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Synthesis and biological evaluation of glycogen synthase kinase 3 (GSK-3) inhibitors: An fast and atom efficient access to 1-aryl-3-benzylureas

Fabio Lo Monte [a,†], Thomas Kramer [a,†], Alexander Boländer [a], Batya Plotkin [b], Hagit Eldar-Finkelman [b], Ana Fuertes [c], Juan Dominguez [c], Boris Schmidt [a,*]

[a] Clemens Schöpf—Institute of Organic Chemistry and Biochemistry, Technische Universität Darmstadt, 64287 Darmstadt, Germany
[b] Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel
[c] Noscira, Tres Cantos, 28760 Madrid, Spain

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ABSTRACT

The glycogen synthase kinase 3 (GSK-3) is implicated in multiple cellular processes and has been linked to the pathogenesis of Alzheimer's disease (AD). In the course of our research topic we synthesized a library of potent GSK-3 inhibitors. We utilized the urea scaffold present in the potent and highly selective GSK-3 inhibitor AR-A014418 (AstraZeneca). This moiety suits both (a) a convergent approach utilizing readily accessible building blocks and (b) a divergent approach based on a microwave heating assisted Suzuki coupling. We established a chromatography-free purification method to generate products with sufficient purity for the biological assays. The structure–activity relationship of the library provided the rationale for the synthesis of the benzothiazolylurea **66** (IC_{50} = 140 nM) and the pyridylurea **62** (IC_{50} = 98 nM), which displayed two to threefold enhanced activity versus the reference compound **18** (AR-A014418: IC_{50} = 330 nM) in our assays.

Alzheimer's disease (AD) is a neurodegenerative disorder defined by progressive memory loss and cognitive impairment.[1] The definite diagnosis of AD is possible postmortem only. It is based on the presence of extracellular plaques of β-amyloid (Aβ), and intracellular neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein.[2] Glycogen synthase kinase 3 (GSK-3) interacts with several neuronal proteins that are directly linked to AD.[3] There are two GSK-3 genes, GSK-3α and GSK-3β. Both are ubiquitously expressed and constitutively active proline-directed serine/threonine kinases.[1] Several GSK-3 inhibitors have been studied in kinase assays and cellular test panels. Among these inhibitors are paullones, indirubin, aminomaleimides and other small molecules. The potent and specific GSK-3 inhibitor AR-A014418 (**18**) was reported by AstraZeneca and its interactions with the essential amino acids of GSK-3β are denoted in Figure 1.[4]

AR-A014418 (**18**) is selective against Cdk2, Cdk5 and 26 other kinases tested. It inhibits GSK-3 activity with a strongly assay dependent IC_{50}, the highest activity was reported as IC_{50} = 104 nM.[4] AR-A014418 constitutes a lead compound in our kinase inhibitor programe targeting neurodegenerative diseases.[5]

There is a need of a robust and inexpensive approach to AR-A014418 (**18**), suitable for combinatorial chemistry, which provides straightforward access to related ureas displaying enhanced GSK-3 inhibition. The generation of chemical diversity and the limitation by commercial building blocks stimulated a search for mild reaction conditions, rapid purification strategy and access to the reactive intermediate isocyanates without destillation.[6] The two step protocol utilized the in situ generation of

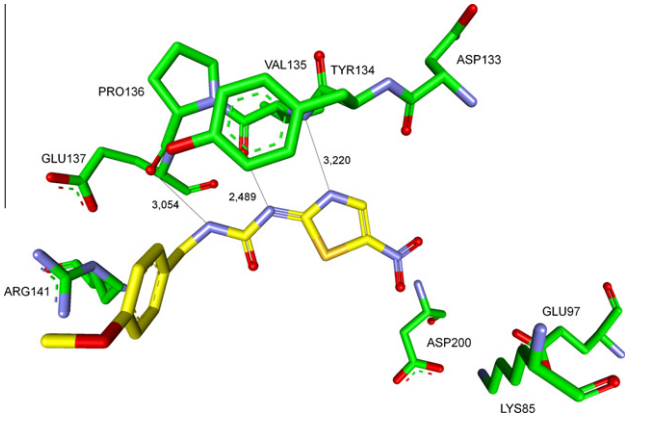

Figure 1. Potent and highly selective GSK-3 inhibitor AR-A014418 (AstraZeneca) in the ATP binding pocket of GSK-3β; important protein–inhibitor interactions are shown. The distance is denoted in Å. PDB code 1Q5K (Accelrys Discovery Studio Visualizer 2.5).

