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Novel CDK inhibition profiles of structurally varied 1-aza-9-oxafluorenes

Burkhardt Voigt,[a] Laurent Meijer,[b] Olivier Lozach,[b] Christoph Schächtele,[c] Frank Totzke[c] and Andreas Hilgeroth[a,*]

[a]*Institute of Pharmaceutical Chemistry, Department of Pharmacy, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle, Germany*
[b]*CNRS, Cell Cycle Group, Station Biologique, BP 74, Roscoff 29682 Cedex, France*
[c]*ProQinase GmbH, Breisacher Strasse 117, 79106 Freiburg, Germany*

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Abstract—A series of 1-aza-9-oxafluorenes with functionally varied 3-substituents have been prepared from *N*-phenoxycarbonyl-4-phenyl-1,4-dihydropyridines and *p*-benzoquinone and biologically evaluated as inhibitors of various cyclin-dependant kinases. The absence of a 3-hydrogen bond acceptor function leads to a complete loss of inhibitory activity. Differing hydrogen bond acceptor functions surprisingly cause significant shifts in the selectivity of inhibition profiles.

Cyclin-dependant kinases (CDKs) play a key role in the cycle of dividing cells.[1] Associated with differing cyclins and by specific natural protein inhibitors they control the progression of cell growth and division.[2–4] Occurring mutations lead to uncontrolled growth as well as cell degeneration process with resulting diseases like cancer or Alzheimer's disease (AD), for which each specific kinases have been characterized as responsible agents.[5,6]

Therefore CDKs like 1, 2 and 4 have been target enzymes for the development of small-sized inhibitors with antiproliferative properties in cancer progression.[5] CDK5/p25 plays a central role in ongoing Alzheimer's disease by phosphorylation of tau-protein leading to the formation of neurofibrillary tangles and neuronal decline. Inhibitors of this kinase have been investigated as potential AD therapeutics.[6]

The main problem in the development of such small-sized inhibitors is the selectivity of the inhibition.[1,5] Among those various reported classes of CDK inhibitors, some show selectivity profiles for CDKs like substituted Flavopiridol-analogues **1**, with some preference for both CDK1 and 2 inhibition beside CDK4, 6 and 7 inhibitory activity, or substituted purine compounds **2**, which also inhibit CDK5/p25[1] (Fig. 1).

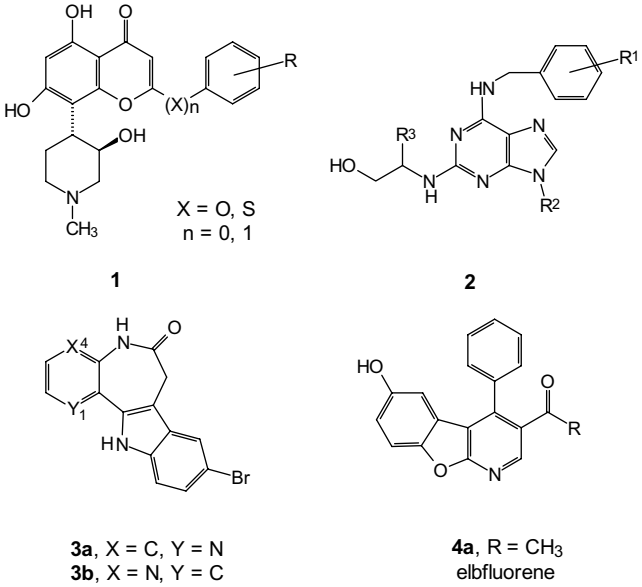

Figure 1.

* Corresponding author. Tel.: +49 345 55 25168; fax: +49 345 55 27026; e-mail: hilgeroth@pharmazie.uni-halle.de

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Reduced CDK-inhibitory activities were recently reported for 1-azakenpaullone **3a** compared to 4-azakenpaullone **3b** with similar activities against CDK1 and 5/p25 as well as glycogen synthase kinase (GSK)-3β,[7] a kinase involved in various cell cycle regulation processes and with dysregulation in AD progression. The resulting observed selectivity of **3a** as GSK-3β inhibitor is caused by the modified binding properties of the 1-azacompound compared to the 4-azacompound because the nitrogen atom responsible for potential hydrogen bond acceptor function to the enzyme backbone is situated at the opposite molecular site.

