Bioorganic & Medicinal Chemistry Letters 20 (2010) 2958–2963

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Nicole Höttecke [a], Miriam Liebeck [a], Karlheinz Baumann [b], Robert Schubenel [b], Edith Winkler [c], Harald Steiner [c], Boris Schmidt [a,*]

[a] Clemens Schöpf-Institute of Chemistry and Biochemistry, Technische Universität Darmstadt, Petersenstr. 22, D-64287 Darmstadt, Germany
[b] F. Hoffmann-La Roche Ltd, Pharmaceuticals Division, Preclinical Research CNS, Bldg. 70/345, CH-4070 Basel, Switzerland
[c] German Center for Neurodegenerative Diseases (DZNE) and Adolf-Butenandt-Institute, Biochemistry, Ludwig-Maximilians-University, Schillerstr. 44, 80336 Munich, Germany

ARTICLE INFO

Article history:
Received 15 January 2010
Revised 26 February 2010
Accepted 27 February 2010
Available online 4 March 2010

Keywords:
Alzheimer's disease
γ-Secretase
Casein kinase 1
Amyloid b secretion

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]

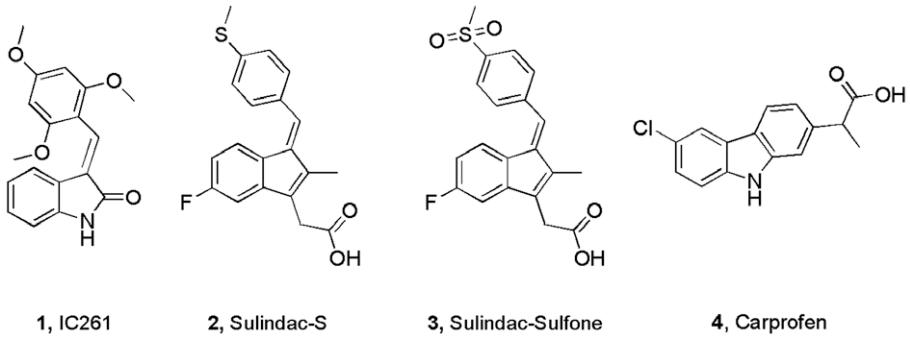
Flajolet et al.[6] reported IC261 (**1**) (Scheme 1), a presumably selective ATP-competitive casein kinase 1ε (CK1ε) inhibitor, which is also an equipotent inhibitor to the CK1δ-isoform (CK1δ) (IC_{50} = 2.57 μM in cells).[19] IC261 exerts rather weak GSI activity in comparison to reported potent GSIs.[6–9] 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.

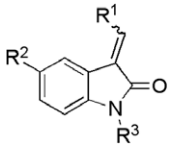
* Corresponding author. Tel.: +49 6151 163075; fax: +49 6151 163278.
E-mail address: schmidt_boris@t-online.de (B. Schmidt).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.02.110



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]

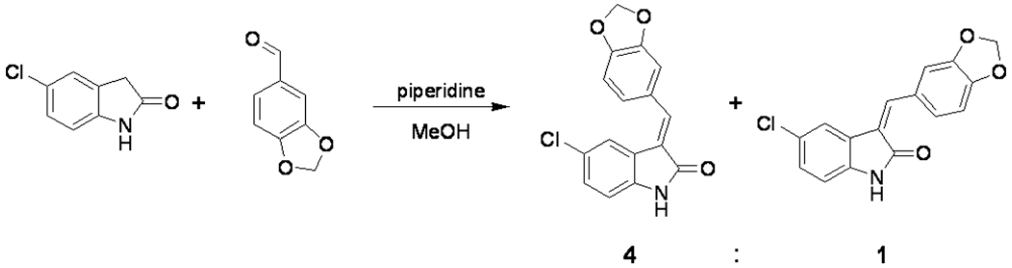
[a] Commercially available.

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.

