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# Synthesis and evaluation of the antiproliferative activity of novel thiazoloquinazolinone kinases inhibitors

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**Abstract**

The microwave-assisted synthesis of a family of 2,8-substituted thiazoloquinazolinones is described. The preliminary evaluation of the antiproliferative activity and the capacity of these molecules to inhibit CDKs and GSK-3 are reported. A lead compound was identified, constituting a scaffold from which more potent inhibitors could be designed.

**Keywords:** *CDK, GSK-3, kinases, breast cancer, quinazolinones, microwave chemistry*



## Introduction

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

Studying the interesting chemistry of 4,5-dichloro-1,2,3-dithiazolium chloride (Appel's salt) [5–7] and its derivatives, we recently described the microwave-assisted multistep synthesis of novel thiazoloquinazolinones [8–9] (**IV** and **V**, Figure 2) which can be considered as hybrid molecules between the purines and the oxindoles mentioned above. The new molecules described in this previous work share some common properties with the majority of CDK inhibitors described in the literature [2–4]; they have low molecular weights ($<600$) and they are flat with an hydrophobic heterocycle core.

Continuing our efforts to optimise the synthesis and to enhance the potential pharmaceutical properties of such molecules, we decided to re-investigate the chemical access of various thiazoloquinazolinones, analogues to **IV** and **V**, with the aim to identify a lead compound for further pharmacomodulation.

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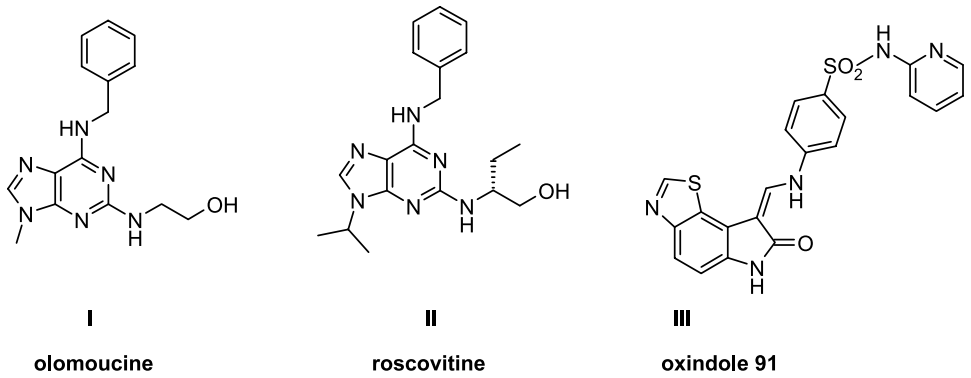



Figure 1. Structure of olomoucine, roscovitine and oxindole 91.



In this article we report the benefits associated with the microwave methodology [10,11] for the preparation of these new products **VI** and **VII** (Figure 2). The effects on CDKs and GSK-3 were investigated and the anti-proliferative effect of selected compounds was also tested.

## Materials and methods

### *Chemistry*

*Instrumentation*. Commercial reagents were used as received without additional purification. Melting points were determined using a Kofler 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 in the "Centre Commun d'Analyses, Université de la Rochelle". Chemical shifts (δ) are reported in part per million (ppm) downfield from tetramethylsilane (TMS) which was used as internal standard. Coupling constants J are given in Hz. 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-Synthetizer™ (Personal Chemistry, AB) 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, on-line 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 T.L.C. after each individual heating period. The CEM 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 compounds **1** and **2**, are consistent with assigned structures as previously described by Alexandre et al. [9].

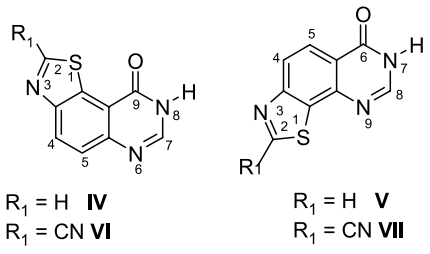



Figure 2. Structures of the studied 8*H*-thiazolo[5,4-*f*]quinazolin-9-ones **IV**, **VI** and the 7*H*-thiazolo[4,5-*h*]quinazolin-6-ones **V**, **VII**.

*Synthesis of N-substituted quinazolinone derivatives*. To a stirred suspension of quinazolinone **1** or **2** (5 mmol) and sodium hydride (6 mmol) (60% dispersion in mineral oil) in DMF (3 mL) was added dropwise 6 mmol of alkylating agent. 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.

