



Library of *N*-(Aryloxyalkyl)-phthalimide Kinase Inhibitors by Parallel Synthesis in Liquid Phase

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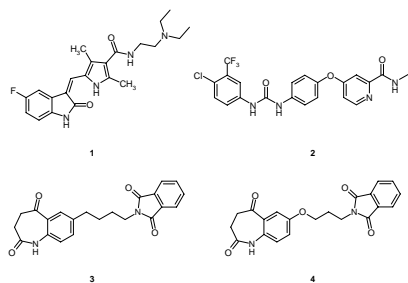
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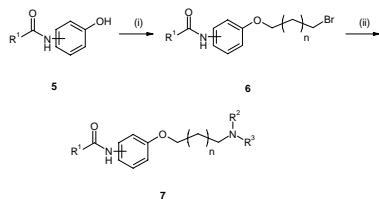
Introduction

Experiences with either low response rates or the development of resistance against the anticancer drugs gefitinib and imatinib led to the idea of using promiscuous agents that inhibit two or more cancer related protein kinases [1,2]. This concept has been established in the drug market by the launch of the multiple kinase inhibitors sunitinib (Sutent; **1**) and sorafenib (Nexavar; **2**) as anticancer drugs. In order to find novel chemotypes representing multiple protein kinase inhibitors we have performed a screening of an in-house compound collection on a panel of cancer-related kinases. As a result of this screening the *N*-substituted phthalimide **3** was found to be an inhibitor of kinases from diverse subpanels. In order to increase the kinase inhibitory properties in the series and to identify structures with antiproliferative activity on cancer cells, a program was initiated for chemical modification of the prototype **3**. In this context the oxadervative **4** was prepared, which showed improved kinase inhibition. However, tests with **4** on a small collection of cultivated human tumor cell lines revealed that it was devoid of noteworthy antiproliferative activity. With the aim to improve the growth inhibitory activity on cancer cells, a program was performed for a parallel synthesis of the derivatives **7** in which the 1*H*-1-benzazepine-2,5(3*H*,4*H*)-dione substructure of **4** was replaced by simple acylaniline moieties, the methylene linker chain was varied between 3 and 4 units, and the phthalimide moiety was replaced by similar cyclic imide substructures.



Chemistry

The synthesis of the *N*-(acylaminoaryloxyalkyl)-imides **7** was carried out following a general procedure shown in Scheme 1. First, a suitable acylamino-phenol **5** was reacted with an excess of either 1,3-dibromopropane or 1,4-dibromobutane in the presence of potassium carbonate in refluxing acetone. [3] Subsequently, the resulting ether **6** was treated with the appropriate potassium or sodium salt of a cyclic imide or, in the case of **7z-7hh**, with sodium saccharine. The structures of the *N*-(acylaminoaryloxyalkyl)-imides **7** are given in Table 1 together with inhibition data for three cancer relevant kinases.



Scheme 1: (i) excess Br-CH₂-(CH₂)_n-CH₂-Br, K₂CO₃, acetone, reflux (ii) sodium or potassium salt of imide, DMSO, 80 °C.

Table 1: Structure of and Kinase Inhibition by *N*-(Acylaminoaryloxyalkyl)-imides **7**

7	R ¹	n	R ²	Remaining kinase activity in the presence of 10 μM 7 ^a		
				IGF1-R	VEGF-R2	SRC
7a	2-acetylamino	1		101	86	96
7b	3-acetylamino	1		86	94	85
7c	3-acetylamino	2		96	96	97
7d	4-acetylamino	1		91	78	86
7e	4-propionylamino	1		87	86	86
7f	4-propionylamino	2		83	109	93
7g	4-benzoylamino	1		77	72	53
7h	4-benzoylamino	2		98	87	74
7i	2-acetylamino	1		12	10	7
7j	3-acetylamino	2		83	85	70
7k	4-acetylamino	1		63	76	31
7l	4-acetylamino	2		51	48	40
7m	4-propionylamino	1		42	54	29
7n	4-propionylamino	2		39	17	10
7o	4-benzoylamino	1		27	21	11
7p	4-benzoylamino	2		40	38	22
7q	2-acetylamino	1		90	96	92
7r	3-acetylamino	1		94	124	101
7s	3-acetylamino	2		106	86	91
7t	4-acetylamino	1		106	105	96
7u	4-acetylamino	2		87	92	91
7v	4-propionylamino	1		88	108	79
7w	4-propionylamino	2		93	94	92
7x	4-benzoylamino	1		89	97	87
7y	4-benzoylamino	2		78	88	72
7z	2-acetylamino	1		89	104	91
7aa	3-acetylamino	1		106	92	93
7bb	3-acetylamino	2		97	101	90
7cc	4-acetylamino	1		71	121	91
7dd	4-acetylamino	2		92	95	87
7ee	4-propionylamino	1		108	85	87
7ff	4-propionylamino	2		93	103	90
7gg	4-benzoylamino	1		84	104	83
7hh	4-benzoylamino	2		86	94	80
7ii	2-acetylamino	1		96	89	78
7jj	3-acetylamino	1		85	88	91
7kk	3-acetylamino	2		87	99	90
7ll	4-acetylamino	1		80	96	93
7mm	4-acetylamino	2		71	107	91
7nn	4-propionylamino	1		101	92	83
7oo	4-propionylamino	2		95	95	92
7pp	4-benzoylamino	1		88	97	84
7qq	4-benzoylamino	2		83	101	76
4	oxadervative (see structure in left column)	1		57	40	25