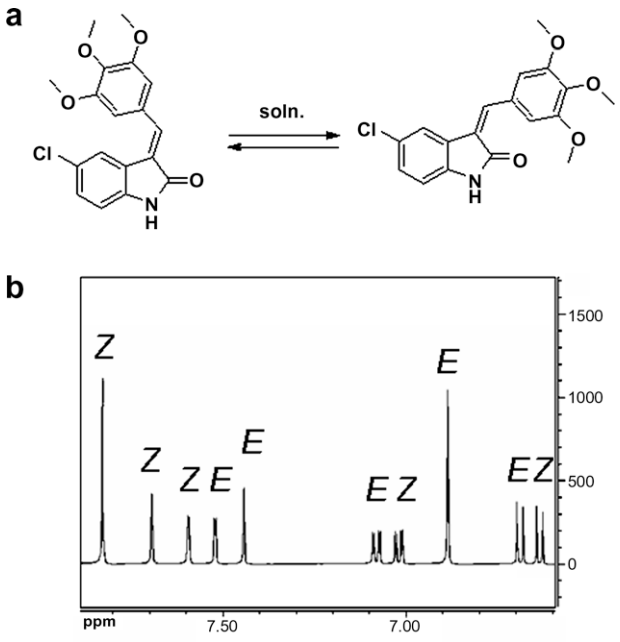
The isomerization of the pure *Z*-isomer to the equilibrium of *E*- and *Z*-isomers was monitored by HPLC–MS for **9** (Scheme 2), ^{1}H NMR-spectroscopy and 2-dimensional-NMR-spectroscopy for **14**[18] (Scheme 3, see Supplementary data) to be complete within 2 days in methanol solution, which compares 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.



Scheme 3. *E*/*Z* isomerization of **14** analyzed by ^{1}H NMR-spectroscopy.

a benzene hydrogen and interactions of the p-methoxy-group with Ser^{22} and Asp^{154} (Fig. 1).

The symmetric substitution pattern with two *o*-methoxy-groups enables interactions of both rotamers. Both the *E*-isomer purity and the atrop-isomerism found in the co-crystal were captured from the equilibrium conformations in solution. The influence of the o-substitution was determined by a derivative bearing just one *o*-methoxy-group (**5**) and several derivatives where the methoxy-groups were replaced by fluorine (**6**), nitro (**7**) or sulfonic acid (**8**).

Just one out of these derivatives (**5** $A\beta_{38}$ = 62%, $A\beta_{40}$ = 76%, $A\beta_{42}$ = 65%) displayed a significant decrease in Aβ-levels comparable to the activity of IC261 ($A\beta_{38}$ = 55%, $A\beta_{40}$ = 77%, $A\beta_{42}$ = 77%, Table 2). The single o-substituent and the absence of a p-substituent may cause these minor changes in potency of **5**. A complete loss of activity was observed for those derivatives which lacked *o*-methoxy-groups. This observation demonstrates the essential role of the *o*-methoxy-group which fits exactly into the ATP-binding-site. This assumption was confirmed by introduction of an benzo[δ][1,3]dioxole (**9**) which did not display any significant activity on Aβ-levels. This group bears oxygen in the *m*-position, but has an additional methylene bridge to another oxygen in the *p*-position. The activity loss can be explained by sterical hindrance of the dioxole group, which may displace Asp^{154} thus disrupting essential hydrogen-bonds. Secondary, m-substitution is detrimental to activity, this was additionally tested by two fluorines (**10**) in the m-positions, which displayed surprisingly increased $A\beta_{42}$ generation of up to 112% at a concentration of 5 μM. This may be due to inverse modulation; such a switch from straight to inverse γ-secretase modulation on a common scaffold was observed previously.[6,20]

Two additional hydrogen-bonds are formed by the *p*-substituted methoxy-group with Ser^{22} and Asp^{154}. Ser^{22} exerts the role of a gate keeper in CK1: Entry of the ATP in the binding-site causes a conformational change into the closed form by Ser^{22}, which locks the binding-site and prevents ATP-diffusion out of the binding-site. This in turn stabilizes CK1 in the active conformation. The ability of 1 to form a hydrogen-bond with Ser^{22} locks the ATP-binding-site permanently. The position of Ser^{22} is fixed in the closed form of the pocket and thereby prevents the replacement of IC261 by ATP.

