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Thiazolo[5,4-*f*]quinazolin-9-ones, inhibitors of glycogen synthase kinase-3

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Abstract—In an effort to identify new protein kinase inhibitors with increased potency and selectivity, we have developed the microwave-assisted synthesis of thiazolo[5,4-*f*]quinazolin-9-ones. The effects of eighteen derivatives on CDK1/cyclin B, CDK5/p25, and GSK-3 were investigated. Several turned out to inhibit GSK-3 in the micromolar range. Molecular modeling studies suggest that the most selective GSK-3 inhibitors **7a**–**d** bind into the ATP-binding site through a key hydrogen bond interaction with Val135 and target the specific hydrophobic backpocket of the enzyme.

Cyclin-dependent kinases (CDKs) constitute a family of highly conserved protein kinases involved in regulating the cell division cycle, apoptosis, numerous neuronal functions, insulin release, and transcription. Glycogen synthase kinase-3 (GSK-3) is a family of kinases involved in cell cycle control, insulin action, apoptosis, neuronal cell death, and developmental regulation. Both families of kinases are implicated in various human diseases such as cancers, Alzheimer's disease, and diabetes, and therefore both have been extensively used as targets to identify small molecular weight pharmacological inhibitors of potential therapeutic interest.[1–4] More than 100 CDK inhibitors and 40 GSK-3 inhibitors have been identified;[2–4] most of which act by competing with ATP binding at the catalytic site of the kinase. Among the numerous inhibitors described, the most studied members contain a purine (e.g., olomoucine **I** and roscovitine **II**) or an oxindole ring (e.g., oxindole 91 **III**) (Fig. 1).

While investigating the chemical interest of 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt)[5–7] and its derivatives, we recently described the microwave-assisted multi-step synthesis of novel thiazoloquinazolinones[8–10] (**IV** and **V**, Fig. 1) which can be considered, at first glance, as hybrid molecules between the purines and the oxindoles mentioned above. After testing the effects of these molecules on CDKs and GSK-3,[10] it was observed that isomer (**V**) is completely inactive whatever substituent is present on the angular structure. Although most synthesized quinazolinones exhibited a moderate to potent GSK-3 inhibitory activity (IC_{50} ranging from 1.3 to 60 μM), one derivative (molecule **7a**, Scheme 1), analogue of **IV**, exerted a selective inhibition toward GSK-3 (IC_{50} = 4.2 μM). This compound bears, at C-2 of the thiazoloquinazolinone, an amidine function incorporated into an imidazoline ring. Considering this product as a possible lead compound, we focused our efforts on the synthesis of various thiazoloquinazolinones, which possess an amidine function on C-2 of the thiazole moiety and various alkyl or aryl groups on N-8 of the quinazoline part of the molecule.

In this article, we report the benefits associated with the microwave methodology[10,11] for the preparation of these new products and their effects on CDKs and GSK-3. Docking studies targeting the ATP-binding site

