Synthesis and in Vitro Characterization of 1-(4-Aminofurazan-3-yl)-5-dialkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Derivatives. A New Class of Selective GSK-3 Inhibitors

Preben H. Olesen,* Anders R. Sørensen, Birgitte Ursø, Peter Kurtzhals, Andrew N. Bowler, Ulrich Ehrbar, and Bo F. Hansen

Discovery, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

Received November 19, 2002

A novel class of GSK-3 inhibitors with favorable water solubility was identified in a HTS screen. SAR studies identified bioisosteric structural moieties in this class of compounds. The compounds were tested in a GSK-3 inhibition assay at 100 μM ATP giving IC_{50}'s in the range from 0.1 to 10 μM. The compounds are ATP competitive inhibitors. They modulate glycogen metabolism and stimulate the accumulation of intracellular β-catenin in whole cell assays with EC_{50}'s in the range from 2 to 18 μM and 4.5−44 μM, respectively. For selected compounds, only a 10-fold lower potency was obtained in cellular assays compared to the potency obtained for inhibition of the isolated enzyme, reflecting a good cell permeability of this compound class. At 10 μM of test compound a 3-fold stimulation of the glycogen synthesis in rat soleus muscle was obtained compared to the level of glycogen synthesis observed at 0.2 nM insulin. This stimulation of glycogen synthesis is comparable to the maximal stimulation by insulin itself.

Introduction

Glycogen synthase kinase-3 (GSK-3) is a protein-serine kinase implicated in the hormonal control of several regulatory proteins. It was first discovered by virtue of its ability to phosphorylate and inactivate glycogen synthase, the regulatory enzyme of glycogen synthesis in mammals. Since then a number of other substrates have been identified, implicating the enzyme in the regulation of several physiological processes.[1−3] Recently a number of papers have appeared suggesting GSK-3 as a target for treatment of type 2 diabetes[2] and Alzheimers disease.[4]

GSK-3 is a key negative regulator of insulin signaling, and upregulation of this enzyme has been associated with insulin resistance and diabetes in both rodents and humans.[5,6] Liver and muscle glycogen synthesis is defective in patients with type 2 diabetes; therefore, activation of glycogen synthase by inhibiting GSK-3 represents a potential new therapeutic target for the treatment of type 2 diabetes.[7] The effect of a novel GSK-3 inhibitor, CHIR98023, on insulin-stimulated glucose metabolism in ZDF rats has recently been described.[8] The observation that GSK-3 inhibition significantly improved oral glucose disposal, mostly by increasing liver glycogen synthesis, further provide support for GSK-3 as a new therapeutic target in the treatment of patients with type 2 diabetes.

A number of different chemical compounds have been identified as inhibitors of GSK-3, e.g., indirubins,[9] paullones,[10] maleimides derivatives,[1] e.g., SB-415286[11] and the marine sponge hymenialdisine,[12] Figure 1. Furthermore, two new classes of compounds, diaminothiazoles and oxalylpyridines, with inhibitory activity for GSK-3, have previously been described from our laboratory.[13,14] Several of these novel classes of GSK-3 inhibitors has recently been reviewed.[15] These structurally distinct compound classes show varying degrees of kinase specificity, but in particular the simultaneous inhibition of cyclin-dependent kinase-2 (CDK-2) and

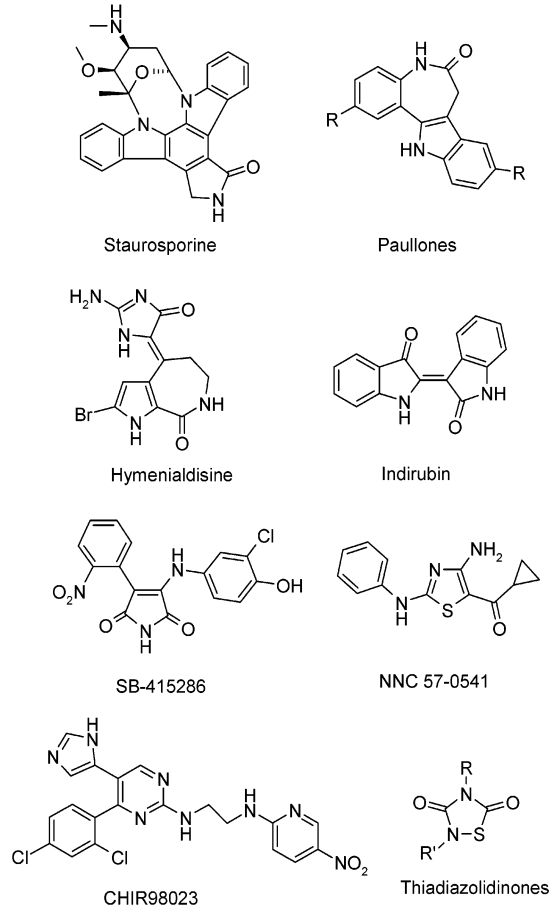
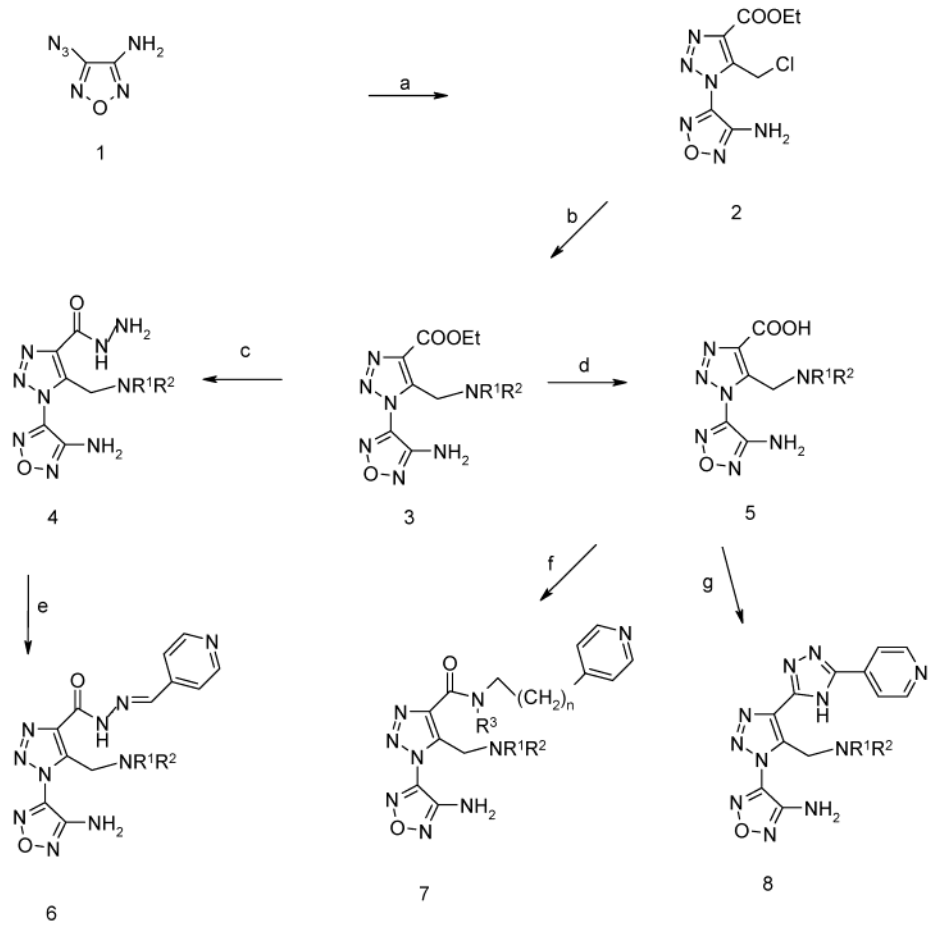


Figure 1. GSK-3 inhibitors.