The interaction of a *p*-substituent with Ser^{22} was investigated by the introduction of both sterically demanding and functional groups (**11**, **12**).[21–23] These groups should prevent the conformational change into the closed form of the ATP-binding-site. The FDA-approved kinase inhibitor Sunitinib (**16**) was included into the investigation for the same rational. 11 did not show any effect on Aβ levels, but an unexpected inverse modulation of the γ-secretase was observed for **12**, which decreased the $A\beta_{38}$ levels and increased $A\beta_{42}$ levels (Fig. 2). The inverse modulation by **12** as well as **10** indicates the mechanism to be different from total inhibition of Aβ generation. The moderate inhibition of Aβ secretion ($A\beta_{38}$ = 26%, $A\beta_{40}$ = 53%, $A\beta_{42}$ = 26%) by **16** was not observed in the purified γ-secretase assay. This indicates an indirect, potentially upstream mode of action.

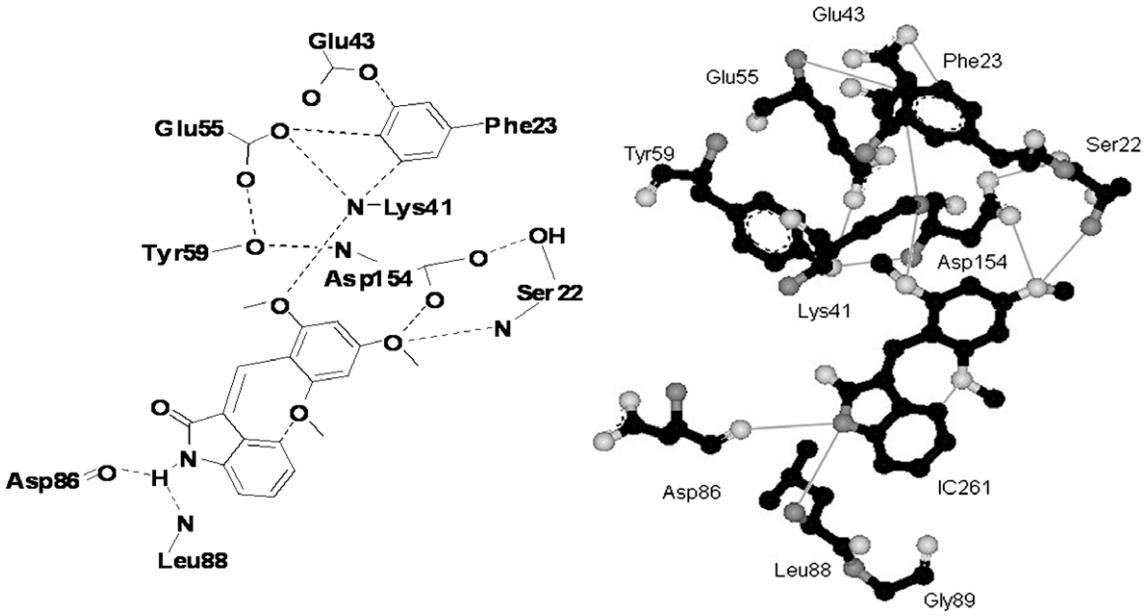


Figure 1. ATP-binding-site interaction of **1** according Mashhoon et al. (PDB: 1EH4).[19]

Table 2
Activity of Aβ generation of oxindoles at a concentration of 10 μM in H4-cells