Abbreviations: CDK, cyclin-dependent kinase; GSK-3, glycogen synthase kinase-3; ATP, adenosine triphosphate; MW, microwave; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; AMP-PNP, 5′-adenylyl-imidodiphosphate; pdb code, RCSB Protein Data Bank.
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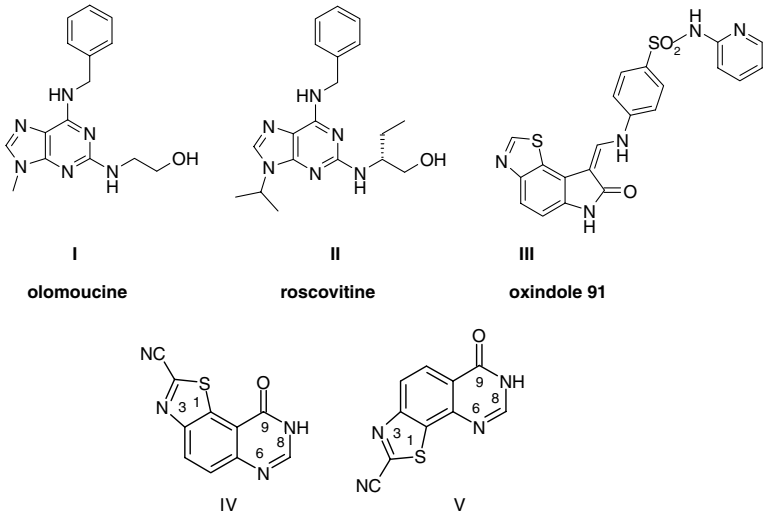
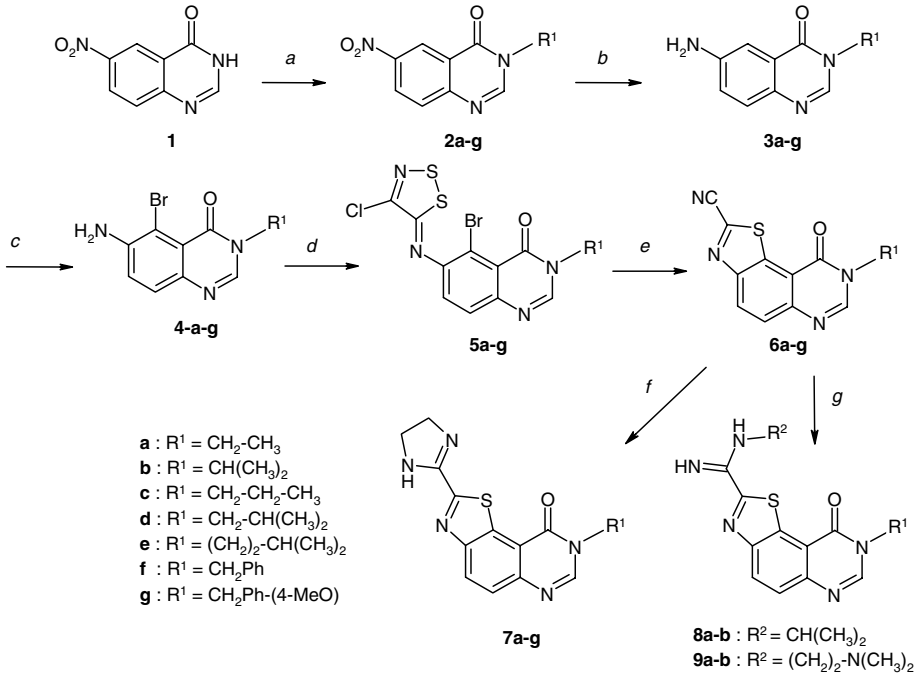


Figure 1. Structure of olomoucine (**I**), roscovitine (**II**), oxindole 91 (**III**), 8*H*-thiazolo[5,4-*f*]quinazolin-9-one (**IV**), and 7*H*-thiazolo[4,5-*h*]quinazolin-6-one (**V**).



Scheme 1. Reagents and conditions: (a) alkyl iodide and benzyl chloride, NaH, DMF, MW, 30 min; (b) HCO_2NH_4, Pd/C, EtOH, MW, 80 °C, 30 min; (c) Br_2, AcOH, rt, 2 h; (d) 4,5-dichloro-1,2,3-dithiazolium chloride, pyridine, CH_2Cl_2, rt, 1 h; (e) CuI, pyridine, MW, 115 °C, 15 min; (f and g) alkylamine or diamine, THF, MW, 80 °C, 30 min.

of a GSK-3β crystal structure were also performed to provide a hypothesis on the possible binding mode of this new class of inhibitors.