* Preben H. Olesen, Novo Nordisk Park, 2760 Måløv, Denmark. Telephone +45 44434886. Fax: +45 44434547. e-mail: phol@novonordisk.com.

10.1021/jm021095d CCC: $25.00
Published on Web 06/10/2003

Scheme 1[a] Synthesis of 1-(4-Aminofurazan-3-yl)-5-dialkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Derivatives



[a] (a) Ethyl chloroacetacetic acid in ethanol; (b) alkylamine in ethanol; (c) hydrazine in ethanol; (d) 1 N hydrochloric acid; (e) 4-formylpyridine in ethanol; (f) (i) EDC/HOBT in DMF, (ii) 4-(aminoalkyl)pyridine; (g) (i) carbonyldiimidazole in THF, (ii) pyridine-4-carboxylic acid amidrazone[19] in THF. For R^1, R^2, and R^3 please refer to Table 1, 2, and 3.

GSK-3 has been difficult to avoid using the hitherto published compounds. All of the above-mentioned compounds have been classified as ATP-competitive inhibitors, but recently a new class of compounds, the thiadiazolidinones (TDZD), has been described as non-ATP competitive GSK-3 inhibitors.[16] Although not as potent as some of the ATP-competitive inhibitors, they have been shown to be very selective for the inhibition of GSK-3 compared to four other protein kinases.

The main challenge identifying new kinase inhibitors is related to achieving kinase specificity. To identify such compounds, the Novo Nordisk library of compounds was screened in a SPA-based high throughput assay (HTS). Further optimization and SAR evaluation of one of the primary hit compounds, identified in this screen, afforded a novel class of compounds with desirable water solubility and a remarkable selectivity for GSK-3.

Chemistry

The route used to synthesize the target compounds is outlined in Scheme 1. Treatment of 3-azido-2-amino-1,2,5-oxadiazole **1**[17,18] with ethyl chloro acetacetic acid gave access to the functionalized [1,2,3]-triazoles **3**. To preclude the purification of the reactive chloromethyl intermediate **2**, the [1,2,3]-triazoles **3** was prepared by a one-pot procedure using an excess of the appropriate amine.

The ester functionality of compound **3** was readily transformed into the hydrazide derivatives **4**, after treatment with hydrazine in ethanol. Condensation of the hydrazide derivatives with the appropriate aldehyde afforded the HTS hit compound **6a** and several analogues.

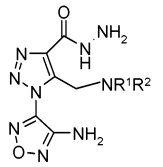
Hydrolysis of the ester functionality of compounds **3** using acidic conditions resulted in the carboxylic acid derivatives **5**. The bioisosteric derivatives of compounds **6** were prepared from these carboxylic acid derivatives after activation with carbodiimide and treatment with the appropriate amine to give amide derivatives of type **7** or activation with carbonyldiimidazole and reaction with amidrazones[19] to give the [1,3,4]-triazole derivatives of type **8**.

Results and Discussion

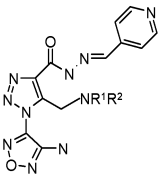
Inhibition of GSK-3 by test compounds was evaluated using human GSK-3β and a glycogen synthase derived substrate, GS2, with the following amino acid sequence YRRAAVPPSPSLSRHSSPHQS(PO_4)EDEEE-NH_2. The compounds were assayed in the presence of 100 μM ATP.

Several ester derivatives **3**, hydrazines **4**, and carboxylic acids **5** were tested in the primary GSK-3 kinase inhibition assay. However, only compounds of type **4**

Table 1. Inhibition of GSK-3 by Hydrazide Derivatives



compound	R^1	R^2	IC_{50},[a] μM
4a	Et	Et	17
4b	-$(CH_2)_5$-		9.6



compound	R^1	R^2	IC_{50}[a] μM
6a	Et	Et	0.41 ± 0.06
6b	-$(CH_2)_5$-		0.10 ± 0.01
6c	-$(CH_2)_4$-		0.10 ± 0.03
staurosporine			0.16 ± 0.05
SB-415286			0.38 ± 0.06

[a] IC_{50} values for inhibition of GSK-3 calculated from three determinations in duplicate. For compound **4a** and **4b**: $n = 1$.

(Table 1) showed minor activity in the GSK-3 assay, indicating the unsubstituted amide functionality as an important factor for inhibitory activity.

In the initial SAR optimization of the hit compound, **6a**, a considerable number of derivatives of type **6** were prepared using commercial by available aromatic and heteroaromatic aldehydes. Data (not shown) obtained from this rapid screen of a large number of compounds supported that the 4-pyridyl moiety was crucial for the inhibition of the GSK-3 enzyme. Replacement of the 4-pyridyl moiety with other aromatic or heteroaromatics resulted in compounds with little or no inhibitory activity.

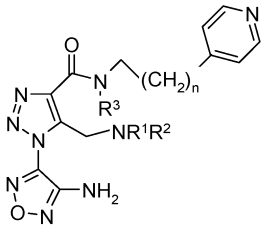
Further optimization of the compounds was focused on the synthesis of derivatives with modification of the aminomethyl substituent ($CH_2NR_1R_2$) and the preparation of compounds with bioisosteric replacements for the potential toxic hydrazide moiety in compound **6a**.

As listed in Table 1, replacement of the diethylamine substituent in **6a** with the ring closed analogues, piperidine **6b** and the pyrrolidine **6c**, increased the inhibitory activity for the compounds by a factor 5 and the potency for these compounds is in the same range as found for staurosporine (0.1−0.2 μM).

The amide derivatives, compound class **7**, showed the same pattern in potency gain after cyclisation of the diethylamino substituent **7a** to the ring-closed analogues **7b** and **7c** (Table 2). The potency of the amides was in general 10 times lower than the potency of the hydrazides comparing compounds **6 a**−**c** with compounds **7 a**−**c**.

Bioisosteric replacement of the amide functionality with a triazole was attempted. This replacement resulted in compound with potency in the same range as the hit compound (**8a** and **8b**, $IC_{50} = 0.25$ μM). In this compound class an even higher difference in potency was observed between the ring open dimethylamino **8d**

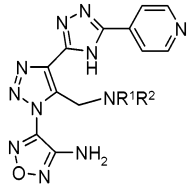
Table 2. Inhibition of GSK-3 by Amide Derivatives



compound	R^1	R^2	R^3	n	IC_{50},[a] μM
7a	Et	Et	H	1	11.7 ± 1.6
7b	-$(CH_2)_5$-		H	1	1.16 ± 0.20
7c	-$(CH_2)_4$-		H	1	2.10 ± 0.14
7d	-$(CH_2)_5$-		H	0	9.30 ± 2.36
7e	-$(CH_2)_5$-		$-CH_3$	1	>250
7f	H	isobutyl	H	0	>250

[a] IC_{50} values for inhibition of GSK-3 calculated from three determinations in duplicate.

Table 3. Inhibition of GSK-3 by Triazole Derivatives



compound	R^1	R^2	IC_{50},[a] μM
8b	-$(CH_2)_5$-		0.28 ± 0.05
8c	-$(CH_2)_4$-		0.24 ± 0.08
8d	$-CH_3$	$-CH_3$	6.92 ± 1.28

[a] IC_{50} values for inhibition of GSK-3 calculated from three determinations in duplicate.

and the ring closed analogues **8b**,**c**, Table 3. The data indicate that some steric bulk is important for activity but also show that the dialkylamino substituent has to be constrained. In line with this observation, the introduction of a monosubstituted alkylamine **7f** was detrimental for the inhibitory activity and resulted in an inactive compound.