Entry	Compds	Code	CK1δ (%)	$A\beta_{38}$ (%)	$A\beta_{40}$ (%)	$A\beta_{42}$ (%)	Tox. (μM)	*c* log *P*[c]
1	**1**	BSc3930 IC261	19	55	77	77	40	2.31
2	**5**	BSc3926	n.t.[b]	62	76	65	40	3.12
3	**6**	BSc3921	n.t.[b]	87	88	91	n.t.[b]	3.40
4	**7**	BSc3923	n.t.[b]	94	88	83	n.t.[b]	3.30
5	**8**	BSc3922	n.t.[b]	101	96	88	n.t.[b]	2.40
6	**9**	BSc3928	n.t.[b]	86	93	90	n.t.[b]	3.02
7	**10**	BSc3914	n.t.[b]	81[a]	106[a]	112[a]	n.t.[b]	3.56
8	**11**	BSc3929	n.t.[b]	99	97	97	n.t.[b]	1.73
9	**12**	BSc3944	91	66	93	123	40	3.93
10	**13**	BSc3890	99	27	46	47	20	2.86
11	**14**	BSc3925	n.t.[b]	54	72	65	80	2.86
12	**15**	BSc3927	87	35	65	65	20	3.10
13	**16**	Sunitinib	n.t.[b]	26	53	26	40	2.99

[a] Activity at a concentration of 5 μM.
[b] n.t. = not tested.
[c] Calculated by ChemDraw.

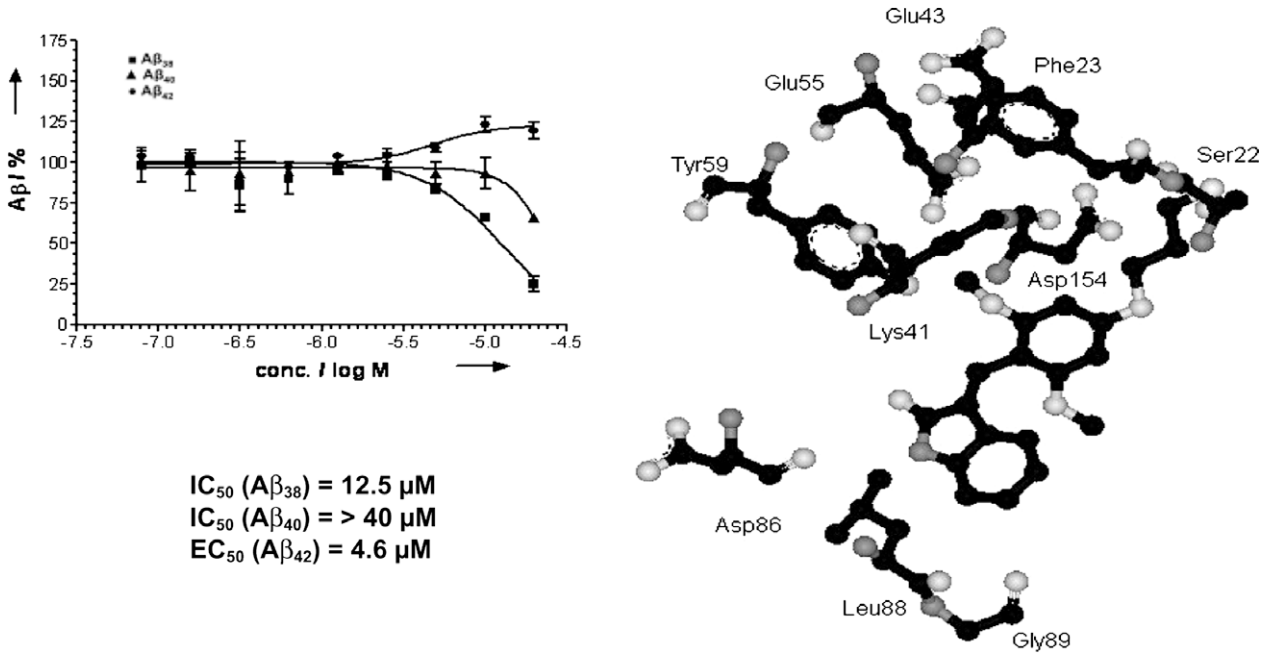


Figure 2. Modelled ATP-binding-site interaction of 12 (based on PDB: 1EH4) and its dose–response curve.