The pharmaceutical interest of the unsubstituted molecule **IV** (Fig. 1) has been judged limited and we demonstrated that N-alkylation of the nude skeleton was not possible in the conditions tested. Then, we decided to perform an N-alkylation of the quinazolinone **1** before fusing the thiazole ring. Selective N-alkylation in position 3 of the quinazolin-4-one skeleton was realized in various yields (44–50%) by treatment of 7-nitroquinazolin-4-one **1**, with sodium hydride and alkylation agents (alkyl iodide or benzyl chloride derivatives). Using ammonium formate for catalytic transfer hydrogenation in ethanol, reduction of the *N*-alkylated 7-nitroquinazolin-4-ones (**2a**–**g**) led to the amino derivatives (**3a**–**g**) in good yields (83–90%) (Scheme 1). Bromination of compounds (**3a**–**g**), in the presence of bromine in acetic acid, gave the *ortho*-brominated imines (**4a**–**g**) (yields: 70–87%). These compounds were condensed with 4,5-dichloro-1,2,3-dithiazolium chloride in dichloromethane at room temperature, followed by addition of pyridine, to give the intermediate imino-1,2,3-dithiazoloquinazolinones (**5a**–**g**) (yields: 60–78%). Final fusion of the thiazole ring onto the quinazoline moiety was realized by a thermolysis

procedure consisting of heating the imines (**5a**–**g**) at 115 °C, in the presence of cuprous iodide, in pyridine. The expected compounds (**6a**–**g**) were obtained in yields superior to 60%. The reactive cyano group of the thiazolo-2-carbonitrile ring was efficiently transformed into various amidines (**7**, **8**, and **9**) by treatment, under microwave irradiation, of compounds **6a**–**g** with ethylene diamine (products **7a**–**g**), isopropylamine (products **8a**,**b**) or *N*,*N*-dimethylethylenediamine (products **9a**,**b**). The basic side chain present on the latest compounds might provide cationic molecules leading to better water solubility and impacting on their biological properties.

Our experience in microwave-assisted chemistry of heterocycles[11] encouraged us to establish an efficient multi-step (five or six steps) synthesis of the 2,8-substituted thiazolo[5,4-*f*]quinazolin-9-one derivatives **6**, **7**, **8**, and **9** (Scheme 1). In all cases, besides resulting in good to excellent yields, our method offers much faster reactions compared to earlier published procedures at atmospheric pressure.

Thiazolo[5,4-*f*]quinazolin-9-one derivatives (**6**, **7**, **8**, and **9**) were tested against three protein kinases, CDK1/cyclin B, CDK5/p25, and GSK-3α/β.[12–14] All assays were run in the presence of 15 μM ATP and appropriate protein substrates (histone H1 for CDKs, GS-1 peptide for GSK-3). IC_{50} values were determined from dose–response curves and are provided in Table 1.

Most synthesized quinazolinones exhibit a moderate to potent GSK-3 inhibitory activity with IC_{50} ranging from 0.56 to 50 μM (Table 1). As we previously observed in our preliminary work,[10] the selectivity for GSK-3 versus CDKs is enhanced for amidine derivatives (**7a**–**d** and **8a**,**b**), compared to their cyano precursors (**6a**–**d**). The most active derivatives (**7a**–**d**) possess an amidine function incorporated into an imidazoline ring. It is also noticeable that N-substitution by an alkyl group seems to have a real influence on the activity of this family. A bulky chain substitution (e.g., *N*-isopentyl or *N*-benzyl derivatives **7e**–**g**) results in a lack of activity, whilst a short chain of 2 or 3 carbons leads to compounds (**7a**–**c**) with similar GSK-3 potency. Interestingly, replacement of the imidazoline ring of **7a**,**b** by a less rigid amidine function gives compounds **8a**,**b** and **9a**,**b** which differ in their activities according to the substituent at the R^2 position. Therefore, introduction of a bulky *N*,*N*-dimethylethylenediamine group leads to compounds **9a**,**b** which show an increased inhibitory activity toward both GSK-3 and CDK1, while a smaller isopropyl group **8a**,**b** enhances selectivity toward GSK-3 versus CDK1, as previously observed for compounds **7a**–**d**.

