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Bioorganic & Medicinal Chemistry Letters 18 (2008) 3661–3666
Bioorganic & Medicinal Chemistry Letters

Novel aryl and heteroaryl substituted *N*-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-oxadiazole-5-carboxamides as selective GSK-3 inhibitors

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Received 2 October 2007; revised 29 November 2007; accepted 30 November 2007
Available online 5 December 2007

Abstract—Synthesis, biological evaluation, and SAR dependencies for a series of novel aryl and heteroaryl substituted *N*-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-oxadiazole-5-carboxamide inhibitors of GSK-3β kinase are described. The inhibitory activity of the synthesized compounds is highly dependent on the character of substituents in the phenyl ring and the nature of terminal heterocyclic fragment of the core molecular scaffold. The most potent compounds from this series contain 3,4-*di*-methyl or 2-methoxy substituents within the phenyl ring and 3-pyridine fragment connected to the 1,2,4-oxadiazole heterocycle. These compounds selectively inhibit GSK-3β kinase with IC_{50} value of 0.35 and 0.41 μM, respectively.

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase that has captured a great attention in modern drug discovery.[1] At least three closely related isoforms of GSK-3 kinase (GSK-3α, GSK-3β, and GSK-3β2) play a major role in Hedgehog and Wnt signaling pathways, regulate cellular mitosis, stem-cell renewal and differentiation, cellular growth, motility and apoptosis, circadian rhythm, transcription, insulin-dependent glycogen synthesis, etc.[2–7] Aberrant regulation of GSK-3 kinases has been implicated in a range of human pathologies including Alzheimer's disease, non-insulin-dependent diabetes mellitus (NIDDM) as well as cancer and inflammatory disorders.[5,8] It has become clear that regulation of GSK-3 activity and the therapeutic potential of small molecule GSK-3 inhibitors constitute the key areas of investigation in the broader realm of medicinal chemistry. At the present day more than 40 inhibitors of GSK-3 kinases have been identified.[9–21] However, there are strong evidences highlighting the significant cell toxicity, various side-effects, poor absorption, distribution, metabolism, and excretion of these inhibitors which essentially restricted their clinical application.

Aryl and heteroaryl substituted 1,2,4-oxadiazole-5-carboxamides represent a relatively little explored class of heterocyclic structures with promising physiological activities. Thus, they were recently reported as antiplatelet, antithrombotic agents and partial serotonin antagonists.[22] Several 1,2,4-oxadiazole- and 1,3,4-oxadiazole-carboxamides containing different lipophilic moieties (i.e., 4-biphenyl-, 1-naphthyl, phenylpropyl-, and *n*-hexyl substituents) and additional basic groups which are mainly alkyl- and aminoalkyl residues have been recently described as antiplatelet and antithrombotic combounds as well as serotonin antagonists.[23]

In this paper, we describe a synthesis and biological evaluation for a series of novel nonpeptide small molecule inhibitors of GSK-3β kinase having general formula **I** (Fig. 1). Structures containing this core frag-

Keywords: Oxadiazole-5-carboxamide; GSK-3β; Inhibitor; Kinase; Combinatorial chemistry; Library.
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doi:10.1016/j.bmcl.2007.11.121