As the IC261 substitution pattern (2,4,6-trimethoxybenzene) was found to be important for the activity, it was systematically varied into 2,3,4- and 3,4,5-trimethoxybenzene (**13**, **14**). These two derivatives also exert a significant reduction in total Aβ. Although the o-positions of **14** ($A\beta_{38}$ = 54%, $A\beta_{40}$ = 72%, $A\beta_{42}$ = 65%) are substituted with hydrogens, it is equipotent to **1** ($A\beta_{38}$ = 55%, $A\beta_{40}$ = 77%, $A\beta_{42}$ = 77%). A distinct rise in activity was observed for **13** ($A\beta_{38}$ = 27%, $A\beta_{40}$ = 66%, $A\beta_{42}$ = 47%) with a methoxy-group in *o*-, *m*- and *p*-position.

The most important interaction was anticipated to be the indole-nitrogen forming two hydrogen-bonds with Asp[86] and Leu[88].[19] This crucial interaction was challenged by a methyl-substitution of the nitrogen, expecting a 90% loss of activity. Surprisingly, a rise in Aβ-inhibitory activity of **15** compared to IC261 was observed. The three Aβ-species were significantly decreased to 35% for $A\beta_{38}$ and to 65% for $A\beta_{40}$ and $A\beta_{42}$ each. Particularly this observation was the first major evidence against the involvement of CK1 in our biological assay.

IC261 and the three most potent derivatives **13**, **15** and **12** were tested for inhibitory activity on CK1δ and 42 other kinases to evaluate the influence of CK1 on γ-secretase (Fig. 3).[24] Surprisingly only IC261 shows a significant inhibition of CK1δ (81% inhibitory activity, Table 1, Fig. 3). As soon as the substitution pattern differs from 2,4,6-trimethoxybenzene, no significant CK1δ inhibition (>80%) was observed. This is shown, for example, in the case of **13** (2,3,4-trimethoxybenzene), which bears a methoxy-group in the m- instead of the second *o*-position. It exerts an inhibition of a mere 1% under the same conditions (Fig. 3). In addition, no significant inhibitory activity on CK1δ was found for **15** and **12**.

Compounds **12** and **13** are inactive on CK1δ yet display significant inhibition (98% and 89%) of the tyrosine kinase Flt3, which plays an important role in leukemia. However, Flt3 is not known to exert effects on APP metabolism.

Only the N-methylated indolinone **15** lacked significant inhibition (inhibitory activity <20%) for all kinases tested, which confirms the relevance of N-methylation of indolinones in kinase