Table 1. Kinase inhibition[a] values (IC_{50} in μM) for compounds **6**–**9**

Compound	CDK1/cyclin B	CDK5/p25	GSK-3α/β
6a	12	27	6.2
6b	>10	>10	>10
6c	>100	>100	6.5
6d	>100	>10	10
6e	>100	>100	50
6f	NT	>10	>10
6g	>10	NT	>10
7a	>100	>100	4.2
7b	>100	>100	1.6
7c	>100	>100	1.6
7d	85	>100	4.6
7e	>10	>10	>10
7f	NT	>10	>10
7g	>10	NT	>10
8a	>100	NT	7.2
8b	>100	NT	9.4
9a	17	NT	1.3
9b	10	4	0.56

[a] Kinase inhibition experiments were carried out as described previously.[12–14] The final ATP-concentration in the test was 15 μM (NT, not tested).

In silico docking[15] gave the structures shown in Figure 2. The most selective GSK-3 inhibitors **7a**–**d** share a similar docking mode and make a critical hydrogen bond between the quinazoline nitrogen N-6 of the molecules and the backbone NH of Val135. According to previous studies,[20,21] all ATP-competitive kinase inhibitors bind in the adenine-binding region and interact with the hinge domain via hydrogen bond interactions. Due to the lengthened structure of these inhibitors, the rigid imidazoline ring targets the hydrophobic backpocket. This region, which is not occupied by ATP, displays structural diversity between members of the kinase family and contains unique residues which could be used to increase selectivity.[21–23] Access to this region is controlled by the so-called 'gatekeeper residue' Leu132, which is replaced by Phe80 in CDK1. Due to the bulkiness of the Phe80 side chain, these compounds may not fit into the hydrophobic pocket, inducing unfavorable contacts in CDK1. Such hypothesis has already been proposed in previous works.[24–26]

Additionally, imidazoline compounds **7e**–**g** bearing a bulky chain at the R^1 position would be expected to

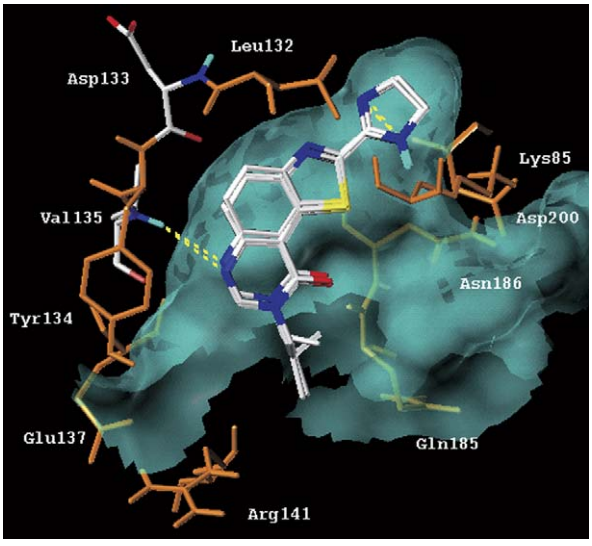


Figure 2. Illustration of the possible binding mode of compounds **7a**–**d** into the ATP-binding site of GSK-3β (based on pdb code: 1UV5), suggested by the molecular docking studies. The active site pocket is highlighted in cyan (MOLCAD surface; program Sybyl 7.0). The hydrogen bonds are indicated as yellow dotted lines.

encounter unfavorable steric interactions with some residues in GSK-3. In order to substantiate this hypothesis, we compared our model with another crystal structure of GSK-3β in complex with the non-hydrolyzable ATP analogue AMP-PNP (pdb code: 1J1B).[17] The three-dimensional structures were superimposed using the α-carbon of residues in the ATP pocket (Fig. 3). Interestingly, the main difference is the side-chain guanidine group of Arg141 which is oriented into a different direction and is closer to the R^1 position of our imidazoline compounds, resulting in a possible steric clash with encumbering substituents.

