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# 1-Azakenpaullone is a selective inhibitor of glycogen synthase kinase-3β

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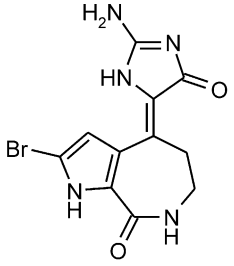
**Abstract**—Kenpaullone derivatives with a modified parent ring system were synthesized in order to develop kinase inhibitors with enhanced selectivity. Among the novel structures, 1-azakenpaullone was found to act as a selective GSK-3β versus CDK1 inhibitor. The charge distribution within the 1-azakenpaullone molecule is discussed as a possible explanation for the enhanced GSK-3β selectivity of 1-azakenpaullone compared to other paullone derivatives.


Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase which is ubiquitously expressed in mammalian tissues and interferes with manifold physiological processes.[1] Two isoforms of GSK-3 exist, designated GSK-3α and GSK-3β, which share a high homology at their catalytic site.[2] Among the proteins which are regulated by phosphorylation through GSK-3 are transcription factors, translation initiation factors, and microtubule-stabilizing proteins. Through the interaction with these targets, GSK-3 is involved in cell differentiation, cellular growth and proliferation, apoptosis control, inflammation and mechanisms involved in neuronal function.[1,3,4] Moreover, GSK-3 is an important factor in the regulation of glucose metabolism being one of the intracellular links downstream the insulin receptor.[5] The inactivation of glycogen synthase by phosphorylation through GSK-3 results in decreased glycogen synthesis. Thus, GSK-3 inhibitors have been suggested as potential antidiabetic drugs.[4,6] The discovery of structural diverse GSK-3 inhibitor classes has been described recently, for example pyridyl-oxadiazoles,[7] thiadiazolidinones,[8] and maleimides.[9,10] The maleimides SB-216763 and SB-415286 have been shown to stimulate glycogen synthesis in human liver cells.[9] Current advances in the search for GSK-3 inhibitors have been reviewed.[11] Since GSK-3 is phylogenetically most closely related to the cyclin-dependent kinases (CDKs),[4] it is not surprising that at least four ATP-competitive kinase inhibitor classes, which extensively have been evaluated for kinase selectivity, were found to act on both GSK-3 and distinct CDKs: hymenialdisine (**1**),[12] the indirubins [e.g., 5-iodo-indirubin-3′-monoxime (**2**)],[13] the aloisines [e.g., aloisine B (**3**)],[14] and the paullones, a family of 7,12-dihydro-indolo[3,2-*d*][1]benzazepin-6(5*H*)-ones.[15]



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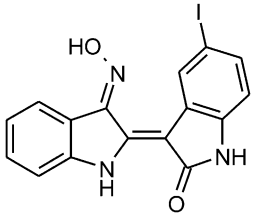

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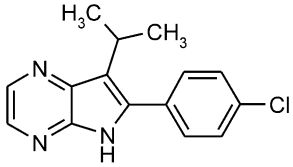

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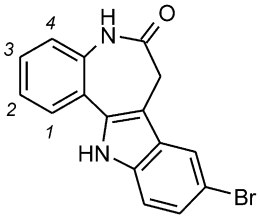