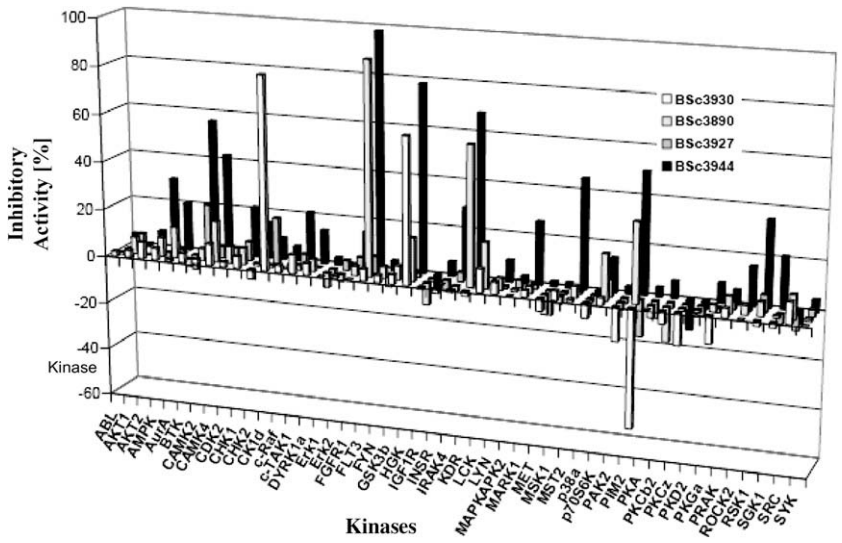


Figure 3. Kinase screening of 43 kinases from compounds **1** (BSc3930), **12** (BSc3944), **13** (BSc3890), **15** (BSc3927). Kinase inhibition is indicated by positive values. Negative values indicate kinase activation.

inhibition. This N–H bond is essential for the inhibition of most kinases by indolinones.

The inhibitory activity of **1**, **12**, **13**, **15** on CK1δ cannot be correlated to the inhibition of γ-secretase activity which is shown in Figure 4 for the $A\beta_{38}$ level reduction at a concentration of 10 μM.

These data agree with three possible explanations: (1) IC261 does not directly affect γ-secretase activity but a degradation product is responsible for γ-secretase inhibition, (2) IC261 is coincidently a weak γ-secretase inhibitor, which independently targets CK1δ or CK1ε activity. (3) Another, yet unidentified kinase is responsible for the cellular activity. To investigate a direct effect on γ-secretase activity, IC261 was tested in a cell-free assay using lipid-reconstituted purified γ-secretase and purified APP C100-His_6 as substrate for Aβ generation,[25] that is, under conditions, where no additional metabolism is present (Fig. 5).

No effect on the Aβ production by γ-secretase was observed at 50 μM, the highest effective concentration used in cultured cells by the study of Flajolet et al.[6] IC261 did also not exert a modulatory effect on the production of $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$ species in the cell-free assay at this concentration. γ-Secretase activity was also not affected at 150 μM IC261, however, γ-secretase inhibition was observed at 250 μM. This may be due to the resemblance of IC261 to known γ-secretase inhibitors derived from NSAIDs, which are characterized by IC_{50}ies around 100–300 μM, for example, **2**.[2,27,28] Consistent with previous results,[25,29] presence of the highly potent GSI L-685,458[26] (structure see Supplementary data) at 0.5 μM completely blocked γ-secretase activity in this assay.

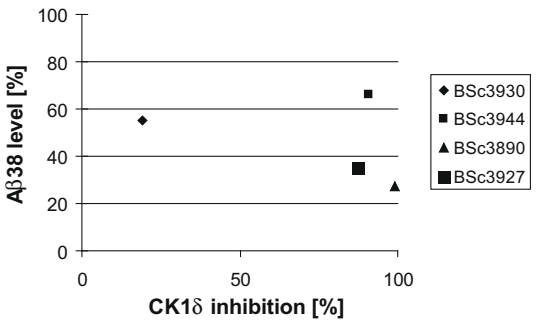


Figure 4. Correlation plot of CK1δ activity inhibition and $A\beta_{38}$ level reduction at a concentration of 10 μM.

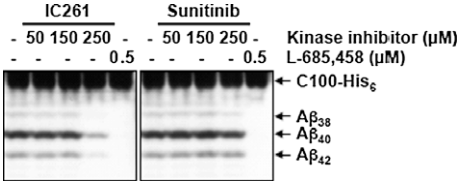


Figure 5. Cell-free γ-secretase assay in the presence of IC261, Sunitinib, or L-685,45826 GSI as control.

