Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 1469–1477
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Novel 9-oxo-thiazolo[5,4-*f*]quinazoline-2-carbonitrile derivatives as dual cyclin-dependent kinase 1 (CDK1)/glycogen synthase kinase-3 (GSK-3) inhibitors: Synthesis, biological evaluation and molecular modeling studies

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Received 26 June 2007; received in revised form 13 September 2007; accepted 18 September 2007
Available online 29 September 2007

Abstract

Continuous efforts in microwave-assisted synthesis and the structure–activity relationships' (SARs) studies of novel modified 9-oxo-thiazolo[5,4-*f*]quinazoline-2-carbonitriles, allowed identification of new amidine and imidate derivatives as potent and dual CDK1/GSK-3 inhibitors. Combination of lead optimization and molecular modeling studies allowed identification of a dual CDK1/GSK-3 inhibitor (compound **13d**) with submicromolar values.
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Keywords: CDK1 inhibitor; GSK-3 inhibitor; Molecular modeling; Microwave-assisted chemistry; Quinazolinones

1. Introduction

Protein kinases have a fundamental role in signal transduction pathways, and aberrant kinase activity has been observed in many diseases. In recent years, kinase inhibition has become a major area for therapeutic intervention and a variety of kinase inhibitor pharmacophores has been described. Most kinase inhibitor molecules currently developed are targeted at the ATP-binding site, an ubiquitous domain in nature, and mimic mainly the H-bonding motif of the ATP aminopyrimidine ring. Among the 518 human kinases [1], two classes have been particularly explored: the cyclin-dependent kinases (CDKs) which are involved in regulating the cell division cycle, apoptosis, neuronal cell physiology, pain signaling, transcription, RNA splicing and insulin release, among other activities [2,3] and the glycogen synthase kinase-3 (GSK-3) which is a family of kinases involved in cell cycle control, insulin action, apoptosis, neuronal cell death and developmental regulation, among other processes [4,5]. Both families of kinases are implicated in various human diseases such as cancers, Alzheimer's disease, diabetes and therefore both have

Abbreviations: CDK, cyclin-dependent kinase; GSK-3, glycogen synthase kinase-3; ATP, adenosine triphosphate; MEPs, molecular electrostatic potentials; MW, microwave; AMP-PNP, 5′-adenylyl-imidodiphosphate; pdb code, RCSB Protein Data Bank.

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doi:10.1016/j.ejmech.2007.09.020

been extensively used as targets to identify small molecular weight pharmacological inhibitors of potential therapeutic interest. A recent mini-review also shows that CDK/GSK-3 inhibitors may emerge as effective therapeutic agents for proliferative renal diseases, furthering the prospect that these inhibitors may emerge as effective therapeutic agents in the near future [6]. Over the past decades, several groups have identified and characterized a fair number of potent CDK inhibitors (olomoucine, roscovitine, purvalanols, indirubins, aloisines, hymenialdisine, etc.). Many of these molecules are derived from marine organisms. The most advanced compound, the purine analogue (*R*)-roscovitine (CYC-202), a CDK2 selective inhibitor, is currently in phase 2 clinical trials against non-small cell lung cancer, breast cancer and various B-cell malignancies, in phase 1 against various kidney inflammations (glomerulonephritis), in pre-clinical, animal testing against Alzheimer's disease and stroke. Interestingly, many inhibitors are in fact dual inhibitors of CDKs and GSK-3, due mainly to the high degree of homology (~86%) between the ATP-binding site of the two kinases.

In an attempt to identify a potent and selective GSK-3 inhibitor focused on 2,8-substituted 9-oxo-thiazoloquinazoline-2-carbonitriles, we unexpectedly discovered a compound, bearing an *N*-isopropyl side chain on the N-8 nitrogen and an amidine function with a bulky *N*,*N*-dimethylethylenediamine group at the C-2 position of the thiazole moiety. This compound showed submicromolar IC_{50} against GSK-3 but also a moderate activity against CDKs (Table 1, entry **15c**) [7]. This article describes our continued efforts in the structure–activity relationships' (SARs) studies and toward the identification of new amidine and imidate derivatives as potent and dual CDK1/GSK-3 inhibitors.

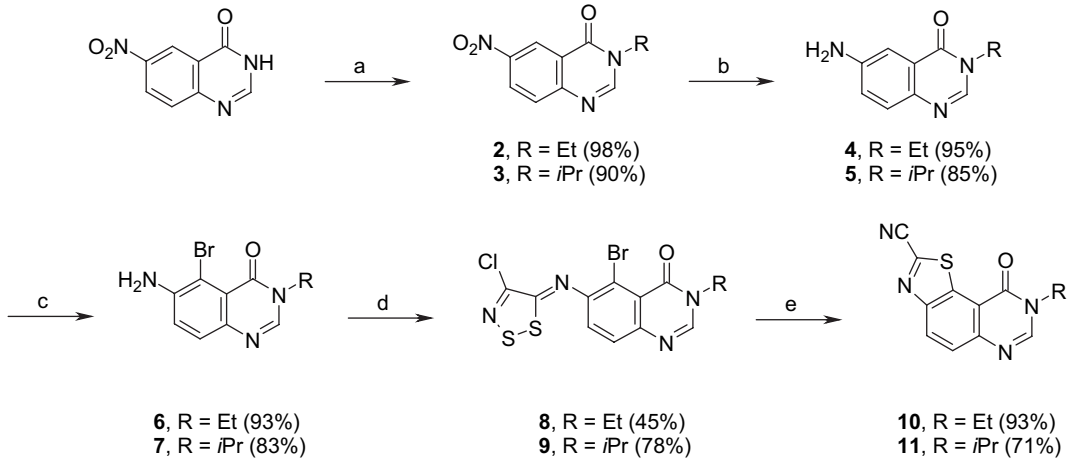
Table 1
Kinase inhibition values (IC_{50} in μM) for compounds **12**–**15** (NT = not tested)