Because of its ready availability, the amide series of compounds was used to explore the constraints for the carboxylic acid functionality. The importance of the unsubstituted amide functionality was confirmed by compound **7e.** Converting the monosubstituted amide functionality in compound **7b** to the *N*,*N*-disubstituted functionality in compound **7e** resulted in a total loss of inhibitory activity. Decreasing the carbon chain spacer from two carbons in **7b** to a single carbon **7d** results in a 10-fold decrease in activity, Table 2. This implies that the distance from the carbonyl oxygen to the nitrogen in the 4-pyridyl moiety compound is important for inhibition of GSK-3.

To address the mechanism of action for the compound class, compound **6a** was tested at ATP concentrations ranging from 5 μM to 100 μM while keeping the substrate concentration constant at 30 μM. The right shift of the dose−response curves with increasing ATP concentration clearly indicate that the compound interact with the enzyme in an ATP dependent manner, Figure 2. Only a limited shift in the dose−response curve was observed varying the substrate concentration keeping the ATP concentration constant at 100 μM,

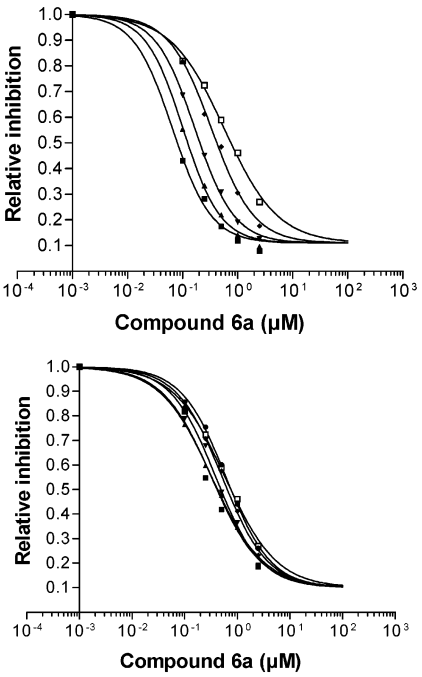


Figure 2. ATP dependent inhibition of GSK-3 by compound **6a**. Top: Inhibition of GSK-3 with substrate concentration GS2 at 30 μm and different ATP concentrations. ■ ATP at 5 μm, ▲ ATP at 10 μm, ▼ ATP at 20 μm, ◆ATP at 50 μm, and □ ATP at 100 μm. Bottom: Inhibition of GSK-3 with ATP concentration at 100 μm and different GS2 concentrations. ■ GS2 at 1 μm, ▲ GS2 at 2.5 μm, ▼ GS2 at 5 μm, ◆ GS2 at 10 μm, ● GS2 at 30 μm, and □ GS2 at 50 μm.

Figure 2. Thus, the ATP assay concentration is very important comparing enzyme inhibition data for ATP competitive inhibitors, and therefore a 100 μM ATP concentration has been used in all the enzyme inhibition assays described here.

To compare the biological effect by exchanging the hydrazine functionality with the amide and triazole functionality, compounds **6b**, **7b**, and **8b** were selected for further evaluation in secondary in vitro assays.

As shown in Figure 3, the compounds were screened for inhibitory activity against a panel of 31 related kinases at 100 μM ATP concentration. Single point determinations were obtained at 10 and 100 μM concentrations of test compound. Only for compound **6b** the data predict an IC_{50} value slightly below 10 μM for inhibition of MSK1 and DYRK1A and especially compound **8b** showed a favorable profile in the kinase screen.

It is interesting to notice the high selectivity for GSK-3 compared to CDK-2 for the test compounds. Because of the close homology between the ATP binding pocket in GSK-3 and CDK-2, it is very common that compounds with inhibitory activity for GSK-3 also show inhibitory activity for CDK-2.[9,20] To confirm the selectivity observed in the primary screen the potency was determined for the compounds ability to inhibit CDK-2. Compounds **6b**−**8b** all gave at least 100 fold selectivity for GSK-3 compared to CDK-2 (Table 4).

Inhibition of the ATP binding site in GSK-3 leads to activation of several intracellular signaling pathways, one other than glycogen synthesis being accumulation of β-catenin.[1,2] To gain insight into the mechanism of action of this new class of compounds, **6b**−**8b** were further tested in cellular assays for the activation of glycogen synthase and accumulation of β-catenin protein.

The potency observed for both β-catenin accumulation and activation of glycogen synthase in the cell lines reflects the potency obtained in the primary enzyme assay, Table 4. In general a 10-fold concentration of test compound is necessary for glycogen synthase activation in the cellular assays compared to the concentration of test compound needed to inhibit the purified kinase. This demonstrates that the cell permeability for the compounds is independent upon the bioisosteric modification introduced into the parent compound. The higher potency of compound **6b**−**8b** compared to a reference compound SB-415286 probably reflects a better solubility and/or a better cell permeability for this new compound class. In line with earlier observations, insulin only activates the glycogen synthase pathway (Table 4).[2]

The activation of both cellular pathways by the inhibition of GSK-3 indicates that compounds **6b**−**8b** are inhibitors of the ATP catalytic binding site. Compound **8b** is 5 times more potent in the stimulation of glycogen synthase compared to β-catenin protein synthesis, whereas SB-415286 is equipotent in the stimulation of both cellular pathways.

To verify the effect of inhibition of GSK-3 in a physiological relevant model the incorporation of radiolabeled D-(U-^{14}C) glucose into glycogen was determined in isolated rat soleus muscle. The compounds were tested at a single dose (10 μM) in the presence of 0.2 nM insulin. Compounds **6b**−**8b** gave a 2−3-fold induction in glycogen synthesis compared to the level of glycogen synthesis at 0.2 nM insulin. This increase in glycogen synthesis is comparable to the maximum effect obtained with 100 nM insulin (Table 4).

Conclusion

A new class of GSK-3 inhibitors was identified after HTS screening of the Novo Nordisk compound library. SAR optimization and bioisosteric modification of functional groups gave access to a number of compounds with improved water solubility and cell permeability compared to reference compounds. The use of bioisosteric replacements has proven it's versatility in the optimization of this class of GSK-3 inhibitors, and it was shown that the hydrazine, amide, and triazole functionality are interchangeable moieties for this new class of GSK-3 inhibitors.

Selectivity among the many kinases is important for developing a therapeutic agent. Although a limited number of 31 kinases were used in the counter screen, the specificity profile obtained among these kinases looks very promising and also surprising in view of the close homology of the ATP binding site for a number of the enzymes noted above. It is interesting to notice the high selectivity for GSK-3 compared to CDK-2 for the test compounds, and compound **8b** is, to our knowledge, the most selective compound described to date.