* Corresponding author. Tel.: +49 6151 164531; fax: +49 6151 163278.
E-mail address: schmidt_boris@t-online.de (B. Schmidt).
† These authors contributed equally to this work.

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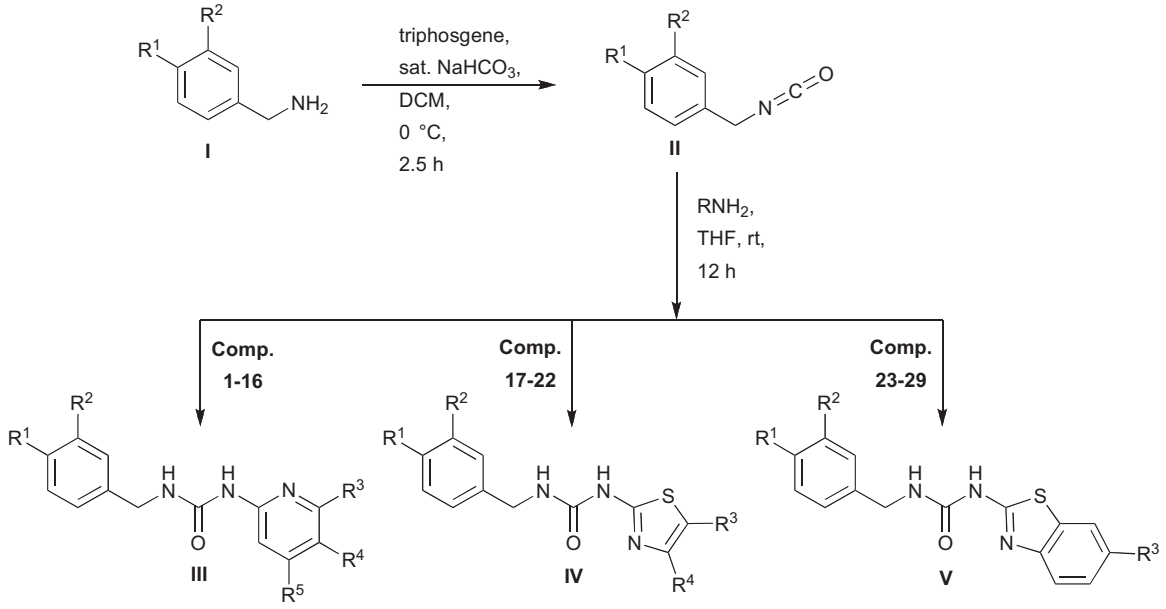


Scheme 1. Mild synthesis and rapidly purified ureas.

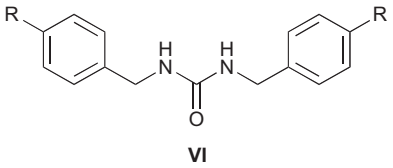
Table 1
Mild synthesis provides crystalline ureas free of chromatography