Compounds	CDK1/cyclin B	CDK5/p25	GSK-3α/β
12a	50	NT	2.1
12b	4.3	NT	0.64
12c	1.4	NT	0.15
12d	1.3	>100	0.17
12e	1.4	4	0.3
13a	13	43	0.15
13b	5.2	NT	0.26
13c	2.4	NT	0.28
13d	0.15	>100	0.13
14a	>100	NT	7.2
14b	>10	>10	2.4
14c	17	NT	1.3
15a	>100	NT	9.4
15b	9	>10	0.52
15c	10	4	0.56
(*R*)-Roscovitine	0.45	0.16	130

2. Chemistry

2.1. Synthesis of the thiazoloquinazolinone core

The expected 9-oxo-thiazolo[4,5-*f*]quinazoline-2-carbonitrile derivatives (**10** and **11**) (Scheme 1) were obtained in six steps from commercially available 5-nitroanthranilic acid. The multistep synthesis was mainly performed under microwaves taking in account of our experience in this domain [8–10]. The experimental conditions and the yields of the different steps are described in Scheme 1. The 6-nitro-3*H*-quinazolin-4-one **1** was synthesized as we previously described by microwave heating, at atmospheric pressure, of 5-nitroanthranilic acid with 5 equiv of formamide (150 °C) [11]. Selective *N*-alkylation in position 3 of the quinazolin-4-one ring was performed at atmospheric pressure by treatment of **1** with sodium hydride and ethyl or isopropyl iodide as alkylating agents. Reduction of the 3-substituted-6-nitroquinazolin-4-ones **2** and **3** led to the 6-amino derivatives **4** and **5** by using ammonium formate for catalytic transfer hydrogenation in ethanol. Compounds **4** and **5** were brominated in the presence of bromine in acetic acid, to give the *ortho* brominated imines **6** and **7**. The latest were condensed, under microwaves, with 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt) [12] in dichloromethane and in a sealed tube. Addition of pyridine led to the desired imino-1,2,3-dithiazoloquinazolinones **8** and **9**. Products **10** and **11** were obtained by microwave-assisted thermolysis consisting of a rapid



Scheme 1. Reaction conditions: (a) alkyl iodide, NaH, DMF, (MW) 140 °C, 5 min; (b) HCO_2NH_4, Pd/C, EtOH, (MW) 80 °C; 15 min; (c) Br_2, AcOH, rt, 2 h; (d) 4,5-dichloro-1,2,3-dithiazolium chloride, pyridine, CH_2Cl_2, (MW) 80 °C, 4 min; (e) CuI, pyridine, (MW) 160 °C, 1 min.

heating of the starting imines (1 min at 160 °C in a sealed tube), in the presence of cuprous iodide in pyridine. Technology used for heating, time of reactions and yields are given in Scheme 1.

2.2. Synthesis of the 8-substituted thiazoloquinazolinones

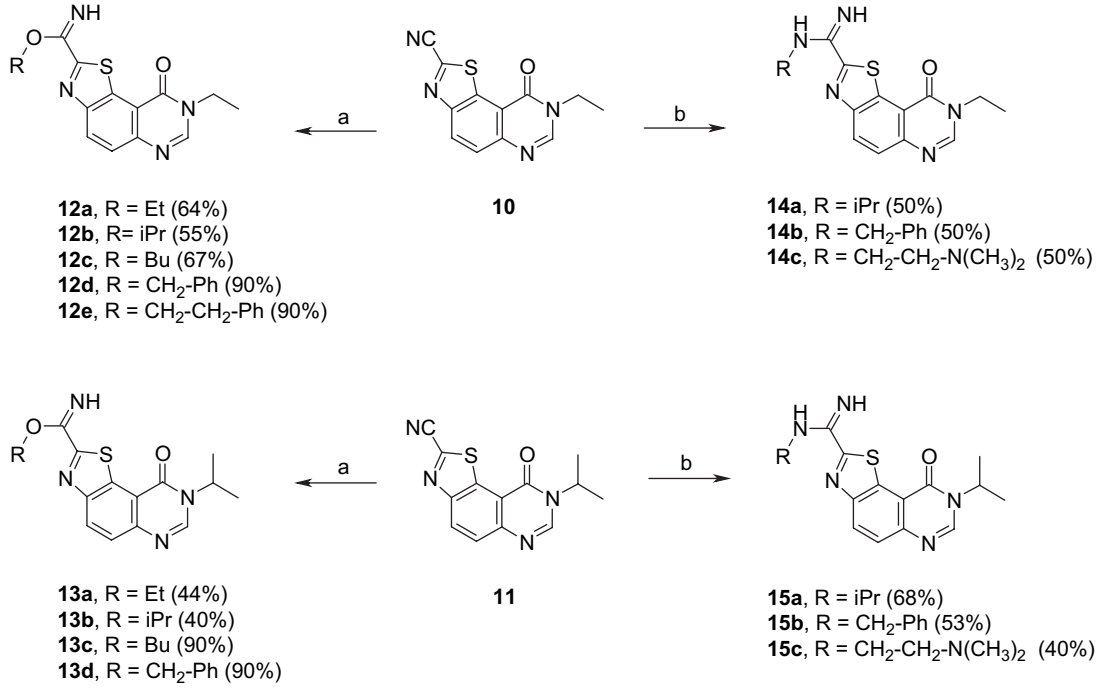
It is known that cyano group in position 2 of benzothiazole ring is very reactive and that its transformation into imidate, imidazoline, amidine and decyanated derivative can be easily realized [12]. Imidates **12a–e** and **13a–d** were, respectively, obtained in good yields from derivatives **10** and **11** by treatment with various alcohols (ethanol, isopropanol, butanol, or benzyl alcohols) in the presence of 1 equiv of NaOH 2.5 N (Scheme 2). The condensation of thiazoloquinazolinone-2-carbonitriles **10** and **11** with the commercially available appropriate amines in various solvents (e.g. ethanol, THF) was studied to give the desired amidines **14a–c** and **15a–c** (Scheme 2).

Thus, employing microwave-assisted organic synthesis allowed us to establish efficient conditions for the preparation of *N*-substituted thiazoloquinazolinones. Our results are all confirming that it is possible to achieve better yields and cleaner reactions by performing a majority of the reactions under microwave irradiation instead of using the purely thermal processes.