*3-Ethyl-6-nitroquinazolin-4-(3H)-one (**3**)*. This compound was prepared from precursor **1**. Yield: 98%, yellow solid, mp = 156°C. (Found $M^+$: 219.0641, $C_{10}H_9N_3O_3$ requires 219.0644); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3093, 2917, 1681, 1606, 1575, 1519, 1481, 1337; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.47 (t, 3H, *J* 7.2 Hz, $CH_3$), 4.12 (q, 2H, *J* 7.2 Hz, $CH_2$), 7.84 (d, 1H, *J* 8.8 Hz, $H_8$), 8.19 (s, 1H, $H_2$), 8.54 (dd, 1H, *J* 2.8 Hz, *J* 8.8 Hz, $H_7$), 9.18 (d, 1H, *J* 2.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.81, 42.63, 122.39, 123.39, 128.20, 129.15, 146.00, 149.16, 152.20, 159.84.

*3-Benzyl-6-nitroquinazolin-4-(3H)-one (**4**)*. This compound was prepared from precursor **1**. Yield: 85%, yellow solid, mp = 164°C, (Found $M^+$: 281.0787, $C_{15}H_{11}N_3O_3$ requires 281.0800); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3088, 1681, 1602, 1571, 1522, 1474, 1344, 1258, 1156, 1076, 940, 848, 751, 716, 696, 630, 515; $^1$H-NMR δ (400 MHz, $CDCl_3$) 5.25 (s, 2H, $CH_2$), 7.37–7.38 (m, 5H, Har), 7.84 (d, 1H, *J* 9.2 Hz, $H_8$), 8.24 (s, 1H, $H_2$), 8.54 (dd, 1H, *J* 2.4 Hz, *J* 9.2 Hz, $H_7$), 9.20 (d, 1H, *J* 2.4 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 50.04, 122.44, 123.57, 127.73, 128.21, 128.38, 128.77, 129.25, 134.83, 146.01, 149.12, 151.99, 159.92.

*3-Ethyl-7-nitroquinazolin-4-(3H)-one (**5**)*. This compound was prepared from precursor **2**. Yield: 50%, yellow solid, mp = 154°C, (Found $M^+$: 219.0641, $C_{10}H_9N_3O_3$ requires: 219.0643); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3101, 3033, 1673, 1604, 1528, 1464, 1336, 1250, 1172, 1093, 935, 835, 794, 746, 695, 476; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.47 (t, 3H, *J* 7.2 Hz, $CH_3$), 4.11 (q, 2H, *J* 7.2 Hz, $CH_2$), 8.17 (s, 1H, $H_2$), 8.27 (dd, 1H, *J* 1.9 Hz, *J* 8.8 Hz, $H_6$), 8.48 (d, 1H, *J* 8.8 Hz, $H_5$), 8.56 (d, 1H, *J* 1.9 Hz, $H_8$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.76, 42.60, 120.92, 123.16, 126.25, 128.67, 148.22, 148.71, 151.38, 159.75.

*3-Benzyl-7-nitroquinazolin-4-(3H)-one (**6**)*. This compound was prepared from precursor **2**. Yield: 41%, yellow solid, mp = 160°C, (Found $M^+$: 281.0787, $C_{15}H_{11}N_3O_3$ requires 281.0800); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3103, 2343, 1679, 1519, 1355, 1291, 1268, 1076, 802, 741, 694, 549; $^1$H-NMR δ (400 MHz, $CDCl_3$) 5.23 (s, 2H, $CH_2$), 7.35–7.38 (m, 5H, Har), 8.22 (s, 1H, $H_2$), 8.27 (dd, 1H, *J* 2.4 Hz, *J* 8.8 Hz, $H_6$), 8.49 (d, 1H, *J* 8.8 Hz, $H_5$), 8.55 (d, 1H, *J* 2.4 Hz, $H_8$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.98, 120.98, 123.17, 126.25, 128.14, 128.65, 128.81, 129.16, 134.89, 148.18, 148.53, 151.41, 159.83.

*Reduction of nitroquinazolinones*. A stirred mixture of nitro precursor **3**, **4**, **5** or **6**(1 mmol), ammonium formate (5 mmol) 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 over magnesium sulfate and concentrated under reduced pressure. The amine was isolated without further purification.