The observation that compound **8b** is 5 times more potent in the stimulation of glycogen synthase compared to β-catenin protein accumulation, whereas SB-415286 is equipotent in β-catenin protein accumulation compared to stimulation of glycogen synthase, implicate

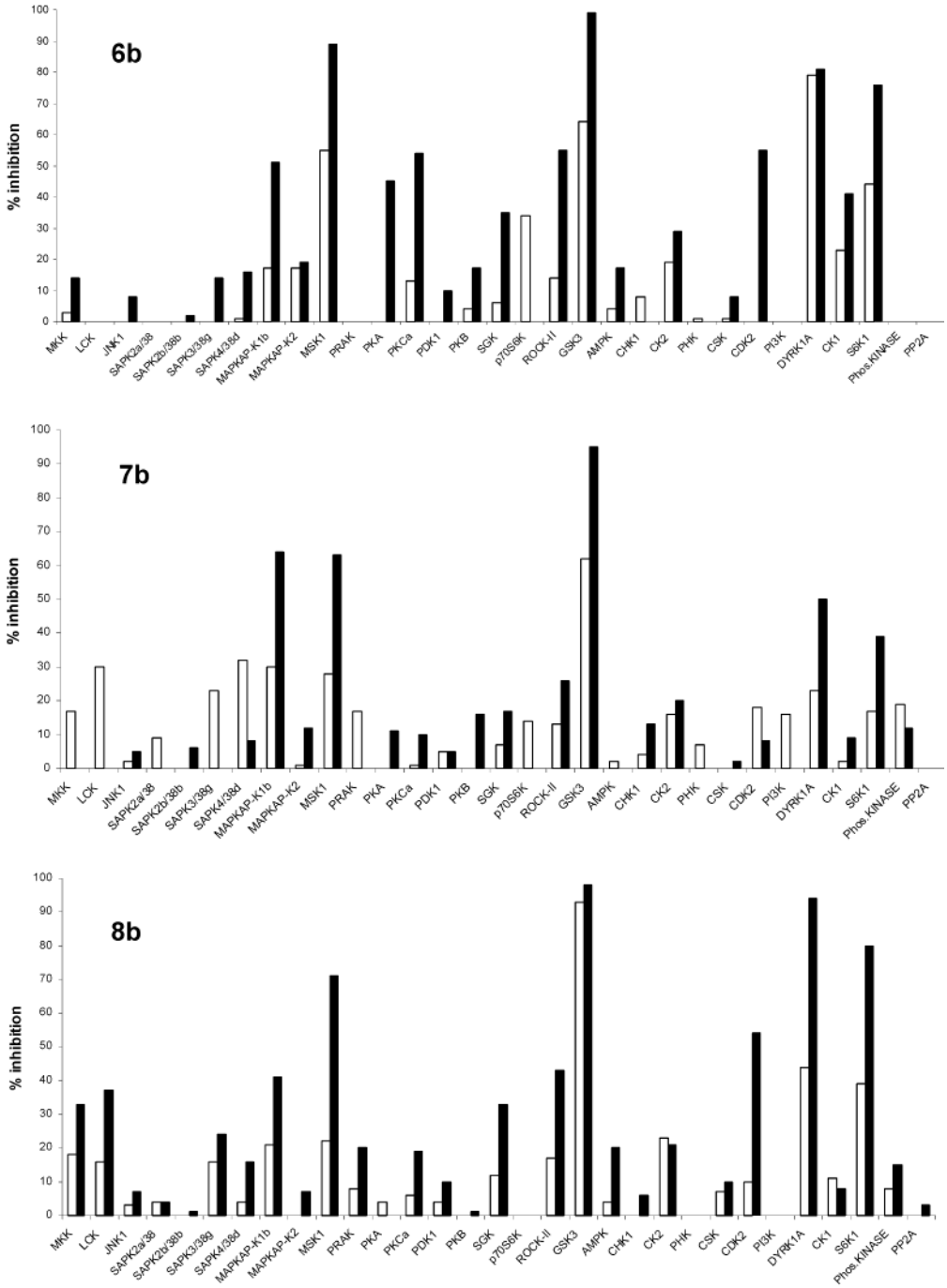


Figure 3. Kinase specificity for compound **6b**−**8b**. Abbreviations for the kinases screened as given in ref 21. Single point determinations. % inhibitions at 10 μM (open bars) and 100 μM (solid bars) of test compound. Screening data for P13K, p70SGK, and PHK was not obtained for compound **8b**.

Table 4. In Vitro Data for Selected Compounds in Secondary Assays

compound	GSK-3, IC_{50}, μM[a]	CDK-2, IC_{50}, μM[b]	glycogen synthase stimulation, EC_{50}, μM[c]	β-catenin synthesis, EC_{50}, μM[d]	glycogen synthesis, fold stimulation at 10 μM[e]
6b	0.10	96	2.85 ± 1.45	4.54 ± 1.06	3.1 ± 0.8
7b	1.17	119	19.07 ± 1.97	57.53 ± 1.01	2.2 ± 0.4
8b	0.28	>250	1.85 ± 1.18	11.05 ± 1.08	3.2 ± 0.4
SB-415286	0.38	0.38	45.60 ± 1.46	30.62 ± 1.07	1.3 ± 0.1
insulin	ND[e]	ND[e]	(0.96 ± 0.24) × 10^{-3}	NS[e]	3.1 ± 0.7 (100 nM)

[a] IC_{50} values for inhibition of GSK-3 calculated from three determinations in duplicate. [b] IC_{50} values for inhibition of CDK-2. These are means of two determinations in duplicate. [c] EC_{50} values for the stimulation of glycogen synthase in the CHO cell line. [c] EC_{50} values for the induction beta-catenin protein expression in the CHO cell line. [d] Fold stimulation of glycogen synthesis in isolated rat soleus muscle on top of 0.2 nM insulin, n=5. [e] ND is not determined and NS is no stimulation.

that it may be possible to obtain some selectivity in the stimulation the two pathways by structurally distinct ATP competitive inhibitors, but further studies with the compounds have to be performed to address this point.

As shown in Figure 1, the compounds previously described as GSK-3 inhibitors are structurally rather diverse, although the cyclic lactam moiety in staurosporine is easily recognized in a number of the structures (paullones, hymenaldisine, indirubins, and SB-415286). For the new compounds and some of the previously described compounds (NNC 57-0541 and CHIR98023), the resemblance to staurosporine is not that immediate. Whether or not this will reflect a difference in the biological activity for these compounds have to be addressed with further comparative studies.

A 3-fold stimulation of glycogen synthesis in rat soleus muscle reflects favorable cell permeability for the compounds and corresponds to the maximal stimulation of glycogen synthesis obtained with insulin under the same assay conditions. The data demonstrates that inhibition of GSK-3 mimics insulin action in the in vitro situation.

On the basis of the data given in Table 4, compounds **6b**−**8b** were selected for further in-vivo studies. The compounds penetrate cells very well and show a suitable water solubility which makes the compounds useful for iv and po dosing. Data from in vivo studies will be reported elsewhere in due course.

Materials and Methods

^{1}H NMR spectra were recorded at 300 MHz on a Bruker AC-300 MHz FT-NMR instrument. Melting points (uncorrected) were determined on a Büchi capillary melting point apparatus. For the HPLC-MS analysis the following instrumentation was used: Hewlett-Packard series 1100 G1312A Bin Pump, column compartment, G13 15A DAD diode array detector. Column: Waters Xterra MS C-18 × 3 mm id. Gradient: 10−100% acetonitrile in 0.01% TFA lineary during 7.5 min at 1.0 mL/min. Detection: UV, 210 nm. MS: ionization mode, API-ES. Elemental analyses were performed by Novo Nordisk micro analytical Laboratory, Denmark. All the reactions described are nonoptimized; therefore, the yields are not always as high as could be otherwise obtained. SB-415286 was prepared as described by Smith et al.[11] Starting materials not described in the Experimental Section were purchased from Chemical-Block: http://www.chemical-block.com. Precautions have to be taken handling 3-amino-4-azidofurazane **1**, as it might be potentially explosive.

Staurosporine was obtained from Sigma and insulin was supplied by Novo Nordisk A/S, Denmark.