Compd	R^1	R^2	R^3	R^4	R^5	Yield[a] (%)	*c* Log *P*[c]
1	F	H	H	I	H	11	3.74
2	F	H	Br	H	H	52	3.48
3	MeO	H	Br	H	H	41	3.25
4	F	H	H	Br	H	54	3.48
5	MeO	H	H	Br	H	48	3.25
6	MeO	H	H	H	Br	75	3.25
7	F	H	H	H	Cl	31	3.33
8	MeO	H	H	H	Cl	34	3.10
9	MeO	H	H	H	CN	63[b]	2.00
10	F	H	H	NO_2	H	21[b]	2.51
11	MeO	H	H	NO_2	H	13[b]	2.28
12	F	H	H	CN	H	63[b]	2.22
13	MeO	H	H	CN	H	58[b]	2.00
14	Br	H	H	CN	H	34[b]	2.94
15	OCF_3	H	H	CN	H	13[b]	3.10
16	$-OCH_2O-$	H	CN	H	19[b]	2.04	
17	F	H	NO_2	H	—	21[b]	2.34
18	MeO	H	NO_2	H	—	26[b]	2.11
19	F	H	COOEt	Me	—	35[b]	3.02
20	MeO	H	COOEt	Me	—	24[b]	2.80
21	F	H	H	4-Me-Ph	—	29[b]	5.00
22	MeO	H	H	4-Me-Ph	—	46[b]	4.77
23	F	H	Br	—	—	24[b]	4.87
24	MeO	H	F	—	—	52[b]	3.92
25	F	H	CF_3	—	—	31[b]	4.96
26	MeO	H	CF_3	—	—	50[b]	4.74
27	MeO	H	NO_2	—	—	26[b]	3.64
28	F	F	NO_2	—	—	36[b]	3.93
29	MeO	H	CN	—	—	70[b]	3.34

[a] Yields of analytically pure products; products recrystallized from methanol or ethanol.
[b] Amines preactivated with *n*-BuLi.
[c] Determined by CS ChemOffice 2008.

isocyanates from benzylamine and triphosgene. These crude intermediates were dissolved in dry *N*,*N*-dimethylformamide or dry tetrahydrofurane and reacted with nonactivated or *n*-buthyllithium (*n*-BuLi) activated amines (Scheme 1 and Table 1).

Initially, the reaction of aminopyridines was dominated by the dimerization of benzylamines. This resulted in the isolation of known benzylamine adducts **30** and **31** (Scheme 2 and Table 2).[7,8]



Scheme 2. Commonly observed dimerization product.

Table 2
Dimerization derived products

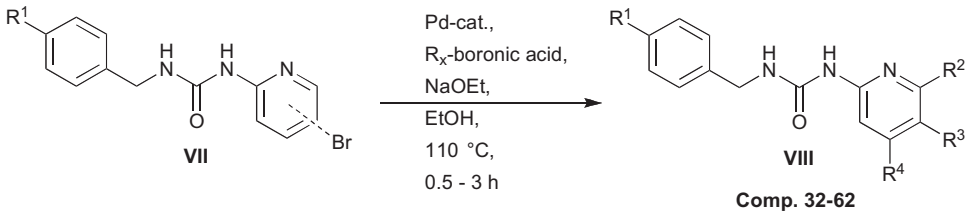
Compd	R	Yield[a] (%)	*c* Log *P*[b]
30[6,7]	4-F	37	3.17
31[6]	4-MeO	43	2.72

[a] Yields of analytically pure products; products recrystallized from methanol.
[b] Determined by CS ChemOffice 2008.

Using *n*-BuLi for the deprotonation of the heterocyclic amines solved this problem. Furthermore, high-pressure liquid chromatography (HPLC) controls showed that the *n*-BuLi preactivated amines reacted with the isocyanates immediately, but nevertheless the reactions were stirred 12 h at room temperature. Afterward the reaction solution was suspended in vigorously stirred water and precipitation occured promptly. The purification of the crude urea was achieved by filtration and recrystallization. These reaction and purification conditions were standardized for a variety of amines, including benzylamines, aminopyridines and aminothiazoles.

AR-A014418 (**18**) was synthesized without heating and chromatographic purification, which were employed by AstraZeneca 2003.[4] The initial yields of the standard methodology were moderate at best, but they did not require chromatography and the yield of compound **6** obtained by the standard protocol was optimized in scaled up experiments to 75%. Thus the atom efficiency of the total process, which includes the purification, is actually sufficiently high as it produces very little waste material.

The required diversity was introduced by the Suzuki-coupling of arylbromides and boronic acids (Scheme 3 and Table 3).



Scheme 3. Suzuki reactions.