*6-Amino-3-ethylquinazolin-4-(3H)-one (**7**)*. This compound was prepared from precursor **3**. Yield: 95%, white solid, mp = 168°C; (Found $M^+$: 189.0898, $C_{10}H_{11}N_3O$ requires 189.0902); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3207, 2956, 1656, 1494, 1382, 1350, 1262; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.40 (t, 3H, *J* 7.6 Hz, $CH_3$), 4.04 (q, 2H, *J* 7.6 Hz, $CH_2$), 7.10 (dd, 1H, *J* 2.8 Hz, *J* 8.8 Hz, $H_7$), 7.49 (d, 1H, *J* 2.8 Hz, $H_5$), 7.53 (d, 1H, *J* 8.8 Hz, $H_8$) 7,87 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.87, 41.95, 108.62, 122.78, 123.20, 128.57, 140.83, 142.93, 145.98, 160.75.

*6-Amino-3-benzylquinazolin-4-(3H)-one (**8**)*. This compound was prepared from precursor **4**. Yield: 98%, white solid, mp = 174°C, (Found $M^+$: 251.1060, $C_{15}H_{13}N_3O$ requires 251.1059); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3200, 3060, 2348, 1667, 1614, 1492, 1352, 1317, 1260, 1161, 928, 882, 832, 695, 612; $^1$H-NMR δ (400 MHz, $CDCl_3$) 4.06 (s, 2H, $NH_2$), 5.18 (s, 2H, $CH_2$), 7.10 (dd, 1H, *J* 2.4 Hz, *J* 8.8 Hz, $H_7$), 7.33–7.34 (m, 5H, Har), 7.49 (d, 1H, *J* 2.4 Hz, $H_5$), 7.53 (d, 1H, *J* 8.8 Hz, $H_8$), 7.92 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.44, 108.84, 122.81, 123.20, 127.84, 128.11, 128.70, 128.90, 135.96, 140.71, 142.93, 146.04, 160.90.

*7-Amino-3-ethylquinazolin-4-(3H)-one (**9**)*. This compound was prepared from precursor **5**. Yield: 85%, white solid, mp = 170°C, (Found $M^+$: 189.0917, $C_{10}H_{11}N_3O$ requires 189.0902); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3200, 1718, 1296, 682, 605, 472; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.40 (t, 3H, *J* 7.2 Hz, $CH_3$), 4.00 (q, 2H, *J* 7.2 Hz, $CH_2$), 4.23 (s, 2H, $NH_2$), 6.79 (d, 1H, *J* 8.8 Hz, $H_6$), 6.81 (s, 1H, $H_8$), 7.95 (s, 1H, $H_2$), 8.10 (d, 1H, *J* 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 15.07, 41.81, 108.94, 113.47, 115.87, 128.43, 146.82.

*7-Amino-3-benzylquinazolin-4-(3H)-one (**10**)*. This compound was prepared from precursor **6**. Yield: 98%, white solid, mp = 175°C, (Found $M^+$: 215.1062, $C_{15}H_{13}N_3O$ requires 251.1059); IR $\upsilon_{max}$

(KBr)/$cm^{-1}$ 3228, 3036, 2952, 1666, 1610, 1496, 1372, 1298, 1173, 832, 762, 704; $^1$H-NMR δ (400 MHz, $CDCl_3$) 4.32 (s, 2H, $NH_2$), 5.14 (s, 2H, $CH_2$), 6.78 (dd, 1H, *J* 2.2 Hz, *J* 8.4 Hz, $H_6$), 6.79 (d, 1H, *J* 2.2 Hz, $H_8$), 7.27–7.34 (m, 5H, Har), 8.00 (s, 1H, $H_2$), 8.09 (d, 1H, *J* 8.4 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.54, 104.24, 114.04, 115.41, 126.99, 128.03, 128.33, 129.03, 135.65, 147.33, 147.56, 149.75, 160.17.

*Bromination of aminoquinazolinones*. Bromine (5.6 mmol) was added dropwise, under an inert atmosphere, to a solution of amine **7**, **8**, **9** or **10** (5.6 mmol) in acetic acid (30 mL). After 2 h stirring at room temperature, 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 with dichloromethane/ethyl acetate (90/10) as eluent to afford the expected compound.