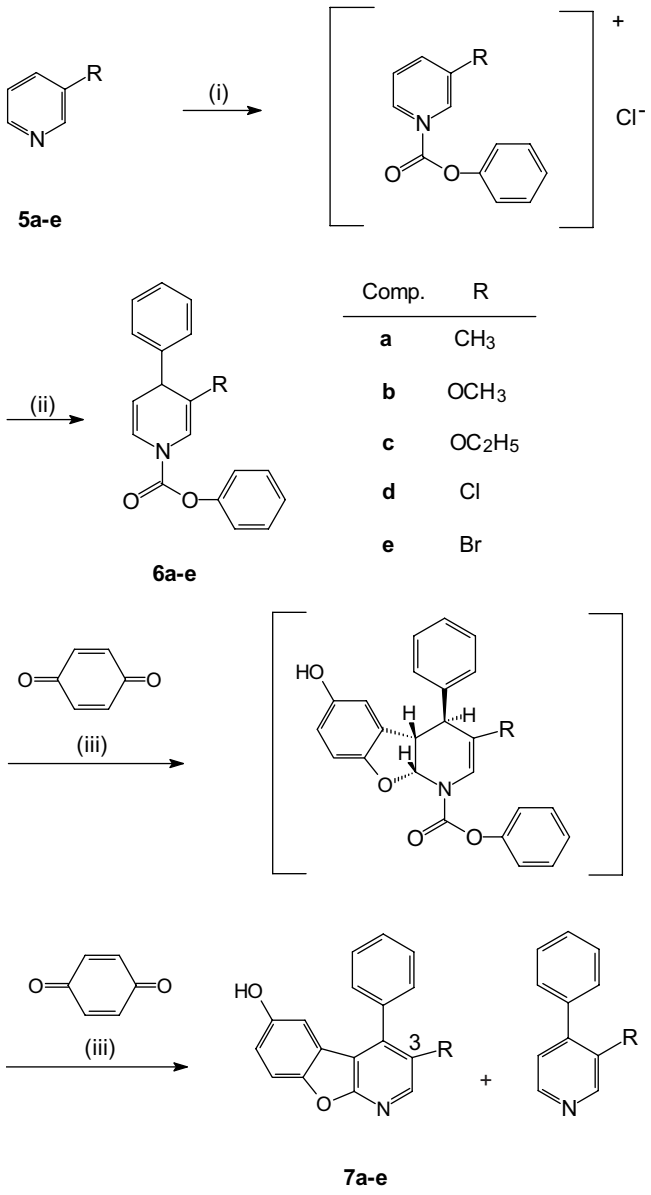
We recently presented 1-aza-9-oxafluorenes **4** as a novel class of small-sized cytostatics.[8] A Compare-analysis of one 4-phenyl derivative with NCI-database compounds gave the first hints to possible kinase inhibitory properties as mode of cytostatic action of the nonplanar molecules. Those 3-acyl substituted candidates turned out primarily as selective CDK1 inhibitors compared to other CDKs like CDK2 and CDK4 or CDK5.[9]

The 6-hydroxy function was found essential for biological activity, with its acetyl ester being inactive. The hydroxy function, the pyridine nitrogen and the 3-acyl substituent may bind to the amino acid background of the ATP-binding pocket of CDK1.

In order to investigate the importance of the nitrogen as potential hydrogen bond acceptor function, its basicity was varied by the introduction of varying electron releasing substituents in the 3-position instead of the electron withdrawing 3-acyl function causing lower basicity and less hydrogen bond acceptor properties.