Table 3
Urea enlargement supported by Suzuki reaction

Compd	R^1	R^2	R^3	R^4	Yield[a] (%)	*c* Log *P*[d]
32	F	Ph	H	H	40[b]	4.58
33	MeO	Ph	H	H	71[b]	4.36
34	F	3-F-Ph	H	H	29[b]	4.75
35	F	3-MeO-Ph	H	H	31[b]	6.37
36	MeO	3-MeO-Ph	H	H	34[b]	6.13
37	F	3-BnO-Ph	H	H	51[b]	6.37
38	F	4-Py	H	H	67[b]	3.26
39	MeO	4-Py	H	H	50[b]	3.04
40	F	H	Ph	H	70[c]	4.37
41	MeO	H	Ph	H	47[c]	4.15
42	F	H	4-Me-Ph	H	34[c]	4.87
43	MeO	H	4-Me-Ph	H	32[c]	4.65
43	MeO	H	4-Me-Ph	H	32[c]	4.65
44	F	H	3-F-Ph	H	68[c]	4.54
45	MeO	H	3-F-Ph	H	33[c]	4.32
46	F	H	3-MeO-Ph	H	37[c]	4.39
47	MeO	H	3-MeO-Ph	H	34[c]	4.17
48	F	H	3-Isopro-poxy-Ph	H	43[c]	5.23
49	MeO	H	3-Isopro-poxy-Ph	H	61[c]	5.01
50	F	H	3-BnO-Ph	H	26[c]	6.16
51	MeO	H	3-BnO-Ph	H	19[c]	5.94
52	MeO	H	3-CF_3-Ph	H	27[c]	5.07
53	F	H	2,3-di-F-Ph	H	83[c]	4.62
54	MeO	H	2,3-di-F-Ph	H	26[c]	4.40
55	F	H	4-Vinyl-Ph	H	85[c]	5.09
56	MeO	H	4-Vinyl-Ph	H	87[c]	4.87
57	MeO	H	H	Ph	77[b]	4.15
58	MeO	H	H	3-F-Ph	57[b]	4.32
59	MeO	H	H	3-MeO-Ph	62[b]	4.17
60	MeO	H	H	2-MeO-Ph	47[c]	3.61
61	MeO	H	H	2-Thio-phen	33[c]	4.06
62	MeO	H	H	2-F-Py	67	3.03

[a] Yields of analytically pure products; products were recrystallized from methanol.
[b] $Pd(OAc)_2$.
[c] $Pd(PPh_3)_4$.
[d] Determined by CS ChemOffice 2008.

The Suzuki coupling of phenylboronic acids and brominated arylureas was catalyzed by $Pd(OAc)_2$ under standard conditions or subtle modifications thereof.[9,10] Some reactions required catalyst exchange to $Pd(PPh_3)_4$. The boronic acids were activated by sodium ethanolate. The starting materials were suspended in dry ethanol and the reactions were carried out in a sealed, argon flushed vessel. Again, the crude products of the Suzuki reaction were purified by atom efficient recrystallization.

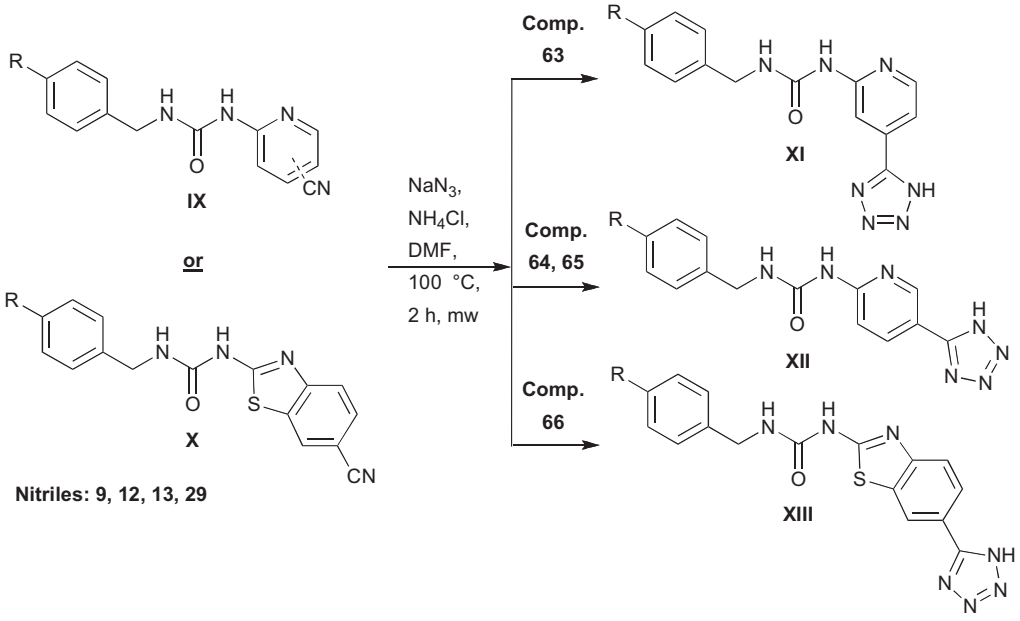
Finally, we obtained four tetrazole derivatives (**63–66**) using microwave radiation in a Biotage Initiator 300. These products were purified by recrystallization and isolated in very good yields requiring no further optimization at this stage (Scheme 4 and Table 4), although the reaction conditions can be improved by using ionic liquids.[11] Be careful while working with azides, they can be explosive and should be handled with great care. During our study we encountered no adverse events.[12]