^a Tests were carried out using the 33PanQinase® Activity Assay on a BeckmanCoulter/Sagian robotic system. ATP concentration = 1 μM.

Biological Evaluation

The initial screening of **7** revealed that only representatives of the tetrachlorophthalimide subgroup inhibited cancer-related kinases by more than 50% (10 μM inhibitor concentration; ATP = 1 μM). The subsequent determination of pIC₅₀ values for three kinases belonging to the survival (IGF1-R), the angiogenesis (VEGF-R2), and the metastasis (SRC) subpanel showed that **7i**, **7m** and **7o** compare favourably to the lead structure **4** (Fig.1). In tests for antiproliferative activity in a panel of 6 human tumor cell lines **7i** demonstrated superior potency compared to the other compounds (Fig. 2). The IC₅₀ values found with **7i** for cultivated cancer cells are given in Table 2.

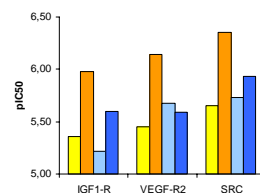


Fig. 1: pIC₅₀ values of compounds **4**, **7i**, **7m**, **7o** for 3 cancer-related kinases (c (ATP) = 1 μM). Tests were carried out using the 33PanQinase® Activity Assay on a BeckmanCoulter/Sagian robotic system. As a statistic quality parameter, the Z'-factors were determined, which did not drop below 0.47 and were above 0.70 in most cases, indicating a very good to excellent assay quality.

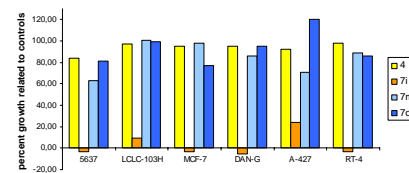


Fig. 2: Growth inhibition exhibited by **4**, **7i**, **7m**, **7o** for 6 human cancer cell lines (Inhibitor concentration = 20 μM). 5637 and RT-4: urinary bladder carcinoma; LCLC-103H: large cell lung carcinoma, MCF-7: breast adenocarcinoma; DAN-G: pancreas carcinoma; A-427: lung carcinoma. Tests were carried out as described in ref. [4].

Cell line	5637	LCLC-103H	MCF-7	DAN-G	A-427	RT-4
IC ₅₀ [μM]	14.0±0.57	10.9±0.43	8.2±0.96	10.1±1.69	13.0±1.30	7.0±0.34

Table 2: Growth inhibition (IC₅₀, [μM]) exhibited by **7i** on 6 human cancer cell lines. 5637 and RT-4: urinary bladder carcinoma; LCLC-103H: large cell lung carcinoma, MCF-7: breast adenocarcinoma; DAN-G: pancreas carcinoma; A-427: lung carcinoma. Tests were carried out as described in ref. [4].

Conclusion

The phthalimide derivative **7i** constitutes a novel chemotype of kinase inhibitors with noteworthy antiproliferative activity for cancer cells.

Acknowledgement

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References

- [1] Espinoza-Fonseca, L. M.: The benefits of the multi-target approach in drug design and discovery. *Bioorg. Med. Chem.* **2006**, *14*, 896-897.
- [2] Daub, H., Specht, K., Ullrich, A.: Strategies to overcome resistance to targeted protein kinase inhibitors. *Nat. Rev. Drug Discov.* **2004**, *3*, 1001-1010.
- [3] Method: Prüfs, C., Technische Universität Braunschweig, unpublished.
- [4] Bracht, K., Boubakari, Grünert, R., Bednarski, J. P.: Correlations between the activities of 19 anti-tumor agents and the intracellular glutathione concentrations in a panel of 14 human cancer cell lines: comparisons with the National Cancer Institute data. *Anti-Cancer Drugs* **2006**, *17*, 41-51.