3. Results and discussion

3.1. Lead optimization

With the thiazolo[5,4-*f*]quinazoline-2-carboxamidine **15c**, as a moderate dual CDK1/GSK-3 lead molecule in hand (IC_{50} values of 10 μM and 0.56 μM, respectively) (Table 1), we first decided to examine the activities of another bulky substituent at the R position of the amidine function. Replacement of the *N*,*N*-dimethylethylenediamine group by a benzyl group gave compound **15b** with comparable potency (IC_{50} values of 9 μM and 0.52 μM, respectively) leading to the assumption that these molecules interact with the ATP-binding site of both enzymes in a very similar manner. In a parallel study, we found that substitution of the amidine function of **15b** by an imidate analogue **13d** considerably improves the efficacy of this compound to reach submicromolar activities in both CDK1 and GSK-3 (IC_{50} values of 0.15 μM and 0.13 μM, respectively). Taken together, these results seem to imply the role of both electrostatic and steric parameters at this level of interactions. In order to confirm the observed structure–activity relationships, we decided to investigate structure modifications on thiazoloquinazolinone nucleus either by introducing *N*-ethyl or *N*-isopropyl side chains on the N-8 nitrogen or by varying the substitution of the imidate side chain. A small number of compounds (**12a–e**;**13a–c**) was thus synthesized and their potency in the purified enzymes was measured. Compounds **12c–e** and **13c** bearing a bulky substituent at the R position of the imidate function (butyl for **12c** and **13c**, benzyl for **12d** and phenyl ethyl for **12e**) maintained a high inhibitory activity against GSK-3 (IC_{50} ranging from 0.15 to 0.30 μM) and were also able to inhibit CDK1, although a 10-fold drop in potency was found, compared to the lead compound **13d** (IC_{50} ranging from 1.30 to 2.40 μM). In contrast, compounds **12a** and **13a** with a smaller ethyl group led to weaker inhibitors of CDK1 (IC_{50} values of 50.0 μM and 13.0 μM, respectively). From a steric point of view, this result is in accordance with the previous observation



Scheme 2. Reaction conditions: (a) NaOH, alcohol, rt, 15 min; (b) amine, THF, (MW) 80 °C, 30 min.

that bulky substituents have a favorable effect on a dual CDK1/GSK-3 inhibitory activity.

3.2. Molecular modeling studies

In attempts to understand the observed structure–activity relationships (SARs), we have carried out molecular modeling studies using automated docking procedure into the ATP-binding site of GSK-3β in complex with the non-hydrolysable ATP analogue AMP-PNP at 1.80 Å resolution (pdb code: 1J1B) [13], following a flexible ligand/rigid receptor docking protocol, as described in Section 5.

The binding pose found by GOLD for imidate inhibitors **12c–e** and **13c,d** is shown in Fig. 1. As can be seen, the docking program positioned these molecules in such a way that the quinazoline nitrogen N-6 makes hydrogen bond with the backbone NH of residue Val135 in the hinge segment. This region of protein kinases makes critical H-bonding contacts to the vast majority of inhibitory molecules that have been published to date. At least, one H-bond is formed in practically all known kinase ligand structures in the Protein Data Bank [14]. Interestingly, due to the bulkiness of the substituents at the R position, these compounds may not fit into the hydrophobic backpocket of GSK-3β (a region which is not conserved and used to gain affinity as well as selectivity [15] but may form polar interactions between the imine nitrogen atom and the side-chain amino group of Lys85. This interaction drew our attention for two reasons. First, the model suggests that this residue plays a significant role in the potency of the compounds because such polar interactions are usually strong. Second, we speculated that it may also play a role in the dual CDK1/GSK-3 inhibitory activity displayed by these compounds since Lys85 (Lys33 in CDK1) is a residue conserved in all kinase enzymes and functionally important in kinase catalysis [1]. Therefore, considering the conserved ranges in CDK1/GSK-3 activities obtained for these molecules, we can hypothesize that an identical interaction could take place in the two enzymes.

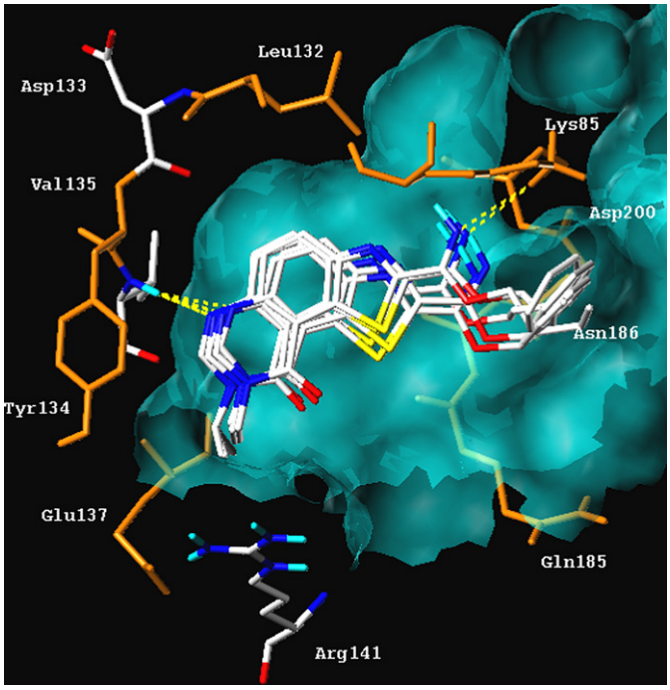


Fig. 1. Proposed binding mode for imidate inhibitors **12c–e** and **13c,d** into the ATP-binding site of GSK-3β starting from 1J1B (PDB code). The active site pocket is highlighted in cyan (MOLCAD surface; program SYBYL 7.2). The hydrogen bonds are indicated as yellow dotted lines. For interpretation of the references to color in the text, the reader is referred to the web version of this article.