The arylureas were evaluated for their inhibition of GSK-3 activity. Several compounds were identified to reduce in vitro GSK-3β activity beneath 50% at a concentration of 10 μM (Table 5).[13]

Two derivatives (**66** and **62**) are more potent than our reference compound AR-A014418 (**18**). Five derivatives (**29**, **63**, **11**, **60, and 13**) display comparable inhibitory activity and four of them are not associated with toxicity alerts (Fig. 2). It should be noted that the tetrazoles, for example, compound **63** with a topological polar surface area of 111.83 Å (ChemOffice2008) may not permeate the blood brain barrier.

A docking study of compound **66** and PDB structure 1Q5K of GSK-3β suggested a binding mode along the hinge region of the ATP-binding pocket approximately like AR-A014418 (**18**) (Fig. 3).[4,14] There are two hydrogen bond interactions between **66** and the amino acid Glu137. Furthermore we assume two other interactions, one between the Asp133 carbonyl and a hydrogen of the phenyl group and the other between the tetrazol moiety and the polar pocket consisting of Lys85, Glu97 and Asp200. We hypothesize that the latter interaction is the reason for the enhanced potency of derivative (**66**).

Via the expeditious structure–activity relationship (SAR) we ascertained that the aminothiazole, used by AstraZeneca, can be



Scheme 4. Microwave reactions.

Table 4
Urea aryl tetrazole formation by microwave reaction

Compd	R	Yield[a] (%)	*c* Log *P*[b]
63	MeO	87	1.75
64	F	81	1.98
65	MeO	88	1.75
66	MeO	91	3.26

[a] Yields of analytically pure products; products recrystallized from methanol.
[b] Determined by CS ChemOffice 2008.

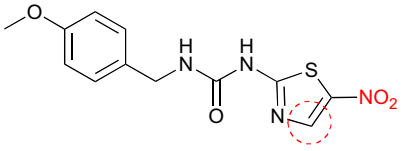
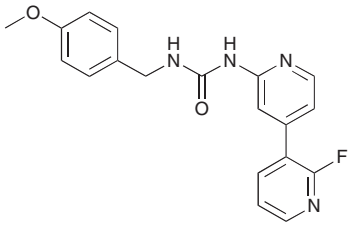
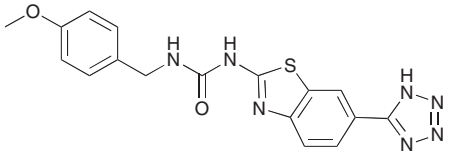
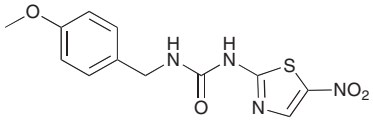


Figure 2. Potential toxic alerts: 1. Nitro group; 2. P450 associated-hepatotoxicity alert.