*6-Amino-5-bromo-3-ethylquinazolin-4-(3H)-one* ***(11)***. This compound was prepared from precursor **7**. Yield: 93%, red solid, mp = 140°C. (Found $M^+$: 266.9996; $C_{10}H_{10}BrN_3O$ requires 267.0007); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3332, 2980, 1652, 1594, 1487, 1381, 1344, 1275, 1012, 935, 844, 544; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.42 (t, 3H, *J* 7.6 Hz, $CH_3$), 4,04 (q, 2H, *J* 7.6 Hz, $CH_2$), 4.60 (s, 2H, $NH_2$), 7.18 (d, 1H, *J* 8.8 Hz, $H_7$), 7.51 (d, 1H, *J* 8.8 Hz, $H_8$), 7.90 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.65, 42.43, 103.43, 120.57, 121.77, 127.86, 142.52, 143.52, 144.48, 158.62.

*6-Amino-3-benzyl-5-bromoquinazolin-4-(3H)-one* ***(12)***. This compound was prepared from precursor **8**. Yield: 70%, red solid, mp = 162°C, (found $M^+$: 329.0164, $C_{15}H_{12}BrN_3O$ requires 329.0164); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3442, 3326, 3200, 1671, 1622, 1483, 1365, 1336, 1281, 1233, 11122, 964, 836, 792, 724, 696, 611; $^1$H-NMR δ (400 MHz, $CDCl_3$) 4.62 (s, 2H, $NH_2$), 5.16 (s, 2H, $CH_2$), 7.18 (d, 1H, *J* 8.8 Hz, $H_7$), 7.35–7.37 (m, 5H, Har), 7.50 (d, 1H, *J* 8.8 Hz, $H_8$), 7.98 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.75, 103.06, 120.69, 121.80, 127.96, 128.01, 128.17, 128.95, 135.73, 142.37, 143.57, 144.66, 159.34.

*7-Amino-8-bromo-3-ethylquinazolin-4-(3H)-one* ***(13)***. This compound was prepared from precursor **9**. Yield: 59%, red solid, mp = 210°C, (Found $M^+$: 267.0005, $C_{10}H_{10}BrN_3O$ requires 267.0007); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3470, 3351, 2980, 1671, 1613, 1486, 1435, 1378, 1284, 1245, 1081, 933, 858, 791, 719, 660, 557; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.40 (t, 3H, *J* 7.2 Hz, $CH_3$), 4.04 (q, 2H, *J* 7.2 Hz, $CH_2$), 4.80 (s, 2H, $NH_2$), 6.89 (d, 1H, *J* 8.8 Hz, $H_6$), 8.06 (d, 1H, *J* 8.8 Hz, $H_5$), 8.11 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.98, 41.99, 104.12, 113.92, 115.35, 126.74, 147.46, 149.60, 160.16.

*7-Amino-3-benzyl-8-bromoquinazolin-4-(3H)-one* ***(14)***. This compound was prepared from precursor **10**. Yield: 90%, red solid, mp = 224°C, (Found $M^+$: 329.0158, $C_{15}H_{12}BrN_3O$ requires 329.0164); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3473, 3340, 3192, 3030, 1652, 1602, 1362, 782, 704; $^1$H-NMR δ (400 MHz, $CDCl_3$) 4.78 (s, 2H, $NH_2$), 5.16 (s, 2H, $CH_2$), 6.89 (d, 1H, *J* 8.8 Hz, $H_6$), 7.34–7.35 (m, 5H, Har), 8.08 (d, 1H, *J* 8.8 Hz, $H_5$), 8.16 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.21, 100.53, 109.02, 113.22, 115.86, 127.86, 128.09, 128.60, 128.91, 136.10, 146.88, 150.04, 152.15.

*Synthesis of iminodithiazole quinazolin-4-ones*. A suspension of amine **11**, **12**, **13** or **14** (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 over magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography with dichloromethane/ethyl acetate (90/10) as eluent to afford the expected compound **7**–**10**.

*5-Bromo-6-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-amino]-3-ethylquinazolin-4-(3H)-one* ***(15)***. This compound was prepared from amine **11**. Yield: 45%, orange solid, mp = 182°C. (Found $M^+$: 401.9030, $C_{12}H_8BrClN_4OS_2$ requires 401.9011); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 2967, 1662, 1594, 1450, 1375, 1269, 1137, 944, 862, 819, 550; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.45 (t, 3H, *J* 7.6 Hz, $CH_3$), 4.05 (q, 2H, *J* 7.6 Hz, $CH_2$), 7.40 (d, 1H, *J* 8.8 Hz, $H_7$), 7.50 (d, 1H, *J* 8.8 Hz, $H_8$), 8.06 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.69, 42.71, 112.97, 124.14, 129.10, 146.24, 147.19, 150.88, 159.11, 162.57.

