Letters

Thienyl and Phenyl α-Halomethyl Ketones: New Inhibitors of Glycogen Synthase Kinase (GSK-3*β*) from a Library of Compound Searching

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Abstract: Glycogen synthase kinase (GSK-3*β*) plays a crucial role in Alzheimer's disease (AD). Its inhibition is a valid approach to the treatment of AD. In this initial letter, some thienyl and phenyl α-halomethyl ketones are described as new non-ATP competitive inhibitors of GSK-3*β*. They are considered as lead compounds for designing and synthesizing new series, to carry out SAR studies, clear up the mechanism of action, and, in general, evaluate their therapeutical usefulness.

Introduction. The progressive aging of the world population brings on the undesired consequence of increasing occurrence of senile dementias such as Alzheimer's disease (AD). It is the most widely spread of these dementias and affects near 50% of the population aged 85 years or more.[1] As longevity increases, this rate may be even higher in a near future.

There are several biochemical processes, connected among them, which are affected in AD patients, but, up to now, acetylcholinesterase inhibitors are the only available drugs in market.[2] At present, most of the research is focused on the search of new agents useful in the treatment of two other pathologies, senile plaques and neurofibrillary tangles (NFT). They constitute the major histological lesions observed in AD brains and are both interconnected in a still unclear relationship.[3] It has also been postulated that both pathological processes are modulated by acetylcholine receptors.[4]

The NFT are formed by paired helical filaments (PHF) whose main component is an extracellular and quite hydrosoluble microtubule-associated protein named tau. In normal cells, tau is essential for the integrity and stability of the neuronal cytoskeleton, but, in neuropathological PHF, it appears abnormally phosphorylated.[5] The first evidence that phosphorilated human tau was related to PHF that accumulate in brain in AD was published in 1988.[6] Several kinases are involved in tau modifications leading to PHF.[7,8] Among them, tau protein kinases I and II are the most relevant.[9] TPK I was found to be identical to glycogen synthase kinase 3*β* (GSK-3*β*)[10] while TPK II consists of a 23 kDa protein activator and a catalytic subunit that is identical with cyclin-dependent kinase 5 (CDK5).[11] GSK-3*β* was initially identified as a regulator of glycogen metabolism, but it proved to have many other physiological functions.[12] Currently, there is a significant evidence, both in vitro and in vivo, that GSK-3*β* plays a crucial role in neurodegeneration in general and AD in particular.[13,14] Because of these data, inhibition of GSK-3*β* is accepted as a promising strategy for the treatment of AD and other neurodegenerative diseases.[15] In addition, a recent work reports that, in a mouse model, GSK-3α is involved in amyloid precursor protein (APP) processing, and generation of amyloid peptides (A*β*) is blocked by lithium trough inhibition of GSK-3α.[16] So, these facts open an attractive approach to prevent the formation of both amyloid plaques and NFT,[17] the two main hallmarks of AD.

Up to the present, few compounds have been reported to inhibit GSK-3*β*: pyrrolopiperazine derivative aloisines,[18] SmithKline Beecham maleimides,[19] an indolobenzazepine group of paullones,[20] the active ingredients of the traditional Chinese medicine indirubins,[21] or the marine sponge constituent hymenialdisine.[22] All of them act by competitive inhibition of ATP binding to the kinase active site. In our group, a series of thiazolidinone derivatives has recently been described[23] as non-ATP competitive inhibitors of GSK-3*β*. Searching for new inhibitors of GSK-3*β*, we considered the library of compounds of our Institute as a source of structures unchecked for this particular activity. As in other research centers, many products were synthesized in the past, screened in search of some biological activity, and then stored. These nearly forgotten compounds have become an important collection of miscellaneous structures. Our initial search was successful, and one compound exhibited a promising activity. The first test was followed by a second group of compounds that shared some chemical features and, after the results were obtained, by the synthesis and biological evaluation of a small series of derivatives. In this paper, we report the biological activity of a group of small molecules, α-halomethyl thienyl or phenyl ketones, as inhibitors of GSK-3*β*.