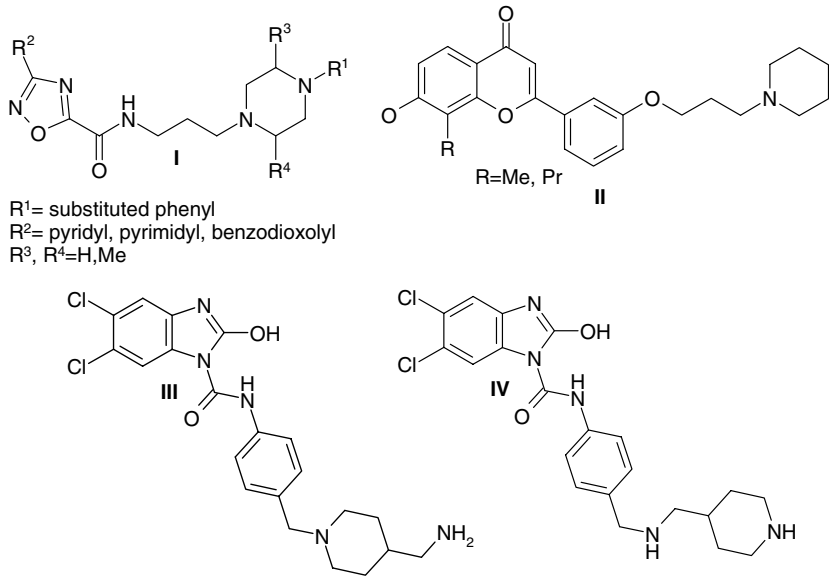


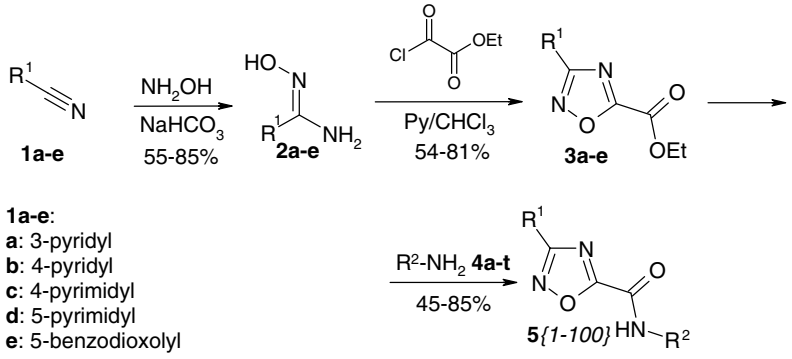
Figure 1. Developed compounds **I** and known GSK-3β inhibitors **II**–**IV**.

ment represent closely related bioisosteric/topological analogues of known inhibitors of GSK-3β kinase (Structures **II**–**IV**)[24,25] and can reasonably be regarded as potential therapeutic agents targeted for the treatment of several cognition and neurodegenerative disorders including Alzheimer's disease.

Our versatile synthesis of target compounds was accomplished according to a sequence of reactions shown in Scheme 1. The applied strategy was based on previously reported methods describing several synthetic routes to 3-substituted ethyl 1,2,4-oxadiazole-5-carboxylates and their carboxamide analogues.[26–28] For example, Santilli and Morris have described a convenient synthesis of 3-[(phenylsulfonyl)methyl]-1,2,4-oxadiazole-5-alkylcarboxamides.[26] In accordance with this approach, a series of heterocyclic compounds containing this core fragment was obtained by the reaction of initial alkyl and halogen substituted 2-[(phenylsulfonyl)methyl]imidamide-*N*′-oximes with ethyl chloroxalate followed by the reaction with various aliphatic amines. Unfortunately, 3-heteroaryl substituted 1,2,4-oxadiazole-5-alkylcarboxamides containing *N*-arylpiperazin fragment have not been described previously in scientific papers.

According to the mentioned approach, commercially available nitriles **1a**–**e** were reacted with hydroxyamine in $EtOH/H_2O$ in the presence of $NaHCO_3$ to give the corresponding amidoximes **2a**–**e** in good yields. The latter were dissolved in $CHCl_3$ and then refluxed for 5 h in pyridine with ethyl chloroxalate to furnish 3-heteroaryl substituted ethyl 1,2,4-oxadiazole-5-carboxylates **3a**–**e**. The obtained esters were then easily converted into the desired heterocyclic compounds **5**{*1–100*} (yield 45–85%) by reaction with *N*-aryl substituted 3-piperazin-1-ylpropan-1-amines **4a**–**t** (Fig. 2).

There are several high throughput- and cell-based biological assays performed for GSK-3β inhibitors.[29–31] For example, for screening against GSK-3β kinase Baki et al have recently reported a high throughput luminescent assay based on the Kinase-Glo system.[29] Cell-based assay describing the ability of Sfrp-1 and Sfrp-2 proteins, attenuators of Wnt signaling pathway, to inhibit the accumulation of β-catenin in LiCl-treated cells has also been reported.[30] In our work all synthesized compounds **5**{*1–100*} were tested for their potency to inhibit GSK-3β catalyzed proteolytic breakdown of its synthetic glycogen-synthase-derived substrate peptide



Scheme 1.