General Method for the Preparation of 1-(4-Aminofurazan-3-yl)-5-alkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Ethyl Esters 3. 1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Ethyl Ester 3b. To a solution of 3-amino-4-azidofurazane **1**[17,18] (10 mmol, 1.26 g) in ethanol (30 mL) were added ethyl 4-chloroacetoacetate (15.0 mmol, 2.46 g) and piperidine (30 mmol, 2.55 g). The reaction mixture was stirred overnight at room temperature and then heated at reflux for 2 h. After cooling, the reaction mixture was evaporated to 5 mL volume, and water (100 mL) was added. The water phase was extracted with ether (2 × 75 mL). The combined ether phase was extracted with 1 N hydrochloric acid solution (3 × 50 mL). The hydrochloric acid extracts were collected and made alkaline with a 30% ammonium hydroxide solution. The title compound separated as oil and was extracted with ether (3 × 50 mL). The ether extracts were dried over magnesium sulfate and evaporated. The crude compound was triturated with hexane, and the crystalline compound was filtered giving the title compound in 750 mg (23%) yield, mp 128−130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.45−1.50 (m, 9H), 2.40−2.50 (m, 4H), 4.15 (s, 2H), 4.40−4.50 (q, 2H), 5.35 (s, 2H). HPLC-MS: m/z = 322 (M^+ + 1); RT = 0.68 min. Anal. ($C_{13}H_{19}N_7O_3$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Ethyl Ester 3a. The title compound was prepared from 3-amino-4-azidofurazan, ethyl 4-chloroacetoacetate, and diethylamine in 52% yield, mp 97−100 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.85−1.95 (t, 6H), 1.40−1.50 (t, 3H), 2.40−2.55 (q, 4H), 4.22 (s, 2H), 4.40−4.55 (q, 2H), 5.13 (s, 2H). HPLC-MS: m/z = 310 (M^+ + 1); RT = 0.77 min.

1-(4-Aminofurazan-3-yl)-5-pyrrolidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Ethyl Ester 3c. The title compound was prepared from 3-amino-4-azidofurazan, ethyl 4-chloroacetoacetate, and pyrrolidine in 97% yield, mp 157−158 °C. ^{1}H NMR $CDCl_3$: δ (ppm) 1.45−1.55 (t, 3H), 1.72−1.85 (m, 4H), 2.10−2.25 (m, 4H), 4.22 (s, 2H), 4.45−4.60 (q, 2H), 5.45 (s, 2H). HPLC-MS: m/z = 308 (M^+ + 1); RT = 0.77 min. Anal. ($C_{12}H_{17}N_7O_3 0.5H_2O$) C, H, N.

General Method for the Preparation of 1-(4-Aminofurazan-3-yl)-5-dialkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Hydrazides 4. 1-(4-Aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic acid Hydrazide 4a. To a solution of 1-(4-aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic acid ethyl ester **3a** (2.5 mmol, 772 mg) in ethanol (30 mL) was added hydrazine hydrate (5 mL). The reaction mixture was stirred at room temperature for 2 h and then evaporated to half the volume. Water (50 mL) was added, and the precipitated compound was filtered washed with water and dried. The title compound was obtained in 626 mg (85%) yield, mp 173−174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 0.70−0.80 (t, 6H), 2.25−2.40 (q, 4H), 4.00 (s, 2H), 4.55 (s, 2H), 6.15 (s, 2H), 10.10 (s, 1H). HPLC-MS: m/z = 296 (M^+ + 1); RT = 0.45 min. Anal. ($C_{10}H_{17}N_9O_2$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Hydrazide 4b. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid ethyl ester **3b** and hydrazine hydrate in 90% yield, mp 181−182 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.40−1.60 (m, 6H), 2.45−2.55 (m, 4H), 4.05 (s, 2H), 4.15 (s, 2H), 5.40 (s, 2H), 9.80 (s, 1H). HPLC-MS: m/z = 308 (M^+ + 1); RT = 0.54 min.

General Method for the Preparation of 1-(4-Aminofurazan-3-yl)-5-dialkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acids 5. 1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Hydrochloride 5b. A solution of 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid ethyl ester **3b** (321 mg, 1.0 mmol) in 20 mL of 1 N hydrochloric acid was heated at reflux for 8 h. The solution was evaporated to dryness and triturated with acetone. The crystalline compound was filtered and dried. The title compound was obtained in 250 mg (90%) yield, mp 199−200 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.44−1.55 (m, 2H), 1.60−1.80 (m, 4H), 3.1−3.3 (m, 4H), 4.25 (s, 2H), 6.7 (s, 2H), 10.8 (s, br, 1H). HPLC-MS: m/z = 294 (M^+ + 1); RT = 0.54 min. Anal. ($C_{10}H_{17}N_9O_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

General Method for the Preparation of 1-(4-Aminofurazan-3-yl)-5-dialkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic acid pyridin-4-ylmethylene Hydrazides 6. 1-(4-Aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]-triazole-4-carboxylic acid pyridin-4-ylmethylene Hydrazide 6a. To a solution of 1-(4-aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic acid hydrazide **4b** (730 mg, 2.5 mmol) in ethanol (30 mL) was added 4-pyridinecarboxaldehyde (294 mg, 2.75 mmol). The reaction mixture was heated at reflux for 1 h. The reaction mixture was evaporated, and ether (30 mL) was added. The crystalline compound was filtered, washed with ether, and dried. The title compound was obtained in 650 mg (68%) yield, mp 188−189 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 0.70−0.80 (t, 6H,), 2.25−2.40 (q, 4H), 4.10 (s, 2H), 6.70 (s, 2H), 7.56−7.70 (d, 2H), 8.60 (s, 1H), 8.65−8.70 (d, 2H), 12.70 (s, 1H). HPLC-MS: m/z = 385 (M^+ + 1); RT = 0.62 min. Anal. ($C_{16}H_{20}N_{10}O_2 \cdot 0.25H_2O$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Pyridin-4-ylmethylene

Hydrazide 6b. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid hydrazide **4b** and 4-pyridinecarboxaldehyde in 85% yield, mp 173−174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.45−1.50 (m, 6H), 2.40−2.50 (m, 4H), 4.15 (s, 2H), 6.20 (s, 2H), 7.65 (d, 2H), 8.55 (s, 1H), 8.63 (d, 2H), 12.45 (s, 1H). HPLC-MS: m/z = 397 ($M^+ + 1$); RT = 0.66 min. Anal. ($C_{17}H_{20}N_{10}O_2$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-pyrrolidin-1-ylmethyl −1*H*-[1,2,3]triazole-4-carboxylic acid pyridin-4-ylmethylene Hydrazide 6c. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-pyrrolidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid hydrazide and 4-pyridinecarboxaldehyde in 78% yield, mp 214−215 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.65−1.80 (m, 6H), 2.30−2.45 (m, 4H), 4.15 (s, 2H), 6.2 (s, 2H), 7.65 (d, 2H), 8.55 (s, 1H), 8.63 (d, 2H), 12.45 (s, 1H). HPLC-MS: m/z = 383 ($M^+ + 1$); RT = 0.53 min. Anal. ($C_{16}H_{18}N_{10}O_2$) C, H, N.