*3-Benzyl-5-bromo-6-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-amino]quinazolin-4-(3H)-one* ***(16)***. This compound was prepared from amine **12**. Yield: 60%, orange solid, mp = 198°C, (Found $M^+$: 463.9168, $C_{17}H_{10}BrClN_4OS_2$ requires 463.9168); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3026, 1688, 1595, 1456, 1370, 1294, 1257, 1152, 965, 871, 839, 661, 605; $^1$H-NMR δ (400 MHz, $CDCl_3$) 5.19 (s, 2H, $CH_2$), 7.38–7.41 (m, 5H, Har), 7.41 (d, 1H, *J* 8.4 Hz, $H_7$), 7.74 (d, 1H, *J* 8.4 Hz, $H_8$), 8.13 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.69, 115.26, 124.17, 128.16, 128.44, 128.56, 129.06, 129.49, 135.50, 146.26, 147.79, 149.63, 159.22, 162.22.

*8-Bromo-7-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-amino]-3-ethylquinazolin-4-(3H)-one (**17**)*. This compound was prepared from amine **13**. Yield: 55%, orange solid, mp = 200°C, (Found $M^+$: 401.9032, $C_{12}H_8BrClN_4OS_2$ requires 401.9011); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 2939, 2355, 1717, 1600, 1517, 1368, 1292, 1232, 1076, 859, 670, 604, 522, 472; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.44 (t, 3H, *J* 7.2 Hz, $CH_3$), 4.09 (q, 2H, *J* 7.2 Hz, $CH_2$), 7.19 (d, 1H, *J* 8.8 Hz, $H_6$), 8.10 (s, 1H, $H_2$), 8.36 (d, 1H, *J* 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.87, 42.20, 112.89, 115.15, 117.81, 120.63, 121.23, 128.10, 147.69, 147.81, 159.98, 162.44.

*3-Benzyl-8-bromo-7-[(4-chloro-5H-1,2,3-dithiazol-5-ylidene)-amino]quinazolin-4-(3H)-one (**18**)*. This compound was prepared from amine **14**. Yield: 65%, orange solid, mp = 202°C, (Found $M^+$: 463.9206, $C_{17}H_{10}BrClN_4OS_2$ requires 463.9168); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3030, 1658, 1566, 860, 786, 726, 519; $^1$H-NMR δ (400 MHz, $CDCl_3$) 5.21 (s, 2H, $CH_2$), 7.18 (d, 1H, *J* 8.8 Hz, $H_6$), 7.33–7.38 (m, 5H, Har), 8.26 (s, 1H, $H_2$), 8.35 (d, 1H, *J* 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.87, 113.08, 117.89, 120.47, 128.01, 128.16, 128.22, 128.37, 128.53, 129.09, 129.70, 135.10, 147.19, 147.59, 147.67, 155.88, 159.96.

*Synthesis of thiazoloquinazolinone-2-carbonitriles*. A suspension of imine **15, 16, 17** or **18** (1 mmol), cuprous 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, and washed with sodium thiosulfate solution (20 mL). The solvent was removed *in vacuo* and the crude residue was purified by column chromatography with dichloromethane/ethyl acetate (80/20) as eluent to afford the expected compound.

*8-Ethyl-9-oxo-8,9-dihydro[1,3]thiazolo(5,4-f(quinazoline-2-carbonitrile (**19**)*. This compound was prepared from imine **15**. Yield: 93%, white solid, mp >260°C. (Found $M^+$: 256.0396, $C_{12}H_8N_4OS$ requires 256.0419); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3055, 2980, 2226, 1675, 1587, 1469, 1381, 1350, 1262, 1150, 969, 837; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.52 (t, 3H, *J* 7.6 Hz, $CH_3$), 4.23 (q, 2H, *J* 7.6 Hz, $CH_2$), 7.99 (d, 1H, *J* 8.8 Hz, $H_4$), 8.29 (s, 1H, $H_7$), 8.54 (d, 1H, *J* 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.92, 42.81, 113.17, 128.08, 130.37, 132.13, 140.19, 147.30, 151.41, 159.44.