In addition, for an explanation of the observed differences in activity between imidates **12d**, **13d** and amidine analogues **14b**, **15b**, which adopted a same orientation in the cavity of GSK-3β (not shown), molecular electrostatic potentials (MEPs) were calculated and projected onto the electron density surface of the inhibitors (in their docked conformations). Knowledge of MEPs is critically important when molecular interactions are to be studied. The results of these calculations are depicted in Fig. 2. Obviously, the molecular shape is rather similar. However, the MEP of inhibitor **15b** is quite electropositive near the NH of its amidine function (red area indicated by an arrow), in contrast to the imidate analogue **13d**, which is having a relatively more electronegative potential (blue region). It is probable that this charge distribution could be affecting more intensely the binding within the ATP cavity of CDK1 than in GSK-3β since amidine **15b** exhibited a dramatic decrease of CDK1 inhibitory potency ($IC_{50} = 9$ μM) as compared to imidate **13d** ($IC_{50} = 0.15$ μM at CDK1). Taking into account that the solvent effect was not considered, we cannot discard the possibility of an unfavorable desolvation with water during the binding process.

4. Conclusion

In conclusion the rapid and efficient synthesis of novel series of 9-oxo-thiazolo[5,4-*f*]quinazoline-2-carbonitrile derivatives was performed and optimized under microwave irradiation. Most of the amidine and imidate derivatives are potent and dual CDK1/GSK-3 inhibitors. Among these molecules, compound **13d** exhibits an efficient capacity to inhibit both CDK1 and GSK-3 (IC_{50} values of 0.15 μM and 0.13 μM, respectively). This work confirms that this family constitutes a promising scaffold from which more potent inhibitors could be designed.

5. Experimental

5.1. Chemistry

Commercial reagents were used as received without additional purification. Melting points were determined using a Köfler melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Paragon 1000PC instrument. ^{1}H and ^{13}C NMR were recorded on a JEOL NMR LA400 (400 MHz) spectrometer (Centre Commun d'Analyses, Université de la Rochelle); chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane

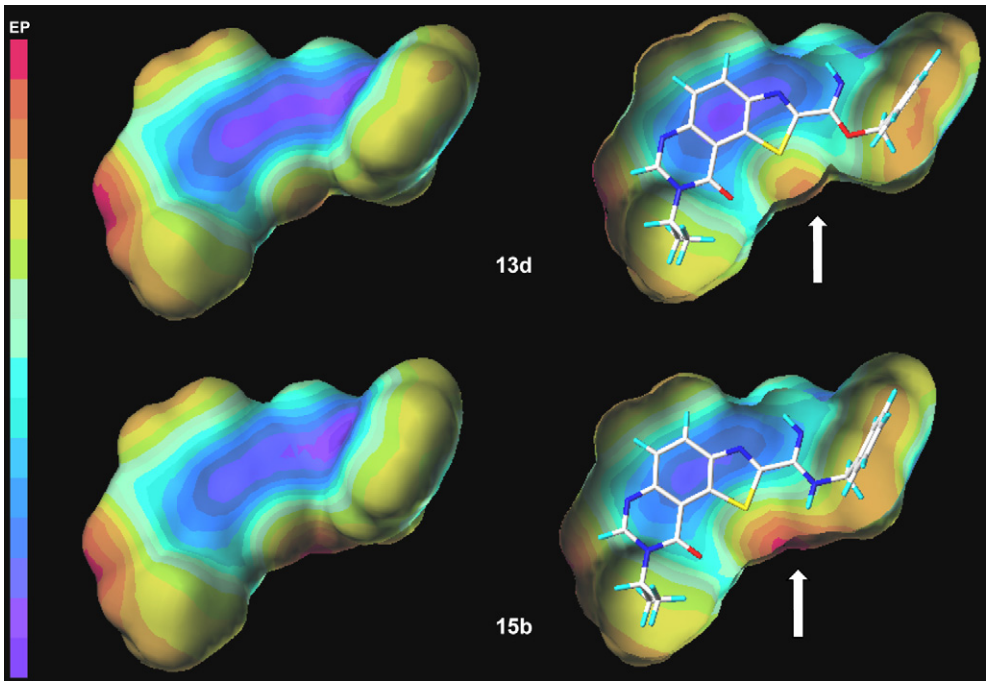


Fig. 2. Visualization of the molecular electrostatic potentials (MEPs) of **13d** and **15b** displayed onto electron density surfaces. Blue areas represent negative electrostatic potentials; red areas represent positive values. (The calculations have been performed using program MOLCAD.) Arrows indicate noteworthy differences in the charge distribution of the two congeners. For interpretation of the references to color in the text, the reader is referred to the web version of this article.

(TMS) which was used as an internal standard. Coupling constants *J* are given in hertz. The mass spectra (HRMS) were recorded on a Varian MAT311 spectrometer in the «Centre Régional de Mesures Physiques de l'Ouest» (CRMPO), Université de Rennes. Column chromatography was performed by using Merck silica gel (70–230 mesh) at medium pressure. Light petroleum refers to the fraction boiling point 40–60 °C. Other solvents were used without purification. Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 aluminium backed plates. Focused microwave irradiations were carried out with a Smith-Synthesizer® (Personal Chemistry AB now Biotage) or a CEM Discover® focused microwave reactor (300 W, 2450 MHz, monomode system). The Smith-Synthetizer® was a single mode cavity, producing controlled irradiation at 2450 MHz. Reaction temperature and pressure were determined using the built-in, online IR and pressure sensors. Microwave-assisted reactions were performed in sealed Smith process vials (0.5–5 mL, total volume 10 mL) under air with magnetic stirring. The software algorithm regulates the microwave output power so that the selected maximum temperature was maintained for the desired reaction/irradiation time. After the irradiation period, the reaction vessel was cooled rapidly to ambient temperature by compressed air (gas-jet cooling). The minimal reaction times were determined by performing sequential series of identical reactions at constant temperature and with continuous heating, but with different irradiation times. Completion of the reaction was estimated by TLC after each individual heating period. The Discover® focused microwave reactor (300 W, 2450 MHz, monomode system) has in situ magnetic variable speed rotation, irradiation monitored by PC computer, infrared measurement and continuous feedback temperature control. Experiments may be performed at atmospheric pressure or in a sealed tube in pressure-rated reaction tubes with continuous pressure measurement.

Spectral data for compound **1** is consistent with assigned structure as previously described by Alexandre et al. [11].