General Procedure for the Preparation of 1-(4-Aminofurazan-3-yl)dialkylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid (2-Pyridin-4-ylethyl)amides 7. 1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]-triazole-4-carboxylic Acid (2-Pyridin-4-ylethyl)amide 7b. To a solution of 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]-triazole-4-carboxylic acid **5b** (100 mg, 0.33 mmol) in DMF (10 mL) were added 1-hydroxybenzotriazole hydrate (45 mg, 0.33 mmol) and *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (77 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 0.5 h, and then 4-(2-aminoethyl)pyridine (61.0 mg, 0.5 mmol) was added. The reaction mixture was stirred at room temperature for 2 h, and then water (50 mL) was added. The water phase was extracted with ether (2 × 50 mL). The organic extracts were dried over magnesium sulfate and evaporated. The crude compound was triturated with diethyl ether and filtered to give the title compound in 85 mg (52%) yield, mp 179−181 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.10−1.20 (m, 6H), 2.15−2.30 (m, 4H), 2.85−2.95 (t, 2H), 3.45−3.55 (m, 2H), 4.02 (s, 2H), 6.15 (s, 2H), 7.30 (d, 2H), 8.45 (d, 2H), 9.0 (t, 1H). HPLC-MS: m/z = 398 ($M^+ + 1$); RT = 0.47 min. Anal. ($C_{18}H_{23}N_9O_2$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid (Pyridin-4-ylmethyl)-amide 7d. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid **5b** and 4-(aminomethyl)pyridine in 20% yield, mp 219−221 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.45−1.55 (m, 6H), 2.45−2.60 (m, 4H), 4.15 (s, 2H), 4.65 (d, 2H), 5.45 (s, 2H), 7.25 (d, 2H), 8.55 (d, 2H), 8.70 (t, 1H). HPLC-MS: m/z = 384 ($M^+ + 1$); RT = 0.49 min. Anal. ($C_{17}H_{21}N_9O_2 \cdot 0.5H_2O$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid Methyl(2-pyridin-4-ylethyl)amide Dioxalate 7e. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]-triazole-4-carboxylic acid **5b** and 4-(2-methylamino)ethyl)-pyridine. The free base was crystallized as the dioxalate salt from acetone in 64% yield. Mp 163−168 °C. ^{1}H NMR (300 MHz, DMSO-d_6, mixture of rotamers): δ (ppm) 1.10−1.20 (m, 2 × 6H), 2.15−2.30 (m, 2×4H), 2.90−3.05 (t, 2 × 2H), 3.05 (s,3H), 3.20 (s, 3H) 3.65 (s, 2 × 2H), 3.75 (t, 2H), 3.95 (t, 2H), 6.65 (s, 2 × 2H), 7.20 (d, 2H), 7.35 (d, 2H), 8.40 (d, 2H), 8.45 (d, 2H). HPLC-MS: m/z = 412 ($M^+ + 1$); RT = 0.85 min. Anal. ($C_{19}H_{25}N_9O_2 \cdot 2C_2H_2O_4$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid (2-Pyridin-4-ylethyl)-amide Dioxalate 7a. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-diethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic acid **5c** and 4-(2-aminoethyl)pyridine in 36% yield. The free base was crystallized as the dioxalate salt from acetone, mp 175−177 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 0.70−0.80 (t, 6H), 2.25−2.40 (q, 4H), 2.85−2.95 (t, 2H), 3.50−3.65 (q, 2H), 4.15 (s, 2H), 6.65 (s, 2H), 7.25−7.30 (d, 2H), 8.45−8.50 (d, 2H), 9.10 (t, 1H). HPLC-MS: m/z = 386 ($M^+ + 1$); RT = 0.64 min. Anal. ($C_{17}H_{23}N_9O_2 \cdot 2C_2H_2O_4$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-pyrrolidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic Acid (2-Pyridin-4-ylethyl)-amide Dioxalate 7c. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-pyrrolidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid and 4-(2-aminoethyl)pyridine in 78% yield. The free base was precipitated as the dioxalate salt from acetone, mp 185−190 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.55−1.65 (m, 4H), 2.10−2.15 (m, 4H), 2.85−2.95 (t, 2H) 3.55−3.65 (q, 2H) 4.40 (s, 2H), 6.70 (s, 2H), 7.25−7.30 (d, 2H), 8.40−8.45 (d, 2H), 9.10 (t, 1H). HPLC-MS: m/z = 386 ($M^+ + 1$); RT = 0.77 min. Anal. ($C_{17}H_{21}N_9O_2 \cdot 2C_2H_2O_4$) C, H, N.

1-(4-Aminofurazan-3-yl)-5-(isobutylaminomethyl)-1*H*-[1,2,3]triazole-4-carboxylic Acid (2-Pyridin-4-ylethyl)-amide Oxalate 7f. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-(isobutylaminomethyl)-1*H*-[1,2,3]-triazole-4-carboxylic acid and 4-(2-aminoethyl)pyridine. The free base was precipitated as the dioxalate salt from ethanol in 7% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 0.83−0.87 (d, 6 H), 1.70−1.85 (m, 1 H), 2.60−2.65 (d,, 2 H), 2.88−3.05 (t, J = 7.03 Hz, 2 H), 3.55−3.65 (m, 2 H), 4.47 (s, 2 H), 6.74 (s, 2 H), 7.29 (d, 2 H), 8.48 (d, 2 H), 9.35 (s, 1 H). HPLC-MS: m/z = 386 ($M^+ + 1$); RT = 0.54 min.

General Procedure for the Preparation of 4-[5-Dialkylaminomethyl-4-(5-pyridin-4-yl-4*H*-[1,2,4]triazol-3-yl)-[1,2,3]triazol-1-yl]furazan-3-ylamines 8. 4-[5-Piperidin-1-ylmethyl-4-(5-pyridin-4-yl-4*H*-[1,2,4]triazol-3-yl)-[1,2,3]triazol-1-yl]furazan-3-ylamine Dihydrochloride 8b. To a suspension of 1-(4-aminofurazan-3-yl)-5-piperidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid, hydrochloride **5b** (990 mg, 3.0 mmol) in dry tetrahydrofuan (80 mL) was added triethylamine (400 mg, 4 mmol), and the reaction mixture was stirred at room temperature for 20 min. Carbonyldiimidazole (450 mg, 3.3 mmol) was added, and the reaction mixture was heated at reflux for 1 h. The reaction mixture was evaporated to dryness, and the crystalline mass was washed with diethyl ether. The crude compound (700 mg, 2.0 mmol) was resuspended in tetrahydrofuran (100 mL) and pyridine-4-carboxylic acid amidrazone[19] (400 mg, 3.0 mmol) was added. The reaction mixture was stirred at room-temperature overnight, and the precipitated compound was filtered and dried. The crude compound (454 mg, 1.1 mmol) was dissolved in absolute ethanol (80 mL), and the reaction mixture was heated at reflux for 6 h. After cooling, the free base of the title compound was precipitated as the dihydrochloric acid salt by the addition of hydrocloric acid to the ethanol solution. Yield 470 mg (34%), mp > 270 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 0.90−1.07 (m, 2 H,), 1.43−1.84 (m, 4 H), 3.35−3.55 (m, 4 H), 5.11 (s, 2 H), 6.84 (s, 2 H), 8.51 (d, 2 H), 8.99 (d, 2 H), 10.49 (m, 1 H). HPLC-MS: m/z = 394 ($M^+ + 1$); RT = 1.19 min. Anal. ($C_{17}H_{19}N_{11}O \cdot 2HCl$) C, H, N.

