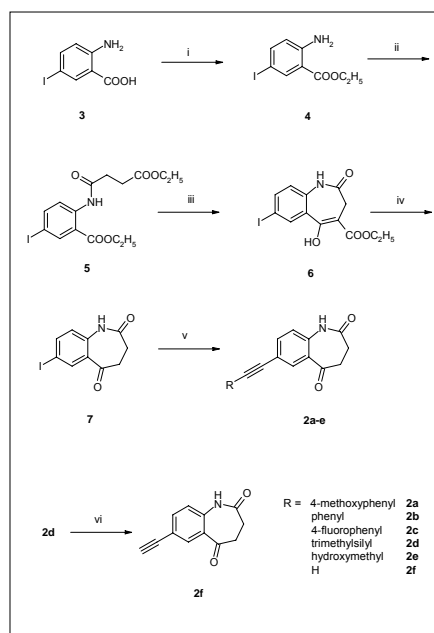


Fig.2: Percent cell growth following a 96 h treatment with 20 μM **2a** (5637, RT-4: bladder cancer; LCLC, A437: lung cancer; MCF-7: breast cancer; DAN-G: pancreas carcinoma)

Antitumor screening

As **2a** showed an interesting inhibition profile on several kinases its antitumor activity was tested on six different cancer cell lines. The results are depicted in Figure 2 giving the percent cell growth following a 96 h treatment with 20 μM substance. Untreated cells showed 100% growth. The cell growth was measured with a microtiter assay based on the staining of cellular material with crystal violet. [3] None of the cell lines was significantly inhibited (Fig. 2).



Scheme 1: (i) IC₂H₅, K₂CO₃, DMF, RT, 2 h (ii) ethyl succinyl chloride, piperidine, toluene, 2 h reflux (iii) potassium hydride, DMF, toluene, N₂, -10 °C → 80 °C, 2 h (iv) DMSO, H₂O, N₂, 150 °C, 4 h (v) RC≡CH, PdCl₂(PPh₃)₂, CuI, Et₃N, N₂, 50 °C (vi) Bu₄NF x 3 H₂O, THF, N₂, RT, 5 min

Conclusion

Except for compound **2a** the other new synthesized analogues did not give convincing results in the kinase assay. Although **2a** showed a better kinase inhibition profile than the lead structure **1** it was devoid of noteworthy antiproliferative activity on tumor cells in vitro.

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