Although we changed just one functional group, while maintaining a hydrogen bond acceptor function, we observed significant shifts in the CDK inhibition profile. Such characteristics have not been previously observed for structurally changed classes of CDK inhibitors.[1]

The novel CDK-inhibition profiles encourage further development of specific AD therapeutics or modifying acting cytostatics.



Scheme 1. Reagents and conditions: (i) PhOCOCl, THF, −20 °C; (ii) PhMgCl, CuI, −20 °C; (iii) dioxane/$HClO_4$ (5%), rt.

The *N*-phenoxycarbonyl-1,4-dihydropyridines **6a**–**e** were prepared from corresponding commercially available pyridine compounds **5a**–**e** by primary acylation using equimolar amounts of phenylchloro formate at low temperature (−20 °C) in dried tetrahydrofurane to intermediate acylpyridinium compounds and consequent regioselective 4-phenylation reaction using equimolar amounts of phenylmagnesium chloride and copper(I) iodide as catalyst.[9,10]

The resulting pure crystalline 1,4-dihydropyridines **6a**–**e** with overall yields of 85% were treated with a 1.2 M excess of *p*-benzoquinone in dioxane containing 5% of perchloric acid as catalyst at room temperature. Primary intermediate tetrahydro-1-aza-9-oxafluorenes were oxidized with excess *p*-benzoquinone added in each 0.3 M portions until no more tetrahydro-intermediate was detectable by TLC (Scheme 1). While resulting 3-methyl, 3-chloro and 3-bromo 1-aza-9-oxafluorenes **7a**,**d** and **7e**[11] were yielded after work up and preparative column chromatography as main products beside small amounts of pyridine oxidation side products (~20%), yields of the 3-methoxy and -ethoxy compounds **7b**,**c**[11] were comparably poor (20% and 25%) with pyridine compounds being the main products formed during the primary cycloaddition procedure.

Biological evaluations as a CDK inhibitor indicated no activity for the 3-methyl compound **7a** with an electron releasing function (+I (inductive)-effect) but without hydrogen bond binding ability of the 3-substitution (Table 1). This results suggest that the 3-acyl function of the previous series **4** is essential for biological activity with a suggested hydrogen bond acceptor function to the ATP-binding pocket backbone.

Table 1. CDK inhibition profiles of novel 1-aza-9-oxafluorenes **7a–e**

Compd	IC_{50} (μM)[a]			
	CDK1/B	CDK5/p25	CDK2/E	CDK4/D
elbfluorene	4.2 ± 1.1	Nd[b]	>100	>100
7a	>100	Na[c]	>100	>100
7b	64.3 ± 16.2	6.3 ± 0.4	80.0 ± 24.3	>100
7c	>100	5.9 ± 1.2	>100	>100
7d	>100	Na[c]	>100	>100
7e	70.7 ± 17.6	Na[c]	3.2 ± 0.3	28.7 ± 4.0

[a] Means of at least two determinations. CDK inhibition experiments were carried out as described.[9]
[b] Nd, not determined.
[c] Na, not active.

However, with maintaining hydrogen bond acceptor function at the 3-position, the 3-methoxy substitution in **7b** with mainly electron releasing effects (−I and +M (mesomeric)-effect) surprises with a loss of CDK1-inhibition (IC_{50} = 64.3 μM) compared to a 3-acetyl substitution in **4a**, elbfluorene, with IC_{50} (CDK1) of 4.2 μM. An increase in CDK5/p25 inhibition with IC_{50} of 6.3 μM was found compared to elbfluorene **4a** with some CDK5/p25 inhibitory activity of 15% observed at 10 μM.[9] CDK2 remains hardly affected by **7b**.

An increase in selectivity of CDK-inhibition was found for the 3-ethoxy derivative **7c** with practically no activity against CDK1, CDK2 and CDK4 and an exclusive activity against CDK5/p25 with an IC_{50} value of 5.9 μM.

As CDK5/p25 as well as GSK-3β are interesting targets for selective inhibitors as potential AD therapeutics we also investigated the inhibition of GSK-3β[12] by **7b**,**c** and observed IC_{50} values of 3.9 ± 1.0 μM (**7b**) and 5.2 μM ± 1.9 μM (**7c**) a partly even better inhibitory activity than against CDK5/p25.