4-[4-(5-Pyridin-4-yl-4*H*-[1,2,4]triazol-3-yl)-5-pyrrolidin-1-ylmethyl-[1,2,3]triazol-1-yl]furazan-3-ylamine Dihydrochloride 8c. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-pyrrolidin-1-ylmethyl-1*H*-[1,2,3]triazole-4-carboxylic acid and pyridine-4-carboxylic acid amidrazone[19] in 25% yield, mp >270 °C.^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 1.92−2.22 (m, 4 H), 5.23 (s, 2 H), 6.84 (s, 2 H), 8.42 (d, 2 H), 8.95 (d, 2 H), 10.74 (s, 1 H). HPLC-MS: m/z = 380 ($M^+ + 1$); RT = 0.95 min. Anal. ($C_{16}H_{17}N_{11}O \cdot 2HCl$) C, H, N.

4-[5-Dimethylaminomethyl-4-(5-pyridin-4-yl-4*H*-[1,2,4]-triazol-3-yl)-[1,2,3]triazol-1-yl]furazan-3-ylamine Dihydrochloride 8d. The title compound was prepared from 1-(4-aminofurazan-3-yl)-5-dimethylaminomethyl-1*H*-[1,2,3]triazole-4-carboxylic acid and pyridine-4-carboxylic acid amidrazone[19] in 41% yield, mp 241−243 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 2.96 (s, 6 H), 5.14 (s, 2 H), 6.84 (s, 2 H), 8.56 (d, 2 H), 8.99 (d, 2 H), 10.75 (m, 1 H). HPLC-MS: m/z = 354 ($M^+ + 1$); RT = 1.09 min. Anal. ($C_{14}H_{15}N_{11}O \cdot 2HCl$) C, H, N.

Biology. GSK-3 Inhibition Assay. Inhibition of GSK-3 by a test compound was evaluated using human GSK-3β and a glycogen synthase derived substrate with the following amino acid sequence: YRRAAVPPSPSLSRHSSPHQS(PO_4)EDEEE-NH_2.

In brief, GSK-3β was incubated with 32 μM substrate and varying concentrations of test compound in a buffer containing 0.1 mM ^{33}P-labeled ATP, 10 mM magnesium acetate, 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.1% dithiothreitol, and 0.03% Triton-X100 for 60 min at room temperature. The reaction was performed using 96-well filter plates and terminated by filtration followed by addition of 25 μL of 2% phosphoric acid to each well. All wells were then washed three times in 0.5% phosphoric acid to remove unincorporated ^{33}P-labeled ATP and dried, and radioactivity was counted in a Packard topcounter. Dose−response profiles were generated, and the IC_{50} value for inhibition of GSK-3 by the test compound was calculated using a four-parameter logistic function.

CDK-2 Inhibition Assay. CDK-2/cyclin A (5 mU) (diluted in 50mM Hepes pH 7.5, 1mM DTT, 0.02% Brij35, 100mM NaCl) was assayed against Histone H1 (1 mg/mL) in a final volume of 25 μL containing 50 mM Hepes pH 7.5, 1 mM DTT, 0.02% Brij35, 100 mM NaCl, 10 mM magnesium acetate, and 0.1 mM [^{33}P-g-ATP](500−1000 cpm/pmol) and incubated for 40 min at room temperature. The reaction was performed using 96-well filter plates. Assays were stopped by addition of 5 μL of 0.5 M (3%) orthophosphoric acid. All wells were then washed three times in 0.5% phosphoric acid to remove unreacted ^{33}P-labeled ATP and dried, and radioactivity was counted in a Packard topcounter. Dose−response profiles were generated, and the IC_{50} value for inhibition of CDK-2 by the test compound was calculated using a four-parameter logistic function.

Selectivity Panel Protein Kinase Assays. The members of the kinase selectivity panel were assessed as described previously.[21] The activated protein kinases were assayed for their ability to phosphorylate the appropriate peptide/protein substrate in the presence of 10 μM and 100 μM of test substance, respectively, at 0.1 mM ATP. Results are given as the mean of duplicate determinations for % inhibition compared to control incubations in which the inhibitors were substituted with DMSO vehicle.

Glycogen Synthase Activity in CHO-hIR Cells. CHO cells expressing approximately 50 000 human insulin receptors were plated in 96-well dishes.[22] Cells were stimulated for 1 h with compound or insulin in the desired concentration. The stimulation was terminated by washing four times in ice-cold PBS (pH 7.4), and cells were lysed in 20 μL of ice-cold homogenization buffer [50 mM Tris, 100 mM NaF, 10 mM EDTA, 2 mM EGTA, 0.1 mM TLCK, 1 mM AEBSF, 1 mM DDT, 5 mM Benzamidine, 0.25 μg/mL Leupeptin, 0.1% Triton X-100, pH = 7.8] by shaking for 1−2h. Protein concentration in the homogenates was measured with the Biorad Bradford assay kit. 40 μL reaction buffer [50 mM Tris, 100 mM NaF, 10 mM EDTA, 20 mg/mL glycogen, 5 mM UDP-glucose, and ^{14}C-UDP-glucose] was added to each well and the reaction allowed to run for 27 min. Reaction was stopped by transfer of samples (50 μL) to Unifilter 350 plates (Frisenette) containing 200 μL of ice-cold 66% ethanol, and glycogen was allowed to precipitate for 1 h at −20 °C. Wells were washed five times with 66% ice-cold ethanol, and plates were dried for 15 min at 52 °C. Subsequently, 75 μL of Microscint 20 (Packard) was added, and plates were sealed and counted in a Packard Topcounter. Glycogen synthase activity was calculated as the incorporation of labeled ^{14}C-UDP-glucose into glycogen per protein at 0.17 mM G6P. EC_{50} values were calculated using a four-parameter logistic function.

***β*-Catenin Protein Expression in CHO-hIR Cells.** CHO-hIR cells (50 000 cells/well) were plated in 12-well dishes and used the following day for experimentation. Cells were stimulated for 3 h with selected compounds and subsequently washed four times in ice-cold PBS. Cells were lysed in 100 μL of lysis-buffer (50 mM Tris-HCL, 100 mM NaF, 10 mM EDTA, 2 mM EGTA, 0,1 mM TLCK, 1 mM AEBSF, 1mM DTT, 5 mM benzamidine, 0,25 μg/mL leupeptin, 0,1% Triton X-100), and the lysates were incubated 20 min on ice. After centrifugation (15 min, 20 000 g at 4 °C), the protein concentration (BioRad) was measured, and lysates were heated (90 °C) for 10 min in 4 x SDS sample buffer. Samples were separated by PAGE using 4−12% BIS TRIS gels and MOPS buffer (KEM-EN-TEC) and transferred to PVDF membranes (Biorad). The membranes were blocked in blocking buffer (5% skim milk and 0.05% Tween 20 in TBS) at RT for 1 h and subsequently incubated with anti-β-catenin antibody (Tranduction Labs) diluted 1:500 overnight at 4 °C. Membranes were then washed four times in TBST and incubated with goat anti-mouse HRP IgG (Bio-Rad) diluted 1:10 000 in blocking buffer. Finally, membranes were washed four times in TBST and bands visualized with ECL. Bands were quantified using the LAS-1000 Fuji-Imager and the Image Gauge 4.0 software. Dose−response profiles were generated, and the EC_{50} value for the stimulation of β-catenin expression by the test compound was calculated using a four-parameter logistic function.