*5.1.1. Synthesis of N-substituted quinazolinones **2** and **3***

Alkylating agent (6 mmol) was added dropwise to a stirred suspension of quinazolinone **1** (5 mmol) and sodium hydride (6 mmol; 60% dispersion in mineral oil) in DMF (3 mL). The mixture was irradiated for 5 min in a sealed tube. The irradiation was programmed to obtain a constant temperature (140 °C). The solvent was removed under reduced pressure, and the residue was hydrolyzed with water and extracted with ethyl acetate. The organic layers dried over magnesium sulfate were evaporated in vacuo. The product was obtained by purification by column chromatography with dichloromethane–ethyl acetate (90/10) as eluent.

*5.1.1.1. 3-Ethyl-6-nitro-3H-quinazolin-4-one (**2**).* Obtained in 98% yield as a yellow solid (mp = 156 °C). Data supporting its chemical structure are reported in Ref. [10].

*5.1.1.2. 3-Isopropyl-6-nitro-3H-quinazolin-4-one (**3**).* Obtained in 90% yield as a yellow solid (mp = 166 °C), IR (KBr) ν_{max}/cm^{-1} 3093, 2979, 1912, 1668, 1621, 1568, 1530, 1467, 1340, 1276, 1176, 1139, 1062, 854, 752, 696, 627; ^1H NMR δ (400 MHz, $CDCl_3$) 1.53 (d, 6H, $J = 6.8$ Hz, $2CH_3$),

5.16–5.23 (m, 1H, CH), 7.83 (d, 1H, $J = 8.8$ Hz, H_8), 8.25 (s, 1H, H_2), 8.54 (dd, 1H, $J = 2.9$ Hz, $J = 8.8$ Hz, H_7), 9.18 (d, 1H, $J = 2.9$ Hz, H_5); ^{13}C NMR δ (100 MHz, $CDCl_3$) 21.95, 46.93, 122.14, 123.64, 128.20, 129.02, 145.97, 146.61, 151.63, 159.64; MS (EI) M^+: 233.

*5.1.2. Synthesis of quinazolinones **4** and **5***

A stirred mixture of 1 mmol of **2** or **3**, 5 mmol of ammonium formate and a catalytic amount of 10% palladium charcoal in 20 mL of ethanol was irradiated for 15 min. The irradiation was programmed to obtain a constant temperature (80 °C) with a maximal power output of 40 W. The catalyst was removed by filtration. The resulting filtrate was dissolved in ethyl acetate, washed with water, dried and concentrated under reduced pressure. The amines **4** or **5** were obtained without further purification.

*5.1.2.1. 6-Amino-3-ethyl-3H-quinazolin-4-one **4**.* Obtained in 95% yield as a white solid (mp = 168 °C). Data supporting its chemical structure are reported in Ref. [10].

*5.1.2.2. 6-Amino-3-isopropyl-3H-quinazolin-4-one **5**.* Obtained in 85% yield as a white solid (mp = 138 °C), IR (KBr) ν_{max}/cm^{-1} 3221, 2977, 2368, 1652, 1496, 1460, 1352, 1316, 1276, 1252, 1176, 1096, 880, 749, 554; ^{1}H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.47 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 5.16–5.23 (m, 1H, CH), 7.10 (dd, 1H, $J = 2.4$ Hz, $J = 8.8$ Hz, H_7), 7.49 (d, 1H, $J = 2.4$ Hz, H_5), 7.53 (d, 1H, $J = 8.8$ Hz, H_8), 7.96 (s, 1H, H_2); ^{13}C NMR δ (100 MHz, $CDCl_3$) 21.96, 45.77, 108.89, 122.84, 122.91, 128.37, 140.18, 140.22, 145.86, 160.47; MS (EI) M^+: 203.

*5.1.3. Synthesis of brominated quinazolinones **6** and **7***

A solution of bromine (5.6 mmol) in dichloromethane (5 mL) was added dropwise, under an inert atmosphere, to a solution of amines **4** or **5** (5.6 mmol), in acetic acid (30 mL). After 2 h of stirring at room temperature, the solvent was removed in vacuo. Then the mixture was dissolved in ethyl acetate and washed with sodium thiosulfate solution (20 mL). The solvent was removed in vacuo and the crude residue purified by column chromatography (dichloromethane–ethyl acetate: 90/10) to afford the expected compound **6** or **7**.

*5.1.3.1. 6-Amino-5-bromo-3-ethyl-3H-quinazolin-4-one **6**.* Obtained in 93% yield as a red solid (mp = 140 °C). Data supporting its chemical structure are reported in Ref. [10].

*5.1.3.2. 6-Amino-5-bromo-3-isopropyl-3H-quinazolin-4-one **7**.* Obtained in 83% yield as a red solid (mp = 140 °C), IR (KBr) ν_{max}/cm^{-1} 3469, 3354, 3158, 2978, 1665, 1619, 1537, 1482, 1414, 1339, 1236, 1096, 936, 828, 632, 548; ^{1}H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.46 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 5.17–5.24 (m, 1H, CH), 7.18 (d, 1H, $J = 8.8$ Hz, H_7), 7.50 (d, 1H, $J = 8.8$ Hz, H_8), 7.96 (s, 1H, H_2); ^{13}C NMR δ (100 MHz, $CDCl_3$) 21.99, 45.95, 103.75, 120.29, 121.85, 127.74, 140.78, 141.98, 144.51, 158.99; MS (EI) M^+: 282.

*5.1.4. General procedure for the synthesis of imino-1,2,3-dithiazoles **8** and **9***

A suspension of the bromo amine **6** or **7** (2.5 mmol), 4,5-dichloro-1,2,3-dithiazolium chloride (2.75 mmol) in dichloromethane (4 mL) was irradiated for 4 min in a sealed tube. The irradiation was programmed to obtain a constant temperature (80 °C). After cooling at room temperature, pyridine (5.5 mmol) was added. The resulting solution was dissolved in ethyl acetate, washed with water, dried and concentrated under reduced pressure. The crude residue was purified by column chromatography (dichloromethane–ethyl acetate: 90/10) to afford the expected compound **8** or **9**.

*5.1.4.1. 5-Bromo-6-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-amino]-3-ethyl-quinazolin-3H-4-one **8**.* Obtained in 45% yield as an orange solid (mp = 182 °C). Data supporting its chemical structure are reported in Ref. [10].