The cellular activity of IC261 and its activity in cell-free assays is inconsistent with an exclusively CK1δ mediated effect on Aβ secretion via γ-secretase inhibition but an influence of other CK1 isoforms, for example, CK1ε or by β-secretase could not be excluded. Another potential mechanism, a Notch mediated effect, was excluded by Flajolet et al.[6] The cell-free assay is neither subject to CK1δ or CK1ε dependent regulation, thus a pleiotropic effect is suggested for the interference of Aβ secretion by IC261: (a) cellular γ-secretase inhibition may occur at high concentration of IC261 or through a metabolic activation of IC261. It is not clear, however, whether the interference of IC261 with γ-secretase activity at high concentration (in the cell-free assay at 250 μM, i.e., five times above the concentration used by Flajolet et al.[6] in the cellular assay) does reflect enzyme inhibition. The observed reduction in γ-secretase activity could also be due to a damage of the lipid environment by this particular compound in the in vitro assays; (b) upstream modulation of Aβ metabolism by an unidentified mode of action, which does not necessarily involve CK1δ at low concentrations, which is also confirmed by Flajolet et al. investigation.[6] Sunitinib, a promiscuous kinase inhibitor, was selected for structural similarity to IC261 and submitted to the assay panel. It reduces Aβ-secretion in the cellular assay. However, Sunitinib did not display an effect on Aβ generation in the cell-free assay suggesting that an unidentified kinase may be involved in Aβ-secretion in the cellular assay.

Even a subtle variation of the IC261 substitution pattern resulted in complete loss of CK1 inhibition but still exerts Aβ lowering capability (e.g., **13**).

In conclusion, our IC261 SAR data are inconsistent with CK1-dependent inhibition of γ-secretase. The most potent compounds are not suitable as lead structure for γ-secretase modulation

in vivo, as long as they display significant inhibition of Flt3. Such off-target activity will impair further development.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.110.