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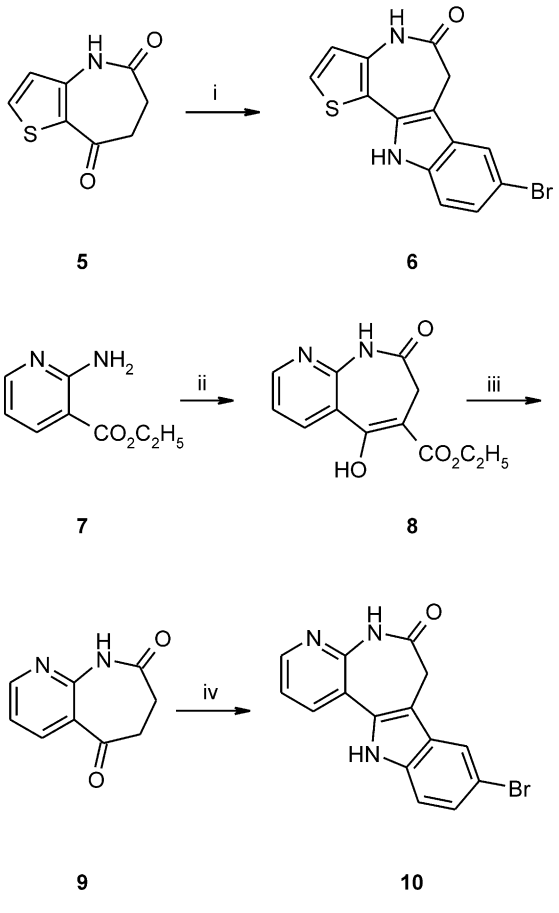
In a recent comparison of commercially available kinase inhibitors, kenpaullone (**4**) in combination with lithium as complementary reagent was suggested for investigations towards the biological effects through GSK-3 inhibition.[16] Although it has been shown that kenpaullone (**4**) exhibits only poor activity on other kinases, its remaining CDK-inhibitory property is still a concern. Even though a large number of paullone derivatives with different substituents on the parent ring system have been synthesized and evaluated, a paullone with both high potency and GSK-3 versus CDK selectivity has not been identified so far. Hence, for a rational design of a paullone with higher GSK-3 selectivity, kenpaullone derivatives with a modified heterocyclic basic structure, namely 8-bromo-6,11-dihydrothieno[3′,2′:2,3]azepino[4,5-*b*]indol-5(4*H*)-one (**6**), 4-azakenpaullone (**10**), and 1-azakenpaullone (**16**), were synthesized. These derivatives show a molecular shape similar to kenpaullone, but due to different charge distribution throughout the molecules a potential to distinguish between the ATP cavities of CDKs and GSK-3 was regarded as possible.

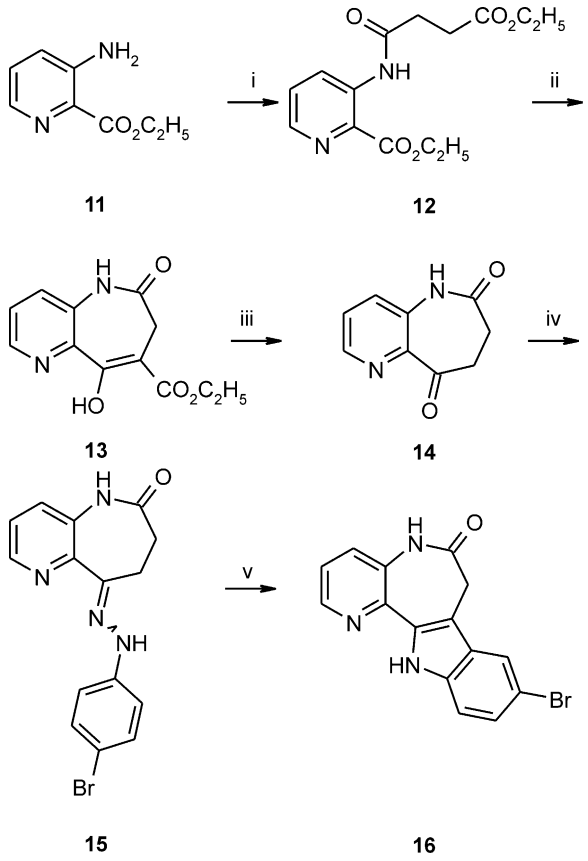
For the preparation of the thieno analogue **6** of kenpaullone, a Fischer indole synthesis with 4*H*-thieno[3,2-*b*]azepine-5,8(6*H*,7*H*)-dione **5**[17] and 4-bromophenylhydrazine catalyzed by sulfuric acid was carried out. The 4-aza-derivative **10** of kenpaullone was synthesized starting from 2-aminopyridine-3-carboxylic acid ethyl ester **7**,[18] which was cyclized by means of succinic acid diethyl ester in the presence of sodium hydride. The resulting enolized β-oxocarboxylic ester **8** was then heated in wet DMSO to yield the cyclic ketone **9**, which afforded **10** by an acid-catalyzed Fischer ring closure (Scheme 1).

The sequence leading to the 1-aza-analogue **16** is outlined in Scheme 2. 3-Aminopyridine-2-carboxylic acid ethyl ester **11** was acylated by ethyl succinyl chloride. Dieckmann cyclization of the resulting amide **12** by means of potassium hydride afforded the enolized β-oxocarboxylic ester **13**, which was subsequently dealkoxycarbonylated by heating in wet DMF. Because the classical Fischer indolization gave unsatisfactory yields when applied to the ketone **14**, the 4-bromophenylhydrazone **15** was prepared, which upon refluxing in diphenyl ether under neutral conditions afforded the 1-azakenpaullone **16** by a thermal indolization reaction.