*5.1.4.2. 5-Bromo-6-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-amino]-3-isopropyl-quinazolin-3H-4-one **9**.* Obtained in 78% yield as an orange solid (mp = 210 °C), IR (KBr) ν_{max}/cm^{-1} 2978, 1664, 1596, 1533, 1457, 1373, 1254, 1156, 940, 864, 813, 764, 663. ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.50 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 5.16–5.23 (m, 1H, CH), 7.39 (d, 1H, $J = 8.8$ Hz, H_7), 7.75 (d, 1H, $J = 8.8$ Hz, H_8), 8.12 (s, 1H, H_2); ^{13}C NMR δ (100 MHz, $CDCl_3$) 22.00, 46.43, 113.07, 121.20, 124.13, 129.12, 143.61, 147.25, 147.40, 150.90, 158.95, 162.16. MS (EI) M^+: 416.

*5.1.5. Synthesis of thiazolo[5,4-f]quinazoline-2-carbonitriles **10** and **11***

A suspension of imine **8** or **9** (1 mmol), copper iodide (2 mmol) in pyridine (4 mL) was irradiated for 1 min in a sealed tube. The irradiation was programmed to obtain a constant temperature (160 °C). After cooling, the mixture was dissolved in ethyl acetate, washed with sodium thiosulfate solution (20 mL). The solvent was removed in vacuo and the crude residue purified by column chromatography (dichloromethane–ethyl acetate: 80/20) to afford the expected compound.

*5.1.5.1. 8-Ethyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carbonitrile **10**.* Obtained in 93% yield as a white solid (mp > 260 °C). Data supporting its chemical structure are reported in Ref. [10].

*5.1.5.2. 8-Isopropyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carbonitrile **11**.* Obtained in 71% yield as a white solid (mp > 260 °C), IR (KBr) ν_{max}/cm^{-1} 2973, 2236, 1674, 1580, 1496, 1357, 1276, 1247, 1174, 839, 581; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.59 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 5.24–5.31 (m, 1H, CH), 8.00 (d, 1H, $J = 8.8$ Hz, H_4), 8.36 (s, 1H, H_7), 8.53 (d, 1H, $J = 8.8$ Hz, H_5); ^{13}C NMR

δ (100 MHz, $CDCl_3$) 22.18, 46.52, 114.59, 126.39, 129.41, 143.73, 147.07, 159.28, 176.49. MS (EI) M^+: 270.

5.1.6. Methods for the synthesis of 2-substituted thiazolo-[5,4-f]quinazolinones

5.1.6.1. Synthesis of imidates. A stirred mixture of carbonitrile **10** or **11** (0.5 mmol) and 2.5 N NaOH (0.5 mmol) in ethanol (5 mL) under argon was stirred at room temperature for 15 min. The resulting precipitate was collected, washed with water and dried to give the imidate **12** or **13** as a white crystalline powder.

*5.1.6.1.1. 8-Ethyl-9-oxo-8,9-dihydro-6-thiazolo[5,4-f]quinazoline-2-carboximidic acid ethyl ester **12a***. Obtained in 64% yield as a white solid (mp = 232 °C). Data supporting its chemical structure are reported in Ref. [10].

*5.1.6.1.2. 8-Ethyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid isopropyl ester **12b***. Obtained in 55% yield as a white solid (mp = 210 °C), IR (KBr) ν_{max}/cm^{-1} 3267, 2984, 1662, 1586, 1497, 1366, 1350, 1337, 1317, 1260, 1144, 1053, 896; ^{1}H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.46 (d, 6H, $J = 6.3$ Hz, $2CH_3$), 1.51 (t, 3H, $J = 7.3$ Hz, CH_3), 4.21 (q, 2H, $J = 7.3$ Hz, CH_2), 5.37–5.40 (m, 1H, CH), 7.90 (d, 1H, $J = 8.8$ Hz, H_4), 8.23 (s, 1H, H_7), 8.45 (d, 1H, $J = 8.8$ Hz, H_5). Found M^+: 316.0974, $C_{15}H_{16}N_4O_2S$ requires 316.0994.

*5.1.6.1.3. 8-Ethyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid butyl ester **12c***. Obtained in 67% yield as a white solid (mp = 160 °C), IR (KBr) ν_{max}/cm^{-1} 3268, 2937, 2877, 1660, 1586, 1498, 1451, 1370, 1350, 1323, 1256, 1131, 1066; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.02 (t, 3H, $J = 7.4$ Hz, CH_3), 1.51 (t, 2H, $J = 7.6$ Hz, CH_3), 1.57 (q, 2H, $J = 7.6$ Hz, CH_2), 1.81–1.88 (m, 2H, CH_2), 4.21 (q, 2H, $J = 7.4$ Hz, CH_2), 4.51 (t, 2H, $J = 7.6$ Hz, CH_2), 7.91 (d, 1H, $J = 8.8$ Hz, H_4), 8.23 (s, 1H, H_7), 8.46 (d, 1H, $J = 8.8$ Hz, H_5), 8.96 (s, 1H, NH). Found M^+: 330.1143 $C_{16}H_{18}N_4O_2S$ requires 330.1150.

*5.1.6.2. 8-Ethyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid benzyl ester **12d***. Obtained in 90% yield as a white solid (mp = 210 °C), IR (KBr) ν_{max}/cm^{-1} 3290, 2971, 1663, 1585, 1497, 1337, 1262, 1149, 1118, 1081, 835, 731; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.50 (t, 3H, $J = 7.2$ Hz, CH_3), 4.20 (q, 2H, $J = 7.2$ Hz, CH_2), 5.51 (s, 2H, CH_2), 7.37–7.54 (m, 5H, Har), 7.90 (d, 1H, $J = 8.8$ Hz, H_4), 8.22 (s, 1H, H_7), 8.45 (d, 1H, $J = 8.8$ Hz, H_5), 9.13 (s, 1H, NH). Found M^+: 364.1000, $C_{19}H_{15}N_4O_2S$ requires 364.0994.