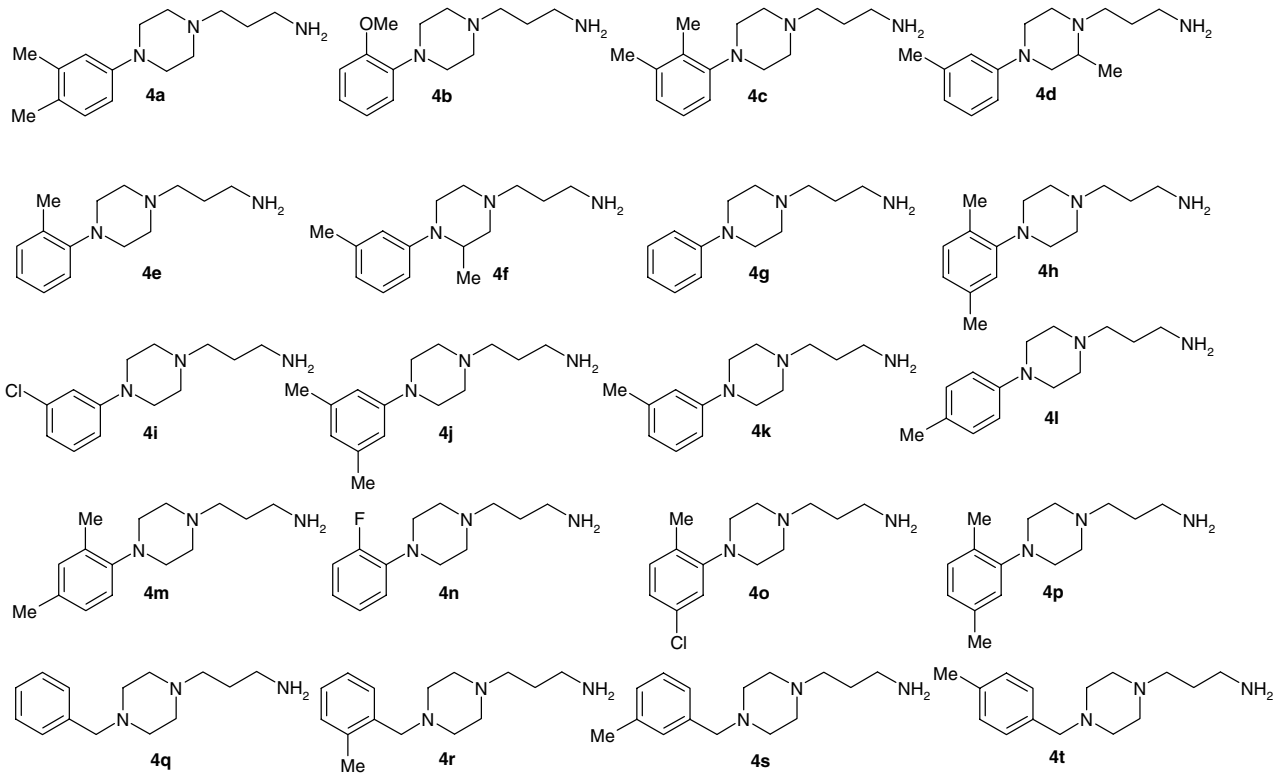


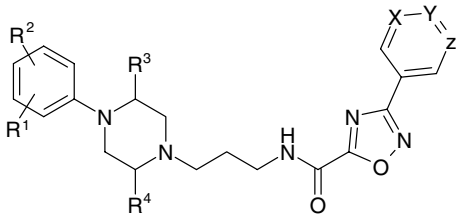
Figure 2. Amines used in this work.

(GSM) in enzymatic ADP Hunter assay and cellular level of β-catenin measured by traditional ELISA procedure in RKO cell-based assay (*See* Supporting Information). Among the tested compounds, several pyridine and pyrimidine substituted *N*-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-oxadiazole-5-carboxamides displayed a moderate to high inhibitory activity in these in vitro kinase assays (Tables 1 and 2). Other compounds from this series have shown insignificant or exhibited no detectable activity except several minor cases.

