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Inhibition of γ-secretase by the CK1 inhibitor IC261 does not depend on CK1δ

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ABSTRACT

CK1 and γ-secretase are interesting targets for therapeutic intervention in the treatment of cancer and Alzheimer's disease. The CK1 inhibitor IC261 was reported to inhibit γ-secretase activity. The question is: Does CK1 inhibition directly influence γ-secretase activity? Therefore we analyzed the SAR of 15 analogues and their impact on γ-secretase activity. The most active compounds were investigated on CK1δ activity. These findings exclude a direct influence of CK1δon γ-secretase, because any change in the substitution pattern of IC261 diminished CK1 inhibition, whereas γ-secretase inhibition is still exerted by several analogues.

Alzheimer's disease is a devastating illness, which robs patients of the ability to manage their lives on their own. This illness is accompanied by protein aggregates in the brain composed of the amyloid-β-peptide (Aβ), which are called amyloid plaques.[1] The amyloid-β-peptide is generated by the subsequent degradation of the amyloid precursor protein (APP), a type I transmembrane protein, by two aspartyl proteases, the β-secretase and the γ-secretase. The γ-secretase is a promising target for therapeutic intervention as it liberates various Aβ-peptides with a length of 38, 40, or 42 amino acids.[2] The toxicity depends on the length: $Aβ_{42}$ is the most toxic species while $Aβ_{38}$ is regarded to be non-toxic as increased production of $Aβ_{38}$ does not diminish cellular viability. Several γ-secretase inhibitors (GSI), which decrease total Aβ levels, and several γ-secretase modulators (GSM), which shift the cleavage-site to the non-toxic $Aβ_{38}$, have been identified so far.[3–5]

Flajolet et al.[6] reported IC261 (**1**) (Scheme 1), a presumably selective ATP-competitive casein kinase 1ε (CK1ε) inhibitor, which is also an equipotent inhibitor to the CK1δ-isoform (CK1δ) (IC_{50} = 2.57 μM in cells).[19] IC261 exerts rather weak GSI activity in comparison to reported potent GSIs.[6–9] IC261 causes a significant reduction of $Aβ_{40}$ (68%) and $Aβ_{42}$ (61%) levels in N2a cells overexpressing constitutively active CK1ε-271 within 5–50 μM concentration at 3 h after incubation. The reported increase of $Aβ_{40}$ secretion by approximately 35% (Fig. 2A in Ref. 6) under overexpression of constitutively active CK1δ in N2a cells and the similarity of IC261 with the known, potent GSM (Sulindac-S (**2**), and Sulindac-sulfon (**3**)) stimulated us to investigate the oxoindole-backbone of IC261 common to many kinase inhibitors and the potential influence of CK1ε/δ inhibitors on γ-secretase activity).

H4-cells do neither express constitutively active CK1ε-271 nor do they overexpress CK1ε, which were postulated to be the regulating CK1 isoforms.[6] Actually, Aβ secretion from the utilized H4 cells responded to IC261 treatment five times stronger, suggesting an CK1ε independent effect. A dual structure–activity relationship analysis (SAR) towards γ-secretase activity in H4-cells and CK1δ activity was carried out by systematical variation of the oxoindole substitution utilizing the CK1/IC261 co-crystallized structure (PDB: 1EH4). (Table 1). The CK1 isoforms differ in the primary structure of the C-terminal non-catalytic domain. However, CK1δ and CK1ε do not differ in the ATP-binding-site for IC261, thus a cell free CK1δ activity assay guided the structure–activity relationship for both CK1 isoforms. This approach is commonly employed for the development of GSK3α/β inhibitors.

This structure guided the variation of the compounds aiming either at enhanced interaction with CK1 or to exclude interaction with CK1 (R^3 = Me, 15). We chose the 5-cloro substituted oxoindole as backbone as it is supposed to show enhanced metabolic stability,[10] which is commonly used in medicinal chemistry, for example, Pfizer's Carprofen (**4**). All compounds were tested in the cellular Aβ generation assay.[11] The four most potent GSIs were subsequently investigated on their CK1δ activity to investigate the influence of CK1δ on the γ-secretase. A further aim of this investigation was the identification of selective GSIs or even GSMs, void off cross-activity on CK1δ or related kinases.