For a biological evaluation the inhibition of the three kinases CDK1/cyclin B, CDK5/p25 and GSK-3β by the new paullone analogues was determined. The CDK1/cyclin B complex [also known as M-phase promoting




**Scheme 1.** (i) (1) 4-Bromophenylhydrazine HCl, NaOAc, HOAc, 70 °C; (2) HOAc, concd $H_2SO_4$, 70 °C; (26%); (ii) succinic acid diethyl ester, NaH, toluene, 90 °C, $N_2$ (39%); (iii) DMSO, $H_2O$, 150 °C, $N_2$ (81%); (iv) (1) 4-bromophenylhydrazine HCl, NaOAc, HOAc, 70 °C; (2) HOAc, concd $H_2SO_4$, 70 °C; (43%); mps: **6**: >330 °C; **8**: 212 °C; **9**: 221 °C; **10**: >330 °C.




**Scheme 2.** (i) (1) Ethyl succinyl chloride, toluene, pyridine, reflux, 2 h (81%); (ii) KH, toluene, DMF, 70 °C, $N_2$ (43%); (iii) DMF, $H_2O$, 150 °C, $N_2$ (95%); (iv) 4-bromophenylhydrazine HCl, NaOAc, HOAc, 70 °C (75%); (v) diphenyl ether, reflux, $N_2$ (81%); mps: **12**: 68 °C; **13**: 221 °C; **14**: 185.5 °C, **16**: >330 °C.

factor (MPF)] is an important regulator of the cell cycle, in which it is required for transition from the G2 to the M-stage.[19] The CDK5/p25 complex is found as a factor of pathological relevance in brain cells of Alzheimer's disease patients, where it leads to hyperphosphorylation of the tau protein.[20,21] In Table 1, the $IC_{50}$ values of **6**, **10** and **16** are given compared with the results for potent GSK-3β inhibitors, which were also evaluated for CDK1 and CDK5 inhibition in our test system before, namely hymenialdisine (**1**), 5-iodo-indirubin-3′-monoxime (**2**), aloisine B (**3**) and kenpaullone (**4**). The selectivity was expressed as the ratio of the $IC_{50}$ values of CDK1/cyclin B and GSK-3β. The three new paullone derivatives exhibited different behavior in the kinase testing. The thieno analogue **6** showed similar properties to kenpaullone, that is a considerable CDK1, CDK5 and GSK-3β inhibitory activity and a moderate selectivity for GSK-3. While the 4-azakenpaullone **10** is a poor inhibitor for the CDKs and GSK-3β, the 1-aza-derivative **16** only lost its CDK-inhibitory activity. Because the GSK-3β inhibitory potency of the parent compound kenpaullone is conserved in 1-azapaullone **16**, it constitutes a novel potent GSK-3β inhibitor with a noteworthy selectivity versus CDKs, expressed by a GSK-3/CDK1 selectivity index of 111 (see Fig. 1).

For an explanation of the observed differences between kenpaullone and the three kenpaullone congeners **6**, **10** and **16**, the structures were constructed in the SYBYL

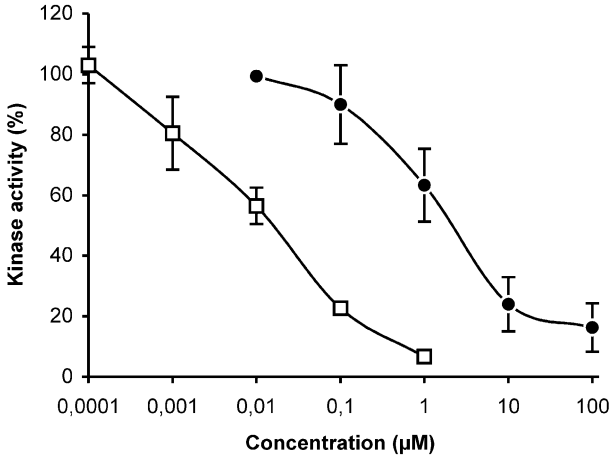



**Figure 1.** Inhibition of CDK1/cyclin B (filled circles) and GSK3-β (open squares) by 1-azakenpaullone (**16**). Vertical bars indicate standard error of the mean (SEM).