The most active compounds within pyridine substituted series have a single methyl/methoxy or two methyl substituents in the phenyl ring. These include dimethylphenyl-piperazines **5**{*1*} and **5**{*3*}, methoxyphenyl-piperazine **5**{*2*}, and methylphenyl-methylpiperazine **5**{*4*} derivatives (Table 1). These compounds have been shown to have a relatively high activity in the enzymatic ADP hunter assay (IC_{50} = 0.35–0.69 μM) and moderate activity in the RKO Cell-based assay (IC_{50} = 16–23 μM). It was disclosed that compounds **5**{*23*} and **5**{*24*} which contain 4-pyridine fragment were at least in two times less active than compounds **5**{*1–15*}, except compound **5**{*22*} which displayed comparable potency to **5**{*11–15*}. Furthermore, these agents were found to partially inhibit GSK-3β kinase activity in enzymatic ADP Hunter assay. In the same way as compounds **5**{*23–24*}, a relatively weak potency has also been demonstrated for pyrimidine derivatives **5**{*16–21*} as compared to 3-pyridine containing compounds **5**{*1–15*}. Among the tested compounds, only four pyridine derivatives were shown to have moderate activity against GSK-3β in cell-based assay (Table 2). Although further experiments have indicated a higher potency of 3,5-pyrimidine derivatives **5**{*16–21*} in contrast to their bioisosteric analogues containing 2,4- and 2,6-pyrimidine fragments in enzymatic assay, whole pyrimidine series exhibited no detectable activity against GSK-3β kinase in the RKO cell-based assay. Unfortunately, several other isosteric modifications of substituents in the pyrimidine ring of tested compounds as well as classical bioisosteric transformation of 1,2,4-oxadiazole into the 1,3,4-oxadiazole resulted in unacceptable loss of their activity. Notable, although the replacement of the phenyl group connected to the nitrogen atom of piperazine moiety by benzyl fragment has also led to complete loss of activity, bioisosteric transformation of pyridine heterocycle by 1,3-benzodioxole in 2-methyl-benzyl substituted derivative resulted in clarification of activity up to IC_{50} = 0.64 μM. However, the inhibition was insufficient because 70% of kinase activity was kept even at 10 μM concentration of the tested compound. Additionally, the replacement of the piperazine cycle by diazepam had a disastrous effect on inhibitory activity. Therefore, it can be tentatively suggested that location of heteroatoms within pyridine and oxadiazole rings as well as character of substitution in the aromatic fragment play an essential role in activity of the studied compounds. In accordance with this suggestion, we have observed a clear correlation between the inhibitory ability and mentioned structural features (see Supporting Information).

To investigate the possible mechanism of action of the tested compounds on GSK-3β kinase, the kinetic evaluation was performed using various concentrations of an

Table 1. In vitro GSK-3β inhibitory potency of pyridine and pyrimidine substituted *N*-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-oxadiazole-5-carboxamides **5**{*1–24*} in the enzymatic ADP Hunter assay



5{*1-24*}

Compound	R^1	R^2	R^3	R^4	X	Y	Z	IC_{50}[a] (μM)
5{*1*}	3-Me	4-Me	H	H	C	C	N	0.35 ± 0.07
5{*2*}	2-OMe	H	H	H	C	C	N	0.41 ± 0.07
5{*3*}	2-Me	3-Me	H	H	C	C	N	0.63 ± 0.1
5{*4*}	3-Me	H	H	Me	C	C	N	0.69 ± 0.1
5{*5*}	2-Me	H	H	H	C	C	N	0.71 ± 0.15
5{*6*}	3-Me	H	Me	H	C	C	N	0.71 ± 0.15
5{*7*}	H	H	H	H	C	C	N	0.86 ± 0.18
5{*8*}	3-Me	6-Me	H	H	C	C	N	0.86 ± 0.17
5{*9*}	3-Cl	H	H	H	C	C	N	0.89 ± 0.18
5{*10*}	3-Me	5-Me	H	H	C	C	N	0.9 ± 0.16 (*partial*)
5{*11*}	3-Me	H	H	H	C	C	N	1.06 ± 0.21
5{*12*}	4-Me	H	H	H	C	C	N	1.33 ± 0.22
5{*13*}	2-Me	4-Me	H	H	C	C	N	1.43 ± 0.22
5{*14*}	2-F	H	H	H	C	C	N	1.48 ± 0.23
5{*15*}	2-Me	5-Cl	H	H	C	C	N	2 ± 0.26 (*partial*)
5{*16*}	3-Me	H	H	Me	N	C	N	2.61 ± 0.27
5{*17*}	2-F	H	H	H	N	C	N	2.99 ± 0.3
5{*18*}	2-Me	4-Me	H	H	N	C	N	3 ± 0.3
5{*19*}	2-Me	5-Me	H	H	N	C	N	3.38 ± 0.31
5{*20*}	2-Me	5-Cl	H	H	N	C	N	3.48 ± 0.31
5{*21*}	3-Me	4-Me	H	H	N	C	N	3.5 ± 0.32
5{*22*}	3-Me	H	Me	H	C	N	C	1.13 ± 0.06
5{*23*}	3-Cl	H	H	H	C	N	C	4.0 ± 0.6 (*partial*)
5{*24*}	H	H	H	H	C	N	C	5 ± 0.9