The different electronic effects of the 3-substituents with (−I) and (+M) effects of the methoxy and ethoxy functions in **7b**,**c** and with only electron withdrawing effects (−I and −M) of the acetyl function in elbfluorene mainly influence potential nitrogen binding ability to the CDK protein backbone and may give a plausible explanation for the observed shift in the CDK inhibition profile.

Surprising differences have been found for the 3-halogen substituted derivatives **7d** and **7e**: While the 3-chloro compound **7d** was found inactive as CDK inhibitor, the bromo derivative shows an inhibition profile shift to CDK2 and CDK4, a set of single CDKs, which are often commonly inhibited by CDK inhibitors.

Electronic effects of the halogen substituents may contribute to the shift in the CDK-inhibition profile. The basicity of the nitrogen in **7d** and **7e** may be different compared to the acyl-substitution in **4a**, as well as to the alkoxy-substitution in **7b** and **7c**, with combined electron withdrawing (−I) and electron releasing (+M) effects of the halogen atoms. However, the difference of activity of the chloro and bromo compound remains an interesting fact, which has to be investigated further.

In summary, structural variations of just one functional group in the class of CDK-inhibitory active 1-aza-9-oxafluorenes causes surprising certain shifts in the selectivity profiles to CDK5/p25 and GSK-3β as well as CDK2/4 and encourages concentration on the development of different therapeutics against AD and CDK-sensitive cancer. Consequent variations of the 4-phenyl substitution will be of interest to improve inhibitory activity and so strengthen selectivity inhibition profiles.

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11. NMR and MS data for target compounds **7a–e**: Compound **7a**: ^{1}H NMR (DMSO-d_6) δ 9.30 (s, 1H), 8.33 (s, 1H), 7.66–7.57 (m, 3H), 7.50 (d, J = 8.8 Hz, 1H), 7.46–7.43 (m, 2H), 6.90 (dd, J = 8.8/2.5 Hz, 1H), 6.28 (d, J = 2.5 Hz, 1H), 2.18 (s, 3H); MS (EI) m/z 275 (M^+). Compound **7b**: ^{1}H NMR (DMSO-d_6) δ 9.34 (s, 1H), 8.25 (s, 1H), 7.61–7.53 (m, 3H), 7.51–7.49 (m, 2H), 7.49 (d, J = 8.9 Hz, 1H), 6.91 (dd, J = 8.9/2.5 Hz, 1H), 6.47 (d, J = 2.5 Hz, 1H), 3.84 (s, 3H); MS (ESI) m/z 292 ($M + H^+$). Compound **7c**: ^{1}H NMR (DMSO-d_6) δ 9.33 (s, 1H), 8.25 (s, 1H), 7.59–7.51 (m, 5H), 7.49 (d, J = 8.9 Hz, 1H), 6.91 (dd, J = 8.9/2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 4.09 (q, J = 7.1 Hz), 1.18 (t, J = 7.1 Hz, 3H); MS (ESI) m/z 306 ($M + H^+$). Compound **7d**: ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 1H), 8.66 (s, 1H), 7.65–7.64 (m, 3H), 7.57 (d, J = 8.7 Hz, 1H), 7.49–7.47 (m, 2H), 6.96 (dd, J = 8.7/2.5 Hz, 1H), 6.24 (d, J = 2.5 Hz, 1H); MS (EI) m/z 295 (M^+). Compound **7e**: ^{1}H NMR (DMSO-d_6) δ 9.45 (s, 1H), 8.57 (s, 1H), 7.67–7.63 (m, 3H), 7.57 (d, J = 8.7 Hz, 1H), 7.54–7.51 (m, 2H), 6.97 (dd, J = 8.7/2.5 Hz, 1H), 6.33 (d, J = 2.5 Hz, 1H); MS (EI) m/z 339 (M^+).
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