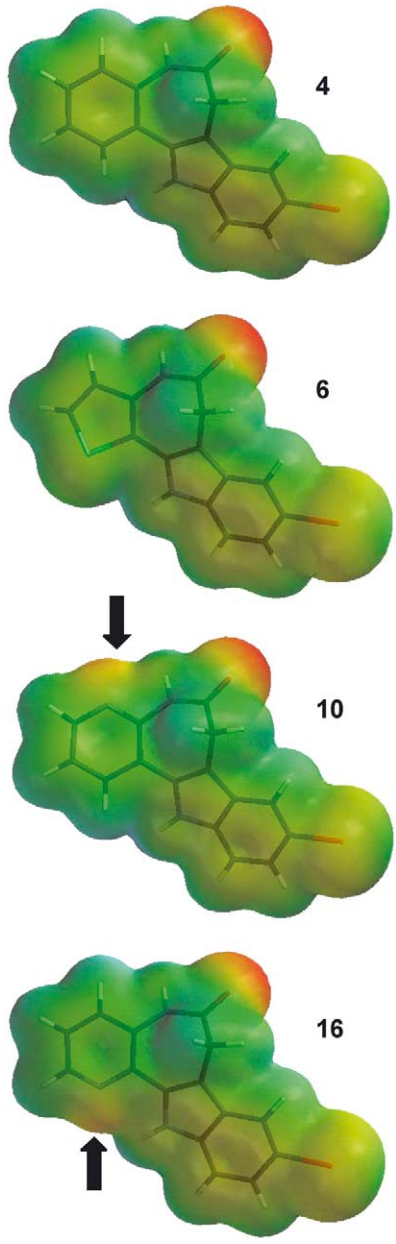



**Figure 2.** Charge distribution on the surface of the kenpaullone molecule **4** and its congeners **6**, **10**, and **16**. Charge is indicated by color: negative: red; positive: blue, neutral: green. Arrows indicate noteworthy differences in the charge distribution of the congeners with respect to kenpaullone.

**Table 1.** Selectivity of kinase inhibition by the new paullone analogues **6**, **10**, **16** compared to dual GSK-3β/CDK inhibitors from the literature

| Entry | | $IC_{50}$ (μM)[a] | | | Selectivity-index GSK-3-CDK1[b] | Ref |
|---|---|---|---|---|---|---|
| | | CDK1/cyclin B | CDK5/p25 | GSK-3β | | |
| **1** | Hymenialdisine | 0.022 | 0.028 | 0.010 | 2.2 | 12 |
| **2** | 5-Iodo-indirubin-3′-monoxime | 0.025 | 0.020 | 0.009 | 2.8 | 13 |
| **3** | Aloisine B | 0.85 | 13.0 | 0.75 | 1.1 | 14 |
| **4** | Kenpaullone | 0.4 | 0.85 | 0.023 | 17.4 | 15 |
| **6** | Thieno analogue of kenpaullone | 0.6 | 4.0 | 0.12 | 5.0 | |
| **10** | 4-Azakenpaullone | 8.4 | 12 | 6.0 | 1.4 | |
| **16** | 1-Azakenpaullone | 2.0 | 4.2 | 0.018 | 111 | |

[a] The kinase inhibition experiments were carried out as described before.[15] The final ATP-concentration in the test was 15 μM.
[b] Selectivity-index GSK-3-CDK1 = $IC_{50}$ CDK1/$IC_{50}$ GSK-3β.

modeling package using the Tripos force field, employing the previously published structure of kenpaullone[22] as template. The structures were then energy minimized by a quantum mechanical method (ab initio Hartree-Fock 3-21G*) using the SPARTAN program.[23] Subsequently, the electrostatic potentials were calculated and projected on the van-der-Waals surfaces of the molecules. The results of these calculations are depicted in Figure 2.

Obviously, the molecular shape of all four molecules is rather similar. Moreover, the charge distribution within the thieno analogue **6** fits very well the picture of kenpaullone, an observation which is reflected in the similar inhibitory activity of the compounds towards CDKs and GSK-3β. However, 4-azakenpaullone (**10**) exhibits a center of negative charge at position 4 (colored red in the molecule image and indicated by an arrow) in contrast to its parent compound. Preliminary docking studies with **10** in a homology model of CDK1[22] showed that the negative partial charge at position 4 leads to an unfavorable electrostatic repulsion to the backbone carbonyl oxygen atom of the amino acid in position 84 in the CDK1 ATP-binding cavity. In contrast, 1-azakenpaullone (**16**) shows a concentration of negative charge around the 1-position (colored red in the molecule image and indicated by an arrow). It can be speculated that this charge distribution disturbs the binding within the ATP cavity of CDKs only, but not the binding to GSK-3β. In order to find out why **16** can distinguish between the binding pockets of CDKs and GSK-3β, detailed docking studies will be carried out in the future.

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