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Examining the correlations between GSK-3 inhibitory properties and anti-convulsant efficacy of valproate and valproate-related compounds

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Abstract—A family of compounds based upon the chemical structure of valproate were synthesized and assayed for their ability to inhibit glycogen synthase kinase (GSK)-3 α and β activity in vitro. This data is correlated to the known anti-convulsant properties of these compounds in order to determine the potential role of GSK-3 inhibition in the therapeutic efficacy of these drugs.

Valproic acid (VPA, 2-propylpentanoic acid) is a potent and widely prescribed drug that acts both as an anti-convulsant in the treatment of epilepsy and as a mood stabilizer to control bipolar disorder.[1,2] The anti-convulsant effects of valproate occur at plasma concentrations over 0.35 mM and toxicity is observed at concentrations over 1.4 mM.[1,3] Exposure to valproate is known to induce a variety of cellular responses, however the mechanism(s) by which valproate alleviates convulsions and modifies behavior is still unclear.[4–6] One potential, physiologically relevant target of valproate is the enzyme glycogen synthase kinase (GSK)-3. The two isoforms, GSK-3 α and β, were initially identified as homologous kinases that phosphorylate the rate-limiting enzyme in glycogen synthesis.[7] Over the last 20 years the list of GSK-3 substrates has grown to encompass a broad array of proteins that are involved in several important regulatory and developmental pathways including translation initiation factor eIF2βε, transcription factors CREB, c-Jun, c-Myc, c-Myb, HSF-1, ATP-citrate lysase and tau.[8] As a result, a great deal of interest has been focused upon GSK-3 as a potential therapeutic target in the treatment of cancer, diabetes, and neurodegenerative diseases.

The ability of valproate to inhibit GSK-3 activity remains controversial.[5,9] A number of compounds that are chemically related to valproate have been shown to exhibit similar anti-convulsant properties in animal models. Very little is known, however, regarding the effects of these compounds on potential cellular targets.

The present report provides insight into the effect of VPA on GSK-3 activity in an attempt to determine the relationship between GSK-3 inhibition and the therapeutic efficacy of VPA as an anti-convulsant. We have prepared a small library of compounds including some with previously determined anti-convulsant activity and tested for their ability to inhibit GSK-3 α and β.

As shown in Scheme 1, commercially available valproic acid (**1**) was converted to its corresponding acid chloride and then treated with either methanolic ammonia to give valpromide (**2**) or hydroxylamine to give the valpropylhydroxamic acid (**3**). Esterification of **1** under Fischer conditions allowed for the preparation of methyl valproate (**4**) which could, in turn, be treated with LDA at −78 °C to give the enolate and converted to either the α-iodo (**5**) or the α-methyl (**6**) systems shown. Exposure of **5** to DBU allowed for elimination to the α,β-unsaturated ester (**7**), which could be readily hydrolyzed to its

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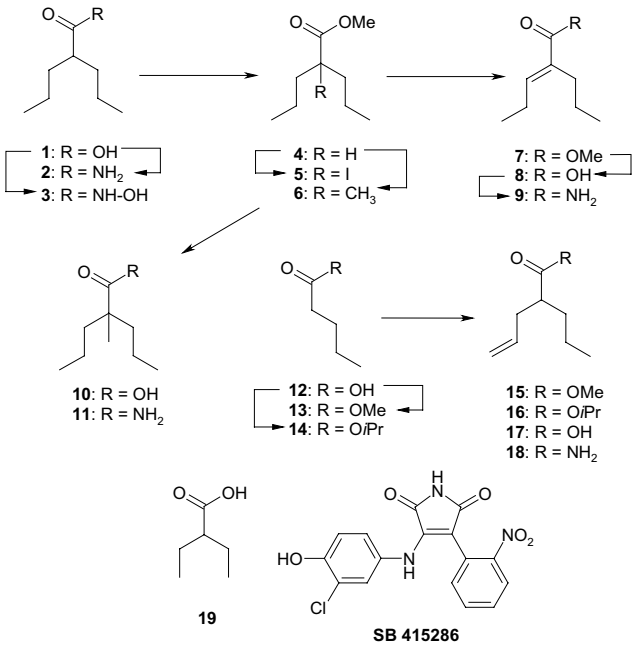

Scheme 1. Synthesis of inhibitor library.