Results and Discussion. Initially, a handful of unrelated structures were selected from our own library of compounds in order to be checked as inhibitors of GSK-3*β*. Commercially available GSK-3*β* was incubated with a phosphate source (ATP) and a substrate (GS-1), in the presence and absence of the corresponding compound to be tested. The inhibitory activity was measured in accordance with a previously described method[24] in which GSK-3*β* activity was expressed in picomoles of phosphate incorporated per 20 min of incubation or in the percentage of maximal activity. Results are given as IC_{50}, defined as the concentration,

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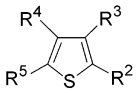
Table 1. Inhibition of GSK-3β by Chloromethyl Thienyl Ketones



compd	R^2	R^3	R^4	R^5	IC_{50} (μM)
1	ClAc[a]	H	Cl	Cl	2.5
2	ClAc	H	Br	H	3.0
3[b]	H	ClAc	Cl	H	25
4	Br	ClAc	Br	Br	1.0
5[b]	Br	ClAc	Cl	Cl	2.0
6	ClAc	H	H	H	50
7[b]	ClAc	H	ClAc	H	1.5
8	ClAc	H	H	Me	>100
9	ClAc	H	ClAc	Me	5.0
10	Me	ClAc	H	Me	>100
11	ClAc	Me	H	H	75
12	ClAc	H	Me	H	>100
13[b]	ClAc	Me	ClAc	H	5.0
14[b]	ClAc	H	H	Cl	10
15[b]	ClAc	H	H	Br	10
16[b]	ClAc	Br	Br	H	0.5
17[b]	ClAc	H	Br	Br	1.0
18[b]	Cl	ClAc	H	Cl	5.0
19	ClAc	H	H	Ac	50
20[b]	ClAc	H	Ac	H	8.0

[a] ClAc: $COCH_2Cl$. [b] Compounds checked as PKA inhibitors. IC_{50} > 100 μM in all cases.

expressed at micromolar units, of the compound that inhibits the enzyme at 50%. All of them were inactive or poorly active except 2-chloroacetyl-4,5-dichlorothiophene **1** that inhibited GSK-3β at a very low concentration.

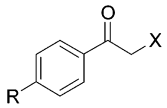
Because of this encouraging result, a second collection of 15 compounds was tested. They were also selected from our library, and all of them bear the common structural feature of an α-carbonylthienyl or phenyl group. The highest activities were again found for the group of chloromethyl halothienyl ketones (chloroacetylhalothiophenes) **2**−**5** (Table 1) while unhalogenated alkyl ketones, esters, and aldehydes did not inhibit GSK-3β at the maximum concentration used (100 μM). Ketones **1**−**5** were long time ago obtained by us as intermediates in the synthesis of potential β-adrenoceptor blocking agents[25] and in a basic chemical study on bromothiophene reactions.[26,27]

Data presented in Table 1 point out the suitably substituted chloroacetylthiophene as the structural feature required for activity. Actually, it is not a surprise because halomethyl ketones are reactive alkylating agents.[28] Their mechanisms of inhibition of serine[29] and cysteine[30] proteases were described some years ago.

New compounds **6**−**20** were synthesized by chloroacetylation of the corresponding thiophene, following the Friedel−Crafts method.[25] Activities of all the 20 α-chloromethyl thienyl ketones tested are presented in Table 1. To have a first impression about the selectivity of these compounds for GSK-3, many of them were tested as inhibitors of protein kinase A (PKA). All of them remained inactive at the highest concentration (100 μM) used.

Some initial ideas may be deduced from Table 1. Besides the chloroacetyl group, a second substituent is required to enhance activity (**6**). According to the alkylating mechanism, electron-donating groups such as methyl clearly reduce activity (**8**, **10**, **11**, **29**) while electron-withdrawing groups enhance the chemical

Table 2. Inhibition of GSK-3β by Halomethyl Phenyl Ketones



compd	X	R	IC_{50} (μM)	compd	X	R	IC_{50} (μM)
21	Cl	H	50	**26**	Br	Me	2.5
22	Cl	Cl	2.5	**27**	Br	MeO	1.0
23	Br	H	5.0	**28**	Br	Ph	2.5
24	Br	Br	0.5	**29**	Br	NO_2	2.0
25	Br	Cl	1.0				