[a] ATP and GSM concentrations were kept at 100 μM and 50 μM, respectively.

Table 2. In vitro GSK-3β inhibitory potency of compounds which exhibit activity in the RKO cell-based assay

Compound	IC_{50} (μM)
5{*1*}	16 ± 2
5{*2*}	23 ± 4
5{*4*}	17 ± 3
5{*6*}	19.15 ± 4
5{*22*}	>50

exemplified compound **5**{*4*}. In the enzymatic ADP Hunter assay the competitive character of inhibition has been demonstrated in the experiments, in which the inhibitory potency was measured in response to increasing concentration of ATP or the peptide substrate up to saturating the enzyme level. The incremental increase in ATP concentration shifted the inhibition curves to the right as one expected for competitive inhibition (Fig. 3). IC_{50} value of compound **5**{*4*} varied from 0.2 to 2.5 μM depending on ATP concentration and not depend on concentration of the peptide substrate.

Also, the competitive character of inhibition has been demonstrated in the experiments, in which the GSK-3β activity was measured in response to a stepwise increasing concentration of ATP or the peptide substrate in the presence of different concentrations of the inhibitor. Transformation of the data in the Lineweaver–Burke coordinates revealed an ATP-competitive type of inhibition (Fig. 4).

As reported in recent studies, the majority of GSK-3 inhibitors are not only acting against GSK-3, but are also affecting many other structurally unrelated kinases. In addition, because of high similarity in amino acid sequence between GSK-3α (51 kDa) and GSK-3β (47 kDa) isoforms, it is difficult to identify an inhibitor that can be selective against GSK-3α or GSK-3β. In spite of this obstacle, previous immunoblot and biological assays revealed that several small molecule agents inhibit selectively the activation of GSK-3β kinase. For example, AR-A014418, a selective GSK-3β inhibitor, prevents the expression of cell-cycle proteins.[32] Notable, as reported in some recent publications, selective inhibition of GSK-3 activity had no effect on cell viability and/or apoptosis in several biological systems.[33]

Selectivity of the most active compounds **5**{*1,2*} and **5**{*4*} within this series was primarily estimated in the