Table 1. GSK-3 Inhibitory Activity

	GSK-3 activity (% control)		Anti-convulsant activity
	α	β	
Control	100 ± 6.6	100 ± 5.4	
1	80.4 ± 8.6[a]	35.5 ± 7.4[a]	++
2	96.6 ± 14.5	83.9 ± 10.4	+++
3	54.2 ± 8.7[a]	49.1 ± 18.5[a]	+++
4	104.5 ± 0.3	81.8 ± 2.2[a]	
6	99.7 ± 13.2	122.5 ± 18.6	
7	61.3 ± 12.9[a]	33.3 ± 10.5[a]	
8	100.0 ± 7.7	91.3 ± 9.4	++
10	67.4 ± 12.9[a]	24.8 ± 11.9[a]	+++
15	99.9 ± 0.4	122.7 ± 23.1	
16	87.9 ± 8.9	110.1 ± 23.9	
17	99.2 ± 4.4	57.7 ± 12.4[a]	+
19 (0.5 mM)	88.3 ± 15.4	56.1 ± 5.2[a]	
19 (4 mM)	0.5 ± 0.3[a]	3.0 ± 0.6[a]	+
LiCl (20 mM)	22.1 ± 6.4[a]	19.5 ± 1.6[a]	
SB415286 (20 μM)	1.5 ± 0.1[a]	4.6 ± 1.5[a]	

4 mM of each compound was tested unless stated otherwise; $n \geqslant 3$.
[a] $P < 0.05$; quantification of anti-convulsant activity is relative to valproate (++) and is based on the findings of Levi et al.,[12] Elmazar et al.,[13] and Keane et al.[14] in animal model systems. Significantly more effective (+++) and less effective (+) anti-convulsants are indicated. No data regarding anti-convulsant activity is known for all other entries.

corresponding acid (**8**) then converted to amide (**9**). In the same way, the α-methyl system (**6**) could be transformed to its corresponding acid (**10**) or amide (**11**). Elaboration of commercially available valeric acid (**12**) allowed for access to the so-called 4-ene-VPA system. Esterification of **12** to either its methyl (**13**) or isopropyl ester (**14**) set the stage for conversion to the appropriate enolate prior to treatment with allyl bromide to provide systems **15** and **16**. Hydroylsis of either ester affords 4-ene-VPA (**17**), which could be converted to its acid chloride then derivatized to amide **18**. 2-Ethylbutyric acid (**19**) was obtained from a commercial source. All compounds were fully characterized using NMR and MS. It should be noted that compounds containing chiral centers were prepared as racemates.

With compounds in hand, GSK-3 α and β activities were determined in vitro by assaying the ability of the recombinant kinase to phosphorylate a synthetic substrate.[10] The inhibitory activity of each of the compounds with respect to GSK-3 is presented in Table 1. The numbers shown represent the percentage activity of GSK-3 in the presence of the compound in relation to the control experiment. Lithium, a selective inhibitor of GSK-3 and a maleimide-based inhibitor, SB415286 were included in the assay as controls.

Our results show that VPA is a direct mmol inhibitor of both GSK-3 α and β. This result is similar to what was observed by Chen et al.[9] and in contrast to the findings of Phiel et al.[5] The reason for this discrepancy is not obvious as similar experimental conditions and substrates were used in all cases. Several of the compounds showed no inhibitory effect on either isoform including **2**, **6**, **8**, **15** and **16**. The most effective GSK-3 inhibitor identified was ethyl butyric acid (compound **19**). Ethyl butyric acid shows significant GSK-3 β inhibitory activity at 0.5 mM. At higher concentrations this compound completely inhibits both isoforms. Interestingly, we have identified two compounds, **4** and **17**, that appear to show significant specificity toward GSK-3 β and have no effect on the α isoform. The trend toward strong inhibitory activity on β was observed in valproate and several other compounds as well. Because the active sites of GSK-3 α and β are highly homologous,[11] the isoform specific nature of this family of inhibitors suggests that they act distal to the substrate-binding site. Further studies are required to determine the specific mode of inhibition for the valproate-related compounds presented in Table 1. Previously identified inhibitors have been shown to compete for GSK-3 binding to ATP (i.e., maleimides, including SB415286) or magnesium (i.e., lithium).[8,11]

Several of the compounds in our library have previously been tested for their ability to act as anti-convulsants in experimental animal models. Thus, by comparing these data sets, we can look for correlations that may indicate a role for GSK-3 inhibitory action in anti-convulsant efficacy. We can conclude from this data that GSK-3 inhibitory function does not correlate with anti-convulsant activity. This is evident from compound **8** that has no GSK-3 inhibitory activity but has been shown to be an effective anti-convulsant.[12,13] Conversely ethyl butyric acid, which is the most effective GSK-3 inhibitor that we have tested, has only limited anti-convulsant properties.[14] To the best of our knowledge, established GSK-3 inhibitors like lithium or SB415286 have not been shown to have any anti-convulsant activities.

In conclusion we have identified novel GSK-3 inhibitors based on the structure of valproate. Our data suggests that GSK-3 inhibition does not play a role in the anti-

convulsant efficacy of this family of compounds. Further analysis is required to determine the mechanism and physiological relevance of GSK-3 inhibition by valproate.

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