Finally, the binding mode of less rigid amidine compounds **8a**,**b** derived from the best docking pose is illustrated in Figure 4. According to this model, these compounds may form interactions similar to those observed for imidazoline compounds **7a**–**d**. In particular, the isopropyl group at the R^2 position is oriented into the hydrophobic backpocket. However, this part of the binding site appears to accommodate only a relatively small substituent since replacement by a bulky *N*,*N*-dimethylethylenediamine group leads to compounds **9a**,**b** which are more potent ATP-competitive inhibitors of GSK-3 (IC_{50} values of 1.30 and 0.56 μM, respectively), but present also a moderate inhibition for CDK1 (IC_{50} values of 17.0 μM and 10.0 μM, respectively). A possible change in binding conformation may occur and this will be the object of future computational studies.

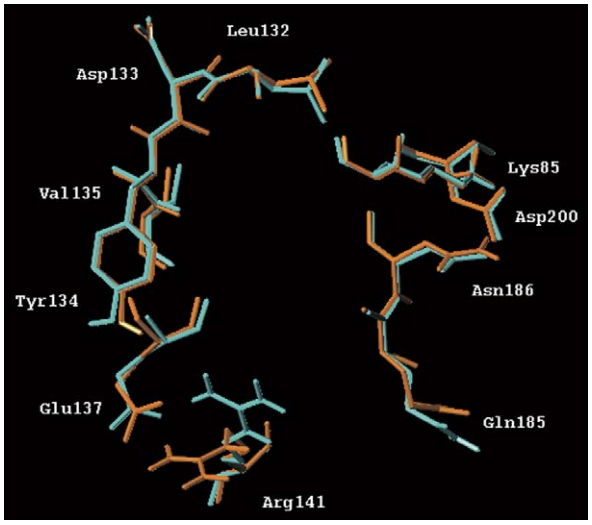


Figure 3. Superimposition of two GSK-3β crystal structures (orange, pdb code: 1UV5 and cyan, pdb code: 1J1B).

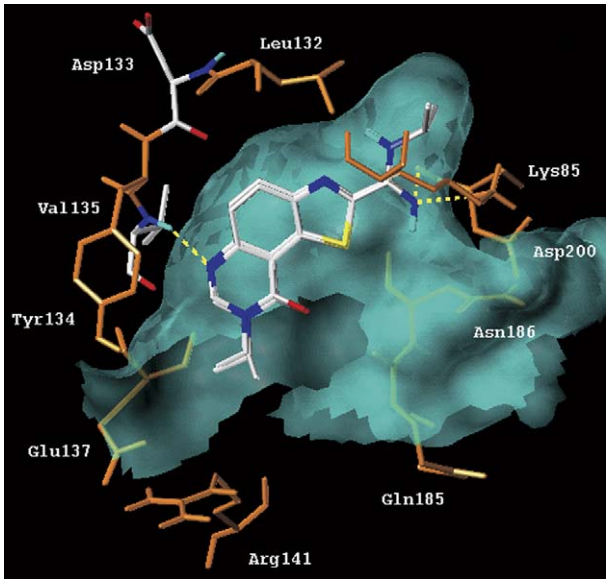


Figure 4. Proposed binding mode of compounds **8a** and **8b** into the ATP-binding site of GSK-3β (based on pdb code: 1UV5).

In conclusion, this work has uncovered a family of 2,8-substituted thiazoloquinazolinones from which selective micromolar GSK-3 inhibitors could be identified (e.g., **7a**–**d** and **8a**,**b**) and has shown how the known hydrophobic backpocket subsite of the enzyme can be exploited to gain affinity as well as selectivity. In two instances, the most GSK-3 active compounds exhibit a moderate inhibitory activity on CDK1 (e.g., **9a** and **9b**). These two molecules suggest that it might be possible to obtain a combined inhibition of GSK-3 and CDKs. In order to improve our knowledge on this series, new amidine and/or imidate derivatives, with various R^1 and R^2 substituents, are currently being evaluated. This family constitutes an interesting scaffold from which more potent inhibitors could be designed, helped by molecular modeling studies.

Acknowledgments

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