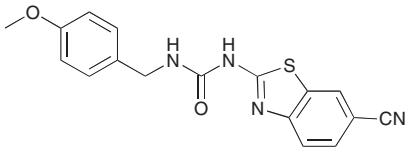
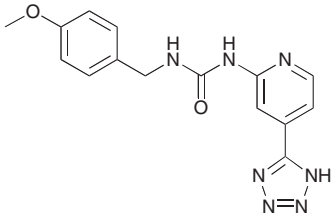
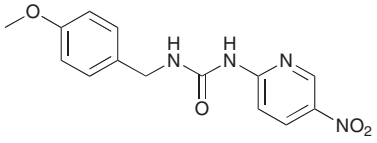
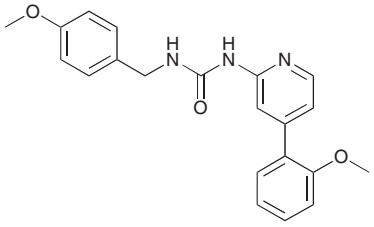
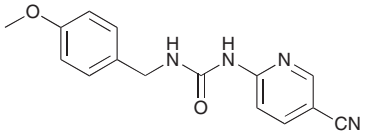
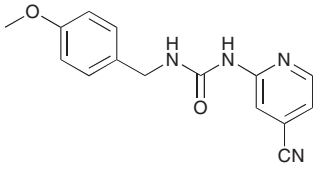
replaced by aminopyridines and aminobenzothiazoles. We found that an elongation in position R^3 and R^4 of structure **III** entailed reduced activity. However, an acceptor like NO_2 or CN is needed in position R^4 of structure **III** as long as no acceptor is allocated in position R^5. Our future research is focused on structure **V** and position R^5 of structure **III** from which we expect to obtain improved activity.

Table 5
GSK-3β inhibitory activity of selected compounds

Compd	Structure	GSK-3β activity in[a] (%)	
		1 μM of compd	10 μM of compd
62		–	12
66		69	18
18		55	20

(*continued on next page*)

Table 5 (*continued*)

Compd	Structure	GSK-3β activity in[a] (%)	
		1 μM of compd	10 μM of compd
29		57	24
63		82	24
11		75	31
60		91	32
13		79	40
9		101	42

[a] Conditions for GSK-3β in vitro assay see Supplementary data.[10]

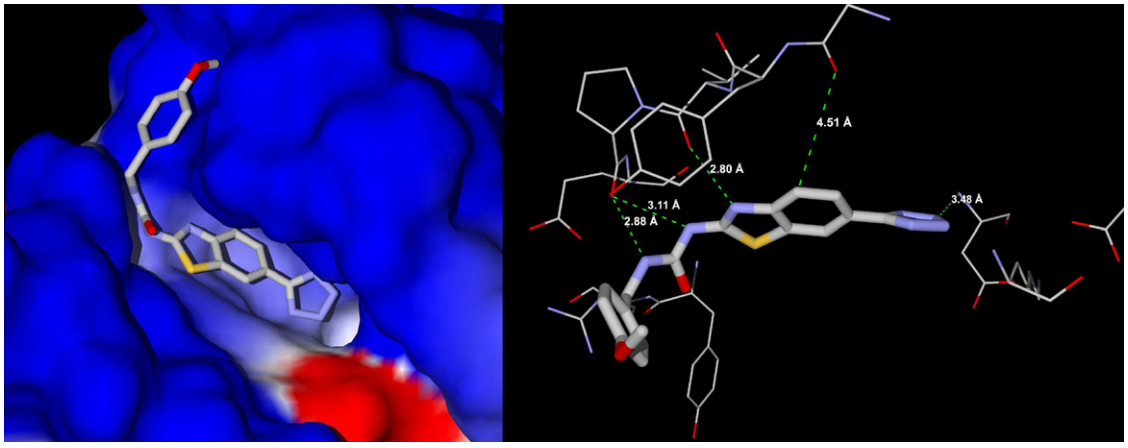


Figure 3. Docking of compound **66** into PDB structure 1Q5K of GSK-3β; Surface illustration of the ATP-binding pocket with **66** (left); Hydrogen bond interactions of **66** with the amino acids of the ATP-binding pocket (right).[4,14]