*8-Benzyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carbonitrile (**20**)*. This compound was prepared from imine **16**. Yield: 80%, white solid, mp = 194°C, (Found $M^+$: 318.0566, $C_{17}H_{10}N_4OS$ requires 318.0575); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3059, 2231, 1667, 1589, 1462, 1358, 1261, 1155, 1069, 940, 856, 730, 695, 506; $^1$H-NMR δ (400 MHz, $CDCl_3$) 5.34 (s, 2H, $CH_2$), 7.38–7.41 (m, 5H, Har), 8.03 (d, 1H, *J* 8.8 Hz, $H_4$), 8.39 (d, 1H, *J* 8.8 Hz, $H_7$), 8.54 (s, 1H, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 50.22, 113.14, 116.33, 128.10, 128.23, 128.85, 129.30, 130.53, 132.37, 134.73, 140.24, 147.33, 148.80, 151.48, 159.43.

*7-Ethyl-6-oxo-6,7-dihydro[1,3]thiazolo[4,5-h]quinazoline-2-carbonitrile (**21**)*. This compound was prepared from imine **17**. Yield: 88%, yellow solid, mp = 210°C, (Found $M^+$: 256.0396, $C_{12}H_8N_4OS$ requires 256.0418); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3065, 2973, 2387, 2235, 1674, 1602, 1552, 1455, 1372, 1351, 1277, 1206, 1153, 1088, 933, 899, 842, 798, 726, 490; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.48 (t, 3H, *J* 7.3 Hz, $CH_3$), 4.16 (q, 2H, *J* 7.3 Hz, $CH_2$), 8.22 (d, 1H, *J* 8.8 Hz, $H_4$), 8.23 (s, 1H, $H_8$), 8.49 (d, 1H, *J* 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.89, 42.81, 112.66, 120.41, 123.20, 126.21, 133.29, 140.28, 148.31, 155.90, 159.98.

*7-Benzyl-6-oxo-6,7-dihydro[1,3]thiazolo[4,5-h]quinazoline-2-carbonitrile (**22**)*. This compound was prepared from imine **18**. Yield: 85%, White solid, mp = 190°C, (Found $M^+$: 318.0566, $C_{17}H_{10}N_4OS$ requires 318.0575); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3078, 2235, 1688, 1600, 1455, 1369, 1350, 708; $^1$H-NMR δ (400 MHz, DMSO) 5.27 (s, 2H, $CH_2$), 7.38–7.39 (m, 5H, Har), 8.23 (d, 1H, *J* 8.8 Hz, $H_4$), 8.28 (s, 1H, $H_8$), 8.49 (d, 1H, *J* 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 50.22, 112.65, 120.45, 123.41, 126.42, 128.20, 128.72, 129.25, 134.99, 140.45, 144.25, 148.42, 156.04, 160.17.

*Synthesis of 2-substituted thiazoloquinazolinone derivatives*

*Synthesis of imidates*. A stirred mixture of thiazoloquinazolinone-2-carbonitrile **19** or **20** (0.5 mmol) and 2.5 M NaOH (0.55 mmol) in anhydrous ethanol (5 mL), under argon, was stirred at room temperature for 15 min. The resulting precipitate was collected by filtration, washed with water and dried over $P_2O_5$ to give the imidate as a white crystalline powder.

*8-Ethyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboximidic acid ethyl ester (**23**)*. This compound was prepared from precursor **19**. Yield: 64%, white solid, mp = 232°C, (Found $M^+$: 302.0837, $C_{14}H_{13}N_4O_2S$ requires 302.0837); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3267, 2978, 1657, 1600, 1499, 1453, 1325, 1267, 1141, 1069, 899, 846, 713, 568, 506; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.49 (t, 3H, *J* 7.2 Hz, $CH_3$), 1.51 (t, 3H, *J* 7.2 Hz, $CH_3$), 4.21 (q, 2H, *J* 7.2 Hz, $CH_2$), 4.51 (q, 2H, *J* 7.2 Hz, $CH_2$), 7.90 (d, 1H, *J* 8.8 Hz, $H_4$), 8.22 (s, 1H, $H_7$), 8.45 (d, 1H, *J* 8.8 Hz, $H_5$), 8.96 (s, 1H, NH); $^{13}$C-NMR δ

(100 MHz, $CDCl_3$) 14.17, 14.91, 42.62, 63.12, 116.79, 126.73, 129.81, 133.13, 146.34, 147.82, 151.86, 159.62, 161.33, 162.35.