Glycogen Synthesis in Soleus Muscle. The effect of a test compound on glucose incorporation into glycogen is determined by measuring incorporation of radiolabeled D-(U-^{14}C) glucose into isolated rat soleus muscle.[23] Briefly, the soleus muscles from male Wistar rats (about 50 g) were removed and transferred to a reaction vessel containing KRH-buffer (5 mM glucose, 20 mM mannitol). The buffer contains 0.2 nM insulin. The muscles were incubated with and without test compound for 120 min and the last 60 min with tracer amounts of radiolabeled D-(U-^{14}C) glucose. The muscles were continuously gassed with 95% O_2/5% CO_2 in a shaking water-bath at 30 °C. After the incubation, the muscles were dried on filter paper, frozen in liquid N_2, weighed, and heated 30 min in 1 M NaOH at 90 °C. The glycogen was precipitated with 96% ethanol in the cold in the presence of carrier glycogen and dissolved in water, scintillation liquid was added, and radioactivity was counted in a β-counter. The results are mean of 5 determinations. For each animal, one soleus muscle with compound and 0.2 nM insulin was compared to the other soleus muscle with only 0.2 nM insulin.

Acknowledgment. The authors wish to acknowledge the technical assistance of Trine Eriksson for the synthesis of SB-415286, Bente Hansen, Lenette Silja Jørgensen, and Pia Jensen for testing of the compounds and Helle Worsaae for running the HTS. We are grateful to the University of Dundee (Division of Signal Transduction Therapy and MCR Protein Phosphorylation Unit) for providing the protein kinase selectivity data.

References

(1) Coghlan, M. P.; Culbert, A. A.; Cross, D. A. E.; Corcoran, S. L.; Yates, J. W.; Pearce, N. J.; Rausch, O. L.; Murphy, G. J.; Carter, P. S.; Cox, L. R.; Mills, D.; Brown, M. J.; Haigh, D.; Ward, R. W.; Smith, D. G.; Murray, K. J.; Reith, A. D.; Holder, J. C. Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem. Biol. (London)* **2000**, *7*, 793−803.

(2) Cohen, P.; Frame, S. The renaissance of GSK3. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 769−776.

(3) Weston, C. R.; Davis, R. J. Signaling Specificity- a Complex Affair. *Science* **2001**, *292*, 2439−2440.

(4) Anderton, B. H.; Dayanandan, R.; Killick, R.; Lovestone, S. Does dysregulation of the Notch and wingless/Wnt pathways underlie the pathogenesis of Alzheimer's disease? *Mol. Med. Today* **2000**, *6*, 54−59.

(5) Nikoulina, S. E.; Ciaraldi, T. P.; Abrams-Carter, L.; Mudaliar, S.; Park, K. S.; Henry, R. R. Regulation of glycogen synthase activity in cultured skeletal muscle cells from subjects with type II diabetes: role of chronic hyperinsulinemia and hyperglycemia. *Diabetes* **1997**, *46*, 1017−1024.

(6) Eldar-Finkelman, H.; Schreyer, S. A.; Shinohara, M. M.; LeBoeuf, R. C.; Krebs, E. G. Increased glycogen synthase kinase-3 activity in diabetes- and obesity-prone C57BL/6J mice. *Diabetes* **1999**, *48*, 1662−1666.

(7) Eldar-Finkelman, H. Glycogen synthase kinase 3: An emerging therapeutic target. *Trends Mol. Med* **2002**, *8*, 126−132.

(8) Cline, G. W.; Johnson, K.; Regittnig, W.; Perret, P.; Tozzo, E.; Xiao, L.; Damico, C.; Shulman, G. I. Effects of a novel glycogen synthase kinase-3 inhibitor on insulin-stimulated glucose metabolism in Zucker diabetic fatty (fa/fa) rats. *Diabetes* **2002**, *51*, 2903−2910.

(9) Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Biernat, J.; Wu, Y. Z.; Mandelkow, E. M.; Eisenbrand, G.; Meijer, L. Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/P25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease − A property common to most cycline-dependent kinase inhibitors? *J. Biol. Chem.* **2001**, *276*, 251−260.

(10) Leost, M.; Schultz, C.; Link, A.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Zaharevitz, D. W.; Gussio, R.; Senderowicz, A. M.; Sausville, E. A.; Kunick, C.; Meijer, L. Paullones are potent inhibitors of glycogen synthase kinase-3 beta and cyclin-dependent kinase 5/p25. *Eur. J. Biochem.* **2000**, *267*, 5983−5994.

(11) Smith, D. G.; Buffet, M.; Fenwick, A. E.; Haigh, D.; Ife, R. J.; Saunders: M.; Slingsby, B. P.; Stacey, R.; Ward, R. W. 3-anilino-4-arylmaleimides: Potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* **2001**, *11*, 635−639.

(12) Meijer, L.; Thunnissen, A. M. W. H.; White, A. W.; Garnier, M.; Nikolic, M.; Tsai, L. H.; Walter, J.; Cleverley, K. E.; Salinas, P. C.; Wu, Y. Z.; Biernat, J.; Mandelkow, E. M.; Kim, S. H.; Pettit, G. R. Inhibition of cyclin-dependent kinases, GSK-3 beta and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.* **2000**, *7*, 51−63.

(13) Bowler, A. N., Engelhardt, S, Sørensen, A. R., Ursø, B, Bödvarsdóttir, T. B., Worsaae, H., Hansen, B. F., and Kutzhalz, P. 2,4-Diaminothiazoles: a Novel class of Glycogen Synthase Kinase-3 (GSK-3) Inhibitors. Presented at the 11th RSC−SCI Medicinal Chemistry Symposium, September 9−12, 2001, Cambridge, UK.

(14) Naerum, L.; Norskov-Lauritsen, L.; Olesen, P. H. Scaffold hopping and optimization towards libraries of glycogen synthase kinase-3 inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1525−1528.

(15) Martinez, A.; Castro, A.; Dorronsoro, I.; Alonso, M. Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Med. Res. Rev.* **2002**, *22*, 373−384.

(16) Martinez, A.; Alonso, M.; Castro, A.; Perez, C.; Moreno, F. J. First non-ATP competitive glycogen synthase kinase 3 beta (GSK-3 beta) inhibitors: Thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. *J. Med. Chem.* **2002**, *45*, 1292−1299.

(17) Tselinskii, I. V.; Mel'nikova, S. F.; Vergizov, S. N. Azidofurazans in the synthesis of condensed systems. *J. Org. Chem. USSR (Engl. Transl.)* **1981**, *17*, 994−995.

(18) Zelenin, A. K.; Trudell, M. L. A 2-step synthesis of diaminofurazan and synthesis of N− monoarylmethyl and N,N-1-diarylmethyl derivatives. *J. Heterocycl. Chem.* **1997**, *34*, 1057−1060.

(19) Kubota, S.; Koida, Y.; Kosaka, T.; Kirino, O. Studies on the Synthesis of 1,3,4-Thiadiazole Derivatives. II. Synthesis of 1,3,4-Thiadiazoline-5-thiones from Amidrazones and Carbon Disulfide. *Chem. Pharm. Bull.* **1970**, *18*, 1696−1698.

(20) Hanks, S. K.; Hunter, T. Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J.* **1995**, *9*, 576−596.

(21) Davies, S. P.; Reddy, H.; Caivano, M.; Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* **2000**, *351*, 95−105.

(22) Hansen, B. F.; Danielsen, G. M.; Drejer, K.; Sørensen, A. R.; Wiberg, F. C.; Klein, H. H.; Lundemose, A. G. Sustained signaling from the insulin receptor after stimulation with insulin analogues exhibiting increased mitogenic potency. *Biochem. J.* **1996**, *315 (Pt 1)*, 271−279.

(23) Olsen, G. S.; Hansen, B. F. AMP kinase activation ameliorates insulin resistance induced by free fatty acids in rat skeletal muscle. *Am J Physiol Endocrinol. Metab.* **2002**, *283*, E965−E970.

JM021095D