*5.1.6.2.1. 8-Ethyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid phenyl ethyl ester **12e***. Obtained in 90% yield as a white solid (mp = 200 °C), IR (KBr) ν_{max}/cm^{-1} 3207, 2930, 1660, 1584, 1498, 1454, 1354, 1277, 1251, 1141, 1112, 1054, 830, 751, 696; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.52 (t, 3H, $J = 7.2$ Hz, CH_3), 3.19 (t, 2H, $J = 6.8$ Hz, CH_2), 4.22 (q, 2H, $J = 7.2$ Hz, CH_2), 4.66 (t, 2H, $J = 6.8$ Hz, CH_2), 7.32–7.38 (m, 5H, Har), 7.89 (d, 1H, $J = 8.8$ Hz, H_4), 8.23 (s, 1H, H_7), 8.44 (d, 1H, $J = 8.8$ Hz, H_5), 8.99 (s, 1H, NH); ^{13}C NMR δ (100 MHz, $CDCl_3$) 14.97, 35.06, 42.58, 67.77, 116.82, 126.54, 126.76, 126.52, 129.09, 129.81, 133.14, 138.03, 146.37, 147.82, 151.80, 159.57, 161.23, 162.06. Found $[M-C_8H_8]^+$: 274.0517, $C_{20}H_{18}N_4O_2S$ requires 274.0524.

*5.1.6.2.2. 8-Isopropyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid ethyl ester **13a***. Obtained in 44% yield as a white solid (mp = 240 °C), IR (KBr) ν_{max}/cm^{-1} 2985, 2322, 1674, 1586, 1500, 1353, 1284, 1120, 1072, 832, 692, 511; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.49 (t, 3H, $J = 6.8$ Hz, CH_3), 1.58(d, 6H, $J = 6.8$ Hz, $2CH_3$), 4.51 (q, 2H, $J = 6.8$ Hz, CH_2), 5.23–5.30 (m, 1H, CH), 7.90 (d, 1H, $J = 8.8$ Hz, H_4), 8.29 (s, 1H, H_7), 8.44 (d, 1H, $J = 8.8$ Hz, H_5), 8.96 (s, 1H, NH). Found M^+: 316.0974, $C_{15}H_{16}N_4O_2S$ requires 316.0994.

*5.1.6.2.3. 8-Isopropyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid isopropyl ester **13b***. Obtained in 40% yield as a white solid (mp = 140 °C), IR (KBr) ν_{max}/cm^{-1} 3278, 2924, 2852, 1655, 1587, 1496, 1452, 1353, 1323, 1317, 1280, 1248, 1160, 110, 1072, 832; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.46 (d, 6H, $J = 6.4$ Hz, $2CH_3$), 1.57 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 5.22–5.28 (m, 1H, CH), 5.37–5.40 (m, 1H, CH), 7.89 (d, 1H, $J = 8.8$ Hz, H_4), 8.29 (s, 1H, H_7), 8.45 (d, 1H, $J = 8.8$ Hz, H_5), 8.95 (s, 1H, NH). Found M^+: 330.1147, $C_{16}H_{18}N_4O_2S$ requires 330.1150.

*5.1.6.2.4. 8-Isopropyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid butyl ester **13c***. Obtained in 90% yield as a white solid (mp = 164 °C), IR (KBr) ν_{max}/cm^{-1} 3274, 2924, 2853, 1656, 1583, 1499, 1354, 1317, 1277, 1248, 1132, 118, 1071, 839; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.02 (t, 3H, $J = 7.2$ Hz, CH_3), 1.54–1.58 (m, 2H, CH_3), 1.58 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 1.83–1.87 (m, 2H, CH_2), 4.43–4.46 (m, 2H, CH_2), 5.26–5.30 (m, 1H, CH), 7.90 (d, 1H, $J = 8.8$ Hz, H_4), 8.30 (s, 1H, H_7), 8.45 (d, 1H, $J = 8.8$ Hz, H_5), 8.94 (s, 1H, NH). Found M^+: 344.1316, $C_{17}H_{20}N_4O_2S$ requires 344.1307.

*5.1.6.2.5. 8-Isopropyl-9-oxo-8,9-dihydro-thiazolo[5,4-f]quinazoline-2-carboximidic acid benzyl ester **13d***. Obtained in 90% yield as a white solid (mp = 214 °C), IR (KBr) ν_{max}/cm^{-1} 3207, 2930, 1660, 1584, 1498, 1454, 1354, 1277, 1251, 1141, 1112, 1054, 830, 751, 696; ^{1}H NMR δ (400 MHz, $CDCl_3$) 1.57 (d, 6H, $J = 7.0$ Hz, $2CH_3$), 5.23–5.30 (m, 1H, CH), 5.52 (s, 2H, CH_2), 7.37–7.54 (m, 5H, Har), 7.91 (d, 1H, $J = 8.8$ Hz, H_4), 8.30 (s, 1H, H_7), 8.46 (d, 1H, $J = 8.8$ Hz, H_5), 9.13 (s, 1H, NH). Found M^+: 378.1137, $C_{20}H_{17}N_4O_2S$ requires 378.1150.

5.1.6.3. Synthesis of amidines. A stirred mixture of carbonitrile **10** or **11** (1 mmol) and amine (5 mmol) in dry THF (10 mL) under argon was irradiated in a sealed tube for 30 min. The irradiation was programmed to obtain a constant temperature (80 °C). The mixture was dissolved in dichloromethane, washed with water. The solvent was removed in vacuo and the crude residue purified by column chromatography (dichloromethane–methanol: 90/10) to afford the amidine **14** or **15**.

*5.1.6.3.1. N-Isopropyl-8-ethyl-9-oxo-8,9-dihydrothiazolo [5,4-f]quinazoline-2-carboxamidine **14a***. Obtained in 50%

yield as white solid (mp = 197 °C), IR (KBr) ν_{max}/cm^{-1} 3394, 3278, 2965, 2932, 1653, 1619, 1587, 1522, 1451, 1365, 1184, 1159, 828; ^1H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.34 (d, 6H, $J = 6.4$ Hz, $2CH_3$), 1.51 (t, 3H, $J = 7.4$ Hz, CH_3), 3.87–3.90 (m, 1H, CH), 4.20 (q, 2H, CH_2), 7.85 (d, 1H, $J = 8.8$ Hz, H_4), 8.20 (s, 1H, H_7), 8.36 (d, 1H, $J = 8.8$ Hz, H_5). Found $[M]^+$: 315.1169, $C_{15}H_{17}N_5OS$ requires 315.1154.