*8-Benzyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboximidic acid ethyl ester (**24**)*. This compound was prepared from precursor **20**. Yield: 60%, white solid, mp = 171°C, (Found $M^+$: 364.1000, $C_{19}H_{16}N_4O_2S$ requires 364.0994); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3282, 2980, 1669, 1581, 1500, 1450, 1331, 1265, 1144, 1062, 1012, 887, 837, 725, 606, 531; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.48 (t, 3H, $\jmath$ 6.8 Hz, $CH_3$), 4.50 (q, 2H, $\jmath$ 6.8 Hz, $CH_2$), 5.33 (s, 2H, $CH_2$), 7.36–7.41 (m, 5H, Har), 7.90 (d, 1H, $\jmath$ 8.8 Hz, $H_4$), 8.29 (s, 1H, $H_7$), 8.45 (d, 1H, $\jmath$ 8.8 Hz, $H_5$), 8.96 (s, 1H, NH); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.14, 50.05, 63.08, 116.74, 126.73, 128.24, 128.60, 129.14, 129.92, 133.24, 135.10, 146.37, 147.59, 151.88, 159.65, 161.18, 162.30.

*Synthesis of imidazolines*. A stirred mixture of thiazoloquinazolinone-2-carbonitrile **19** or **20** (1 mmol) and ethylenediamine (40 mmol) in dry THF (4 mL) was irradiated in a sealed tube for 4 min. The irradiation was programmed to obtain a constant temperature (130°C). The solvent was removed in vacuo and water (5 mL) was added to the crude residue. The precipitated solid was dissolved in dichloromethane, washed with water and dried over magnesium sulfate. The solvent was removed in vacuo and the crude residue purified by column chromatography with dichloromethane/methanol (90/10) as eluent to afford the imidazoline.

*2-(4,5-dihydro-1H-imidazol-2-yl)-8-ethyl[1,3]thiazolo[5,4-f]quinazolin-9-(8H)-one (**25**)*. This compound was prepared from precursor **19**. Yield: 70%, white solid, mp = 190°C, (Found $M^+$: 299.0841, $C_{14}H_{13}N_5OS$ requires 299.08408); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 2933, 2332, 1657, 1586, 1293, 1262, 1124, 836, 676, 622; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.50 (t, 3H, $\jmath$ 7.2 Hz, $CH_3$), 3.72 (bs, 2H, $CH_2$), 4.20 (q, $\jmath$ 7.2 Hz, 4H, 2x$CH_2$), 5.73 (s, H, NH), 7.86 (d, 1H, $\jmath$ 8.8 Hz, $H_4$), 8.20 (s, 1H, $H_7$), 8.39 (d, 1H, $\jmath$ 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 15.02, 42.44, 45.08, 56.39, 116.76, 126.45, 129.38, 132.90, 146.38, 147.64, 152.15, 159.51, 159.89, 162.62.

*8-Benzyl-2-(4,5-dihydro-1H-imidazol-2-yl)[1,3]thiazolo[5,4-f]quinazolin-9-(8H)-one (**26**)*. This compound was prepared from precursor **20**. Yield: 40%, yellow solid, mp = 194°C, (Found $M^+$:361.0976, $C_{19}H_{15}N_5OS$ requires 361.0997); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3055, 2930, 2867, 1669, 1600, 1519, 1450, 1344, 1287, 1162, 1081, 981, 831, 712, 506; $^1$H-NMR δ (400 MHz, $CDCl_3$) 3.68 (t, 2H, $\jmath$ 9.6 Hz, $CH_2$), 4.15 (t, 2H, $\jmath$ 9.6 Hz, $CH_2$), 5.32 (s, 2H, $CH_2$), 5.76 (s, 1H, NH), 7.29–7.42 (m, 5H, Har), 7.85 (d, 1H, $\jmath$ 8.8 Hz, $H_4$), 8,.23 (s, 1H, $H_7$), 8.38 (d, 1H, $\jmath$ 8.8 Hz, $H_5$).

*Synthesis of amidines*. A stirred mixture of carbonitrile **19** or **20** (1 mmol) and *N,N*-dimethylethylenediamine (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 and dried over magnesium sulfate. The solvent was removed in vacuo and the crude residue was purified by column chromatography with dichloromethane/methanol (90/10) as eluent to afford the amidine.