References and notes

1. Jakob-Roetne, R.; Jacobsen, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 3030.
2. Beher, D.; Wrigley, J. D.; Owens, A. P.; Shearman, M. S. *J. Neurochem.* **2002**, *82*, 563.
3. Gasparini, L.; Ongini, E.; Wenk, G. *J. Neurochem.* **2004**, *91*, 521.
4. Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Bulter, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*, 212.
5. Weggen, S.; Eriksen, J. L.; Sagi, S. A.; Pietrzik, C. U.; Ozols, V.; Fauq, A.; Golde, T. E.; Koo, E. H. *J. Biol. Chem.* **2003**, *278*, 31831.
6. Flajolet, M.; He, G.; Heiman, M.; Lin, A.; Nairn, A. C.; Greengard, P. *PNAS* **2007**, *104*, 4159.
7. Baumann, S.; Hoettecke, N.; Schubenel, R.; Baumann, K.; Schmidt, B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6986.
8. Cozza, G.; Gianoncelli, A.; Montopoli, M.; Caparrotta, L.; Venerando, A.; Meggio, F.; Pinna, L. A.; Zagotto, G.; Moro, S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5672.
9. Narlawar, R.; Perez Revuelta, B. I.; Baumann, K.; Schubenel, R.; Haass, C.; Steiner, H.; Schmidt, B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 176.
10. Reinecke, D. M.; Ozga, J. A.; Magnus, V. *Phytochemistry* **1995**, *40*, 1361.
11. Narlawar, R.; Perez Revuelta, B. I.; Haass, C.; Steiner, H.; Schmidt, B.; Baumann, K. *J. Med. Chem.* **2006**, *49*, 7588.
12. Bouerat, L. M. E.; Fensholdt, J.; Nielsen, S. F.; Liang, X.; Havez, S. E.; Andersson, E. C.; Jensen, L.; Hansen, J. R. 2005 (Leo Pharma A/S, Den.), WO2005058309, p 198.
13. Yang, C.; Xie, P.; Duan, J.; Miao, H.; Song, X. 1996 (Hematology Institute, Chinese Academy of Medical Sciences, Peop. Rep. China), CN1115640, p 21.
14. Kobayashi, M.; Chen, T. 2007 (Oncorex Co., Ltd, Japan), JP2007084494, p 59.
15. Balderamos, M.; Ankati, H.; Akubathini Shashidhar, K.; Patel Anish, V.; Kamila, S.; Mukherjee, C.; Wang, L.; Biehl Edward, R.; D'Mello Santosh, R. *Exp. Biol. Med. (Maywood)* **2008**, *233*, 1395.
16. Angell, R.; Reynolds, K.; Lumley, J. 2004 (Arrow Therapeutics Limited, UK), WO2004069998, p 67.
17. Zhang, W.; Go, M. L. *Bioorg. Med. Chem.* **2009**, *17*, 2077.
18. Cincinelli, R.; Cassinelli, G.; Dallavalle, S.; Lanzi, C.; Merlini, L.; Botta, M.; Tuccinardi, T.; Martinelli, A.; Penco, S.; Zunino, F. *J. Med. Chem.* **2008**, *51*, 7777.
19. Mashhoon, N.; DeMaggio, A. J.; Tereshko, V.; Bergmeier, S. C.; Egli, M.; Hoekstra, M. F.; Kuret, J. *J. Biol. Chem.* **2000**, *275*, 20052.
20. Narlawar, R.; Pickhardt, M.; Leuchtenberger, S.; Baumann, K.; Krause, S.; Dyrks, T.; Weggen, S.; Mandelkow, E.; Schmidt, B. *ChemMedChem* **2008**, *3*, 165.
21. Apodaca, R.; Dvorak, C. A.; Xiao, W.; Barbier, A. J.; Boggs, J. D.; Wilson, S. J.; Lovenberg, T. W.; Carruthers, N. I. *J. Med. Chem.* **2003**, *46*, 3938.
22. Ekambome Bassene, C.; Suzenet, F.; Hennuyer, N.; Staels, B.; Caignard, D.-H.; Dacquet, C.; Renard, P.; Guillaumet, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4528.
23. Mahindroo, N.; Wang, C.-C.; Liao, C.-C.; Huang, C.-F.; Lu, I. L.; Lien, T.-W.; Peng, Y.-H.; Huang, W.-J.; Lin, Y.-T.; Hsu, M.-C.; Lin, C.-H.; Tsai, C.-H.; Hsu, J. T. A.; Chen, X.; Lyu, P.-C.; Chao, Y.-S.; Wu, S.-Y.; Hsieh, H.-P. *J. Med. Chem.* **2006**, *49*, 1212.
24. Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science* **2002**, *298*, 1912.
25. Winkler, E.; Hobson, S.; Fukumori, A.; Dumpelfeld, B.; Luebbers, T.; Baumann, K.; Haass, C.; Hopf, C.; Steiner, H. *Biochemistry* **2009**, *48*, 1183.
26. Shearman, M. S.; Beher, D.; Clarke, E. E.; Lewis, H. D.; Harrison, T.; Hunt, P.; Nadin, A.; Smith, A. L.; Stevenson, G.; Castro, J. L. *Biochemistry* **2000**, *39*, 8698.
27. Fraering, P. C.; Ye, W.; Strub, J. M.; Dolios, G.; LaVoie, M. J.; Ostaszewski, B. L.; van Dorsselaer, A.; Wang, R.; Selkoe, D. J.; Wolfe, M. S. *Biochemistry* **2004**, *43*, 9774.
28. Takahashi, Y.; Hayashi, I.; Tominari, Y.; Rikimaru, K.; Morohashi, Y.; Kan, T.; Natsugari, H.; Fukuyama, T.; Tomita, T.; Iwatsubo, T. *J. Biol. Chem.* **2003**, *278*, 18664.
29. Li, Y. M.; Lai, M. T.; Xu, M.; Huang, Q.; DiMuzio-Mower, J.; Sardana, M. K.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. *PNAS* **2000**, *97*, 6138.