5.1.6.3.2. N-Benzyl-8-ethyl-9-oxo-8,9-dihydrothiazolo[5,4-f]quinazoline-2-carboxamidine ***14b***. Obtained in 50% yield as white solid (mp = 128 °C), IR (KBr) ν_{max}/cm^{-1} 3447, 3324, 2932, 1646, 1588, 1502, 1450, 1344, 1261, 1165, 1106, 967, 823, 742; ^1H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.50 (t, 3H, $J = 7.2$ Hz, CH_3), 4.20 (q, 2H, CH_2), 4.58 (s, 2H, CH_2), 7.31–7.40 (m, 5H, CHar), 7.85 (d, 1H, $J = 8.8$ Hz, H_4), 8.20 (s, 1H, H_7), 8.36 (d, 1H, $J = 8.8$ Hz, H_5). Found $[M]^+$: 363.1144, $C_{19}H_{17}N_5OS$ requires 363.1154.

5.1.6.3.3. N-(2-Dimethylamino-ethyl)-8-ethyl-9-oxo-8,9-dihydrothiazolo[5,4-f]quinazoline-2-carboxamidine ***14c***. Obtained in 50% yield as a white solid (mp = 144 °C). Data supporting its chemical structure are reported in Ref. [10].

5.1.6.3.4. N-Isopropyl-8-isopropyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboxamidine ***15a***. Obtained in 68% yield as white solid (mp = 217 °C), IR (KBr) ν_{max}/cm^{-1} 3389, 3276, 2979, 2925, 1649, 1621, 1584, 1519, 1453, 1349, 1174, 827, 808; ^1H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.38 (d, 6H, $J = 6.4$ Hz, $2CH_3$), 1.57 (d, 6H, $J = 7.2$ Hz, $2CH_3$), 4.06–4.08 (m, 1H, CH), 5.26–5.31 (m, 1H, CH), 7.88 (d, 1H, $J = 8.8$ Hz, H_4), 8.29 (s, 1H, H_7), 8.38 (d, 1H, $J = 8.8$ Hz, H_5). Found $[M]^+$: 329.1295, $C_{16}H_{19}N_5OS$ requires 329.1310.

5.1.6.3.5. N-Benzyl-8-isopopyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboxamidine ***15b***. Obtained in 53% yield as white solid (mp > 240 °C), IR (KBr) ν_{max}/cm^{-1} 3456, 3317, 3176, 2981, 1651, 1584, 1495, 1451, 1347, 1279, 1174, 1092, 824, 738, 697; ^1H NMR δ (400 MHz, DMSO-$d_6 + D_2O$) 1.50 (d, 6H, $J = 7.2$ Hz, $2CH_3$), 3.15 (s, 2H, CH_2), 5.04–5.08 (m, 1H, CH), 7.24–7.47 (m, 5H, Har), 7.83 (d, 1H, $J = 8.8$ Hz, H_4), 8.42 (d, 1H, $J = 8.8$ Hz, H_5), 8.59 (s, 1H, H_7). Found $[M]^+$: 377.1330, $C_{20}H_{19}N_5OS$ requires 377.1310.

5.1.6.3.6. N-(2-Dimethylamino-ethyl)-8-isopopyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboxamidine ***15c***. Obtained in 40% yield as a white solid (mp = 148 °C), IR (KBr) ν_{max}/cm^{-1} 2978, 2380, 2323, 1656, 1588, 1348, 1281, 1021, 831, 732, 634, 540; ^1H NMR δ (400 MHz, $CDCl_3 + D_2O$) 1.55 (d, 6H, $J = 6.8$ Hz, $2CH_3$), 2.50 (s, 6H, $2CH_3$), 2.89 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 5.27–5.30 (m, 1H, CH), 7.84 (d, 1H, $J = 8.8$ Hz, H_4), 8.26 (s, 1H, H_7), 8.36 (d, 1H, $J = 8.8$ Hz, H_5). Found $[M-C_4H_8N]^+$: 288.0943, $C_{17}H_{22}N_6OS-C_4H_8N$ requires 288.0919.

5.2. Biological evaluation

Modified 9-oxo-thiazolo[5,4-*f*]quinazoline-2-carbonitrile derivatives and (*R*)-roscovitine were tested against three protein kinases, CDK1/cyclin B, CDK5/p25 and GSK-3α/β [16–18]. 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.

5.3. Molecular modeling

5.3.1. Docking studies

Molecular modeling studies were performed with the commercially available SYBYL 7.2 software package running on a Silicon Graphics Octane workstation [19]. The three-dimensional structure of all compounds were built from a standard fragments library, and their geometry was subsequently optimized using the Tripos force field [20] including the electrostatic term calculated from Gasteiger and Hückel atomic charges. Powell's method available in Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol Å. Atomic coordinates for the GSK-3β in complex with AMP-PNP have been deposited in the Protein Data Bank with resolution of 1.80 Å (PDB code: 1J1B) [13] and was used as the target structure for docking. The original ligand as well as the water molecules were removed from the coordinates set. Flexible docking of molecules into the ATP-binding site was performed using GOLD software [21]. For each compound, the most stable docking model was selected according to the best scored conformation predicted by the GoldScore [21] and X-Score [22] scoring functions. The complexes were energy-minimized using Powell's method available in Maximin2 procedure with the Tripos force field and a dielectric constant of 4.0 until the gradient value reached 0.1 kcal/mol Å.

5.3.2. Molecular electrostatics potentials (MEPs)

Molecular electrostatic potentials (MEPs) for the most active compound imidate **13d** and its amidine analogue **15b** (in their docked conformations) were displayed using the program MOLCAD in SYBYL 7.2. Partial atomic charges were computed with the quantum chemistry package program MOPAC version 6.0 [23] using the semi-empirical molecular orbital method AM1. Partial charges were derived using Mulliken population analysis. Electrostatic potential was mapped onto the electron density surface for both inhibitors.

Acknowledgements

We thank the ''Comités de Charente et de Charente-Maritime de la Ligue Nationale Contre le Cancer'' for financial support. The work presented here was also supported by a grant from the EEC (FP6-2002-Life Sciences & Health, PRO-KINASE Research Project) (L.M.) and the Cancéropôle Grand-Ouest.

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