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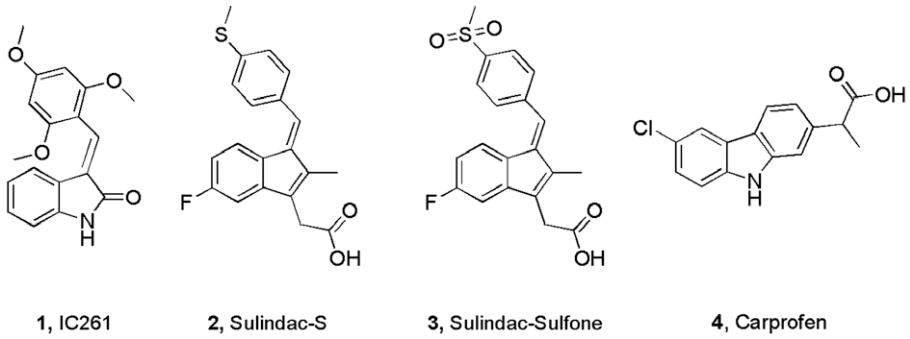
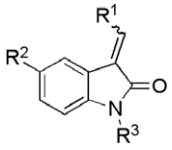


Scheme 1. Structural similarity of IC261 and the GSM of the Sulindac-series (**2**, **4**).

Table 1
Compounds **1, 5–16**



Entry	Compds	Code	R^1	R^2	R^3	Ref.
1	**1**	BSc3930 IC261	2,4,6-Trimethoxy-benzene	H	H	6
2	**5**	BSc3926	2-Methoxybenzene	H	H	12
3	**6**	BSc3921	2-Fluorobenzene	Cl	H	—
4	**7**	BSc3923	2-Nitrobenzene	Cl	H	13
5	**8**	BSc3922	2-Benzene sulfonic acid	Cl	H	—
6	**9**	BSc3928	Benzo[δ][1,3]dioxole	Cl	H	14
7	**10**	BSc3914	3,5-Difluorobenzene	Cl	H	—
8	**11**	BSc3929	4-Benzeneacetamide	Cl	H	—
9	**12**	BSc3944	4-Chloropropoxy-benzene	Cl	H	—
10	**13**	BSc3890	2,3,4-Trimethoxy-benzene	Cl	H	[a]
11	**14**	BSc3925	3,4,5-Trimethoxy-benzene	Cl	H	15
12	**15**	BSc3927	2,4,6-Trimethoxy-benzene	Cl	H	16
13	**16**	Sunitinib	*N*-(2-(Diethylamino)-ethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide	H	Me	[a]

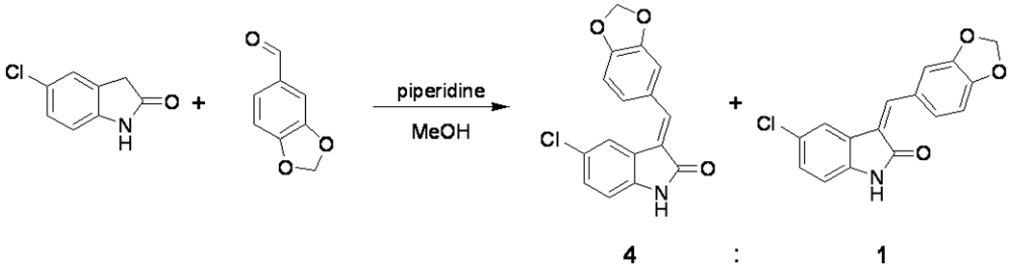
[a] Commercially available.

The Knoevenagel condensation of these IC261-derivatives utilizes an oxoindole-derivative, a respective aldehyde and piperidine as a base. The reaction is carried out under microwave irradiation at 100 °C for 30 min to provide the products in moderate to good yields.[17] The *Z*-isomer was enriched in the subsequent re-crystallization. The proportion of the *E*/*Z* isomers was analyzed by HPLC–MS signal integration and the HPLC signals were definitely assigned to the molecular mass. (Scheme 2, see Supplementary data) The assignment of the two isomers to the two HPLC signals was established by ^{1}H NMR-spectroscopy.

The isomerization of the pure *Z*-isomer to the equilibrium of *E*- and *Z*-isomers was monitored by HPLC–MS for **9** (Scheme 2), ^{1}H NMR-spectroscopy and 2-dimensional-NMR-spectroscopy for **14**[18] (Scheme 3, see Supplementary data) to be complete within 2 days in methanol solution, which compares to the assay conditions: buffered H_2O, 24 h. Thus the cellular data are obtained for *E*/*Z* mixtures regardless of the purity of the initial isomer.

IC261 (**1**) is a competitive ATP-binding-site inhibitor. The interaction with this binding-site was reported by Mashhoon et al. based on the co-crystallization (PDB: 1EH4) of CK1 with IC261.[19]

1 features both a hydrogen-bond-donor in form of the indole-amin and three methoxy-substituents as hydrogen-bond-acceptors, which can be divided in two *o*- and one *p*-substitution. The structure analysis suggests two hydrogen-bonds of the indole-amine with Asp[86] and Leu[88].[19] Notable interactions were assigned to the *o*-methoxy-group and Lys[41] and an intermolecular interaction with



Scheme 2. *E*/*Z* isomerization in solution.