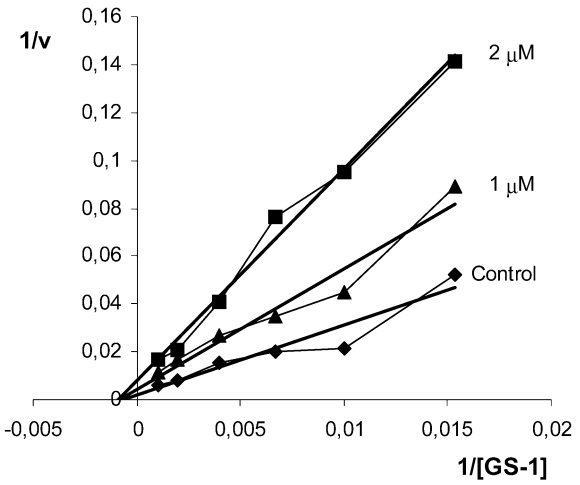


Figure 1. Double reciprocal plot of kinetic data from assays of GSK-3b activity at different concentrations of **17**. GS-1 concentrations in the reaction mixture varied from 6.5 to 100 μM. **17** concentrations are 1 and 2 μM, and the concentration of ATP was kept constant at 15 μM. V is picomoles of phosphate/20 min.

reactivity of the halomethyl ketone. A second chloroacetyl group produces a positive effect (**7**, **9**, **13**), but it may be due to the chloro atom, its electron-attracting features, or both. Until now, the group of polyhalogenated chloroacetylthiophenes have afforded the best results.

In addition, some commercial 4-substituted chloro- and bromoacetylphenyl derivatives **21**−**29** were also checked. They all showed an interesting range of activities that are recorded in Table 2, but there is not an obvious correlation between the para-substituent features and their biological activities. Compounds **22**−**29** were also evaluated as PKA inhibitors. They all were found inactive (IC_{50} >100 μM) except **29**, which showed a moderate activity (IC_{50} = 25 μM).

To investigate the mechanism of α-halomethyl ketones action on GSK-3β, several kinetic experiments were performed. First, we varied the concentrations of both GS-1 (6.5, 10, 15 25, 50, and 100 μM) and **17** (1 and 2 μM), keeping constant the concentration of ATP (15 μM). Double reciprocal plotting of the data (Figure 1), in with each point is the mean of three different experiments, suggest that **17** acts as a noncompetitive inhibitor of GS-1 binding

Moreover, kinetic experiments varying both ATP (6.5, 10, 15 25, 50, and 100 μM) and **17** (1 and 2 μM) levels were performed. Double reciprocal plotting of the data (Figure 2), in which each point is mean of three different experiments, confirms that **17** acts as a noncompetitive inhibitor of ATP binding.

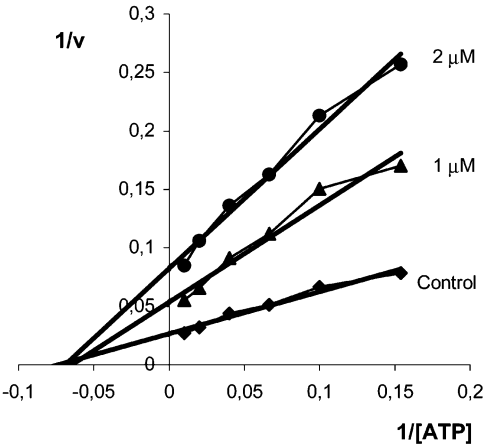


Figure 2. Double reciprocal plot of kinetic data from assays of GSK-3β activity at different concentrations of **17**. ATP concentrations in the reaction mixture varied from 6.5 to 100 μM. **17** concentrations are 1 and 2 μM, and the concentration of GS-1 was kept constant at 15 μM. V is picomoles of phosphate/20 min.

Conclusions. In summary, this letter presents the first steps of a new line, as a part of the research work of our group, with the final objective of a therapeutical treatment of AD. Here, we describe the biological properties of some thienyl and phenyl α-halomethyl ketones as non-ATP competitive inhibitors of GSK-3β. We regard this group of small molecules as lead compounds of a new series. More work is currently in progress, focused on the synthesis and evaluation of analogues bearing punctual structural variations of several parts of the molecule. A structure/activity relationship study will be carried out with this new series.

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Supporting Information Available: Analytical data of new compounds and experimental method to measure inhibition of GSK-3β. This material is available free of charge via the Internet at http://pubs.acs.org.

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