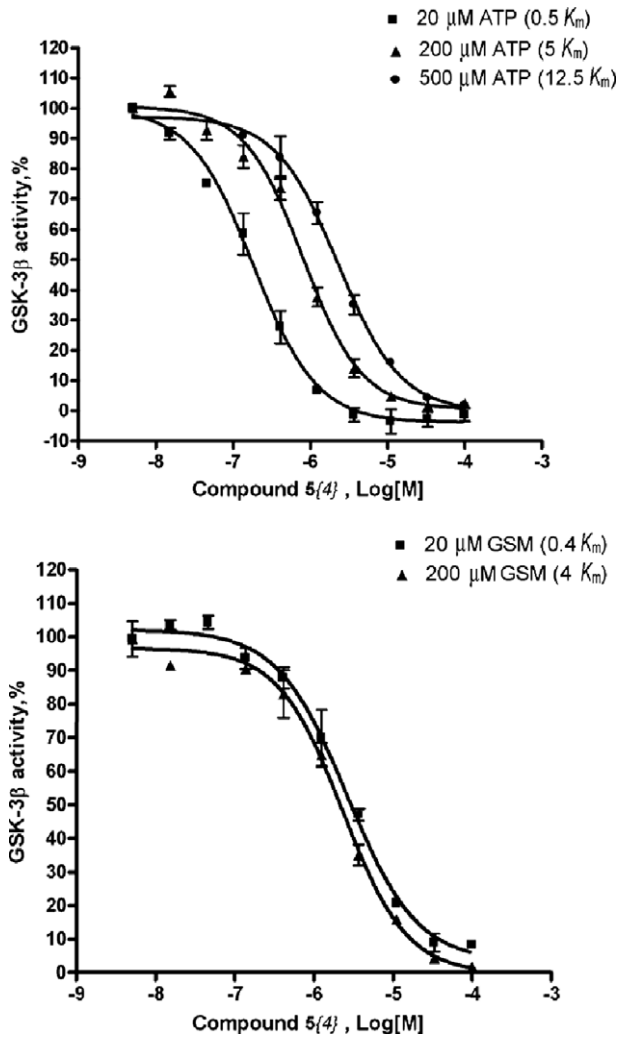


Figure 3. Effect of compound **5**{*4*} on GSK-3β activity. The kinase activity was assayed in the presence of indicated concentrations of ATP and GSM peptide substrate. When ATP concentration was varied, GSM was kept constant at 500 μM and vice versa.

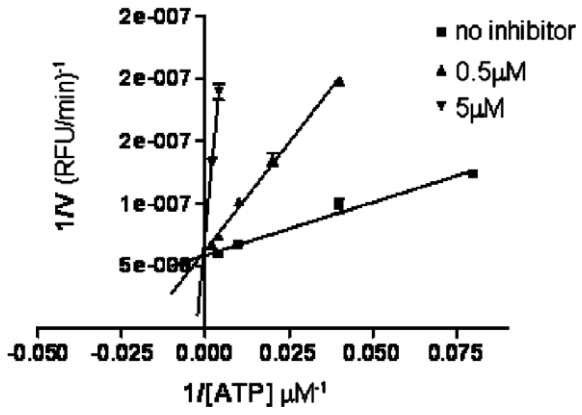


Figure 4. Lineweaver-Burke transformation of the ATP concentration curve for GSK-3β kinase at different concentrations of compound **5**{*4*}. The plot demonstrates competitive character of inhibition, as the inhibitor increased K_m without affecting the apparent V_{max}. GSM concentration was kept constant at 500 μM.

reactions with a panel of active recombinant human protein kinases and the specific substrates for each individual enzyme. These include a set of 4 serine/threonine (Aurora A, Aurora B, Pim-1, NUAK1) and 4 tyrosine (JAK2, IGF1R, EphB4, c-Met) kinases, phylogenetically and structurally unrelated to GSK-3β. The activity of eight selected kinases was evaluated in the presence of 10 μM of tested compounds. It has been shown that these agents exhibited a weak inhibition activity against Pim-1 and no detectable inhibition toward other classes of kinases. Thus, compound **5**{*2*} was the most potent inhibitor of Pim-1 kinase (IC_{50} = 8 μM), but the inhibition was partial and required higher concentrations of the tested compound. Therefore, it can be strongly suggested that examined compounds are selective inhibitors of GSK-3β. Selectivity of the most active compounds from this series is currently being evaluated against the additional subset of kinases including CDKs, PKCα/γ, PDK, Akt, etc.

In summary, we synthesized a library of novel *N*-[3-(4-phenylpiperazin-1-yl)propyl]-1,2,4-oxadiazole-5-carboxamides and tested them in a series of biological experiments. Most of the obtained compounds are efficient GSK-3β inhibitors. Compounds **5**{*1*} and **5**{*2*} with 3,4-*di*-methyl and 2-metoxy substituents in aromatic ring are the most potent compounds from this series (IC_{50} = 0.35 and 0.41 μM, respectively). Our primary data suggest ATP-competitive character of GSK-3β inhibition. The selected compounds **5**{*1–4*} showed a high selectivity against GSK-3β. Further biological studies are continuing.

Acknowledgments

The authors thank Dr. Konstantin V. Balakin and Dr. Yan A. Ivanenkov (Department of Computational Medicinal Chemistry, ChemDiv, Inc.) for SAR analysis and preparation of manuscript.

Supplementary data

The rigorous experimental section and analytical data for intermediates and final products (^{1}H NMR, ^{13}C NMR, HRMS), experimental procedures for the biological tests, inhibition kinetics and selectivity data for compounds **5**{*1–4*}, and data concerning the effect of substituents in the phenyl ring and the key role of terminal heterocyclic systems on the GSK-3β inhibitory activity are available free of charge via Internet at www.elsevier.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.121.

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