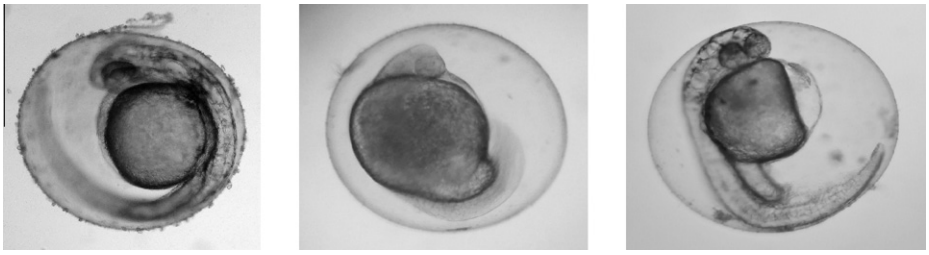


Figure 4. Exposure of zebrafish embryos to 1% DMSO (left), 100 mM **18** (AR-A14418, middle), and 100 mM **66** (right). The embryos were collected and maintained in E2 medium at 30 °C, compounds were added at 8–15 somites, and the phenotypes were compared after 25 h.

Table 6
GSK3 selectivity panel

Compound	IC_{50} values (mM)					
	GSK-3β	GSK-3α	Cdk5/p35	CKIε	AurKA	PKCα
18 (AR-A014418)	0.33	0.07	>100	>100	>100	>100
9	1.43	1.12	>100	>100	>100	>100
27	3.64	0.22	>100	>100	>100	>100
63	0.51	0.37	>100	>100	>100	>100
66	0.14	0.13	26.4	15.3	4.8	24.2

To evaluate the specificity of the family against GSK-3β, four of the synthesized compounds (**9**, **27**, **63** and **66**) were selected and tested against five human protein kinases (GSK-3α, PKCα, AurKA, Cdk5/p35 and CKIε) (Table 6).

Compound **66** with an IC_{50} of 140 nM for GSK3β was the only one able to inhibit all of the kinases tested (PKCα, AurKA, Cdk5/p35 and CKIε) but the IC_{50} values were one or two orders of magnitude above those found for the GSK-3 isoforms, indicating a fair degree of selectivity for this compound towards these latter enzymes. Such selectivity is even larger for the other three compounds tested, which were inactive (or poorly active) against the former kinases when tested up to 100 μM.

In order to demonstrate the utility and nontoxicity of the pyridine and benzothiazole moiety in whole organisms, we performed a zebrafish embryo phenotype assay.[15,16] We exposed the zebrafish embryos to the compounds **66**, **18** (AR-A014418) and **63** at early stages of development (8 till 15 somites, Fig. 4).

Controls after 18 h and 25 h showed that **18** (AR-A014418) is indeed toxic. This toxicity could be diminished by replacement of the thiazole with a benzothiazole (**66**) or pyridine (**63**) moiety (CHIR98014 is not toxic and compound **19** precipitates—data not shown). We observed some deformation in the fishtail at 100 μM (**66**, **63** and at 25 μM of CHIR98014—data not shown) and after eclosion we observed these fishes were swimming in circles only. This correlates with the observation that Wnt signaling, and thus GSK-3β, plays a crucial role in the development of metazoan and that known GSK-3 inhibitors like LiCl and the ruthenium complex (**R**)-**7** perturbe the zebrafish development.[15]

We have established a fast and atom efficient approach to generate kinase targeting urea derivatives. Hereby, it is feasible to diversify the scaffold in a time efficient manner. Thereby we expect to identify novel GSK-3 inhibitors displaying enhanced activity and selectivity. A preview of the biological activity and selectivity of the synthesized compounds is depicted in Tables 5 and 6. They are of comparable potency and selectivity to the reference compound **18** (AR-A014418). The aminothiazole used by AstraZeneca was replaced by other heterocycles. We have also shown that a selection of our compounds compared to **18** (AR-A014418) were less toxic in a zebrafish embryo phenotype assay, albeit some of these are not expected to be brain penetrant. This is subject of ongoing and future research in animal models of Alzheimer's disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.131.

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