*N-[2-(dimethylamino)-ethyl]-8-ethyl-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboxamidine (**27**)*. This compound was prepared from precursor **19**. Yield: 50%, yellow solid, mp = 144°C, (Found $[M\text{-}C_4H_8N]^+$: 274.0784, ($[M\text{-}C_4H_8N]^+$ requires 274.0762); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3312, 2834, 1660, 1600, 1456, 1347, 1256, 1115, 966, 825, 760, 603, 570; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.49 (t, 3H, $\jmath$ 6.8 Hz, $CH_3$), 2.74 (t, 2H, $\jmath$ 6.4 Hz, $CH_2$), 3.50 (t, 2H, $\jmath$ 6.4 Hz, $CH_2$), 4.19 (q, 2H, $\jmath$ 6.8 Hz, $CH_2$), 7.84 (d, 1H, $\jmath$ 8.8 Hz, $H_4$), 8.19 (s, 1H, $H_7$), 8.37 (d, 1H, $\jmath$ 8.8 Hz, $H_5$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.99, 42.45, 45.46, 58.89, 116.78, 126.19, 129.36, 133.28, 146.16, 147.41, 152.10, 159.61, 169.28.

*8-Benzyl-N-[2-(dimethylamino)-ethyl]-9-oxo-8,9-dihydro[1,3]thiazolo[5,4-f]quinazoline-2-carboxamidine (**28**)*. This compound was prepared from precursor **20**. Yield: 70%, white solid, mp = 174°C, (Found $[M\text{-}C_4H_{12}N_2]^+$: 318.0566, $[M\text{-}C_4H_{12}N_2]^+$ requires 318.05753); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3461, 3294, 2956, 2822, 1745, 1728, 1668, 1590, 1452, 1344, 1255, 1020, 800, 737, 696, 607; $^1$H-NMR δ (400 MHz, DMSO) 2.22 (bs, 4H, 2x$CH_2$), 3.35 (s, 6H, 2x$CH_3$), 5.32 (s, 2H, $CH_2$), 5.76 (s, 1H, NH), 7.30–7.44 (m, 5H, Har), 7.85 (d, 1H, $\jmath$ 8.8 Hz, $H_4$), 8.43 (d, 1H, $\jmath$ 8.8 Hz, $H_5$), 8.78 (s, 1H, $H_7$).$^{13}$C-NMR δ (100 MHz,DMSO) 39.91, 45.51, 49.77, 58.95, 116.69, 126.13, 128.21, 128.49, 129.08, 129.39, 133.47, 135.25, 146.14, 147.13, 152.11, 159.62.

*Decyanation of thiazoloquinazolinone-2-carbonitrile*. A stirred solution of thiazoloquinazolinone-2-carbonitrile derivatives **19** or **20** (1 mmol) in 48% aqueous HBr (10 mL) was irradiated for 30 min. The irradiation was programmed to obtain a constant temperature (115°C) with a maximal power output of 60 W. The solvent was removed in vacuo and water (5 mL) was added to the crude residue. The crude material dissolved in water was treated with 10% aqueous sodium hydroxyde and extracted with dichloromethane. The residual oily solid obtained after removal of the solvent was purified by column chromatography with dichloromethane as eluent to afford the desired compound.

*8-Ethyl[1,3]thiazolo[5,4-f]quinazolin-9-(8H)-one (**29**)*. This compound was prepared from precursor **19**. Yield: 74%, white solid, mp = 180°C, (Found $M^+$: 231.0452, $C_{11}H_9N_3OS$ requires 231.0466); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 2927, 1662, 1604, 1447, 1376, 1347, 1217, 1088, 976, 835, 801; $^1$H-NMR δ (400 MHz, $CDCl_3$) 1.51 (t, 3H, *J* 7.6 Hz, $CH_3$), 4.21 (q, 2H, *J* 7.6 Hz, $CH_2$), 7.91 (d, 1H, *J* 8.8 Hz, $H_4$), 8.21 (s, 1H, $H_7$), 8.50 (d, 1H, *J* 8.8 Hz, $H_5$), 9.23 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 14.97, 42.52, 116.63, 126.07, 129.38, 130.40, 146.00, 147.15, 152.53, 157.75, 159.77.

*8-Benzyl[1,3]thiazolo[5,4-f]quinazolin-9-(8H)-one **30***. This compound was prepared from precursor **20**. Yield: 93%, white solid, mp = 154°C, (Found $M^+$: 293.0898, $C_{16}H_{11}N_3OS$ requires 293.0623); IR $\upsilon_{max}$ (KBr)/$cm^{-1}$ 3068, 1662, 1594, 1450, 1350, 1156, 981, 837, 712; $^1$H-NMR δ (400 MHz, $CDCl_3$) 5.32 (s, 2H, $CH_2$), 7.33–7.42 (m, 5H, Har), 7.89 (d, 1H, *J* 8.8 Hz, $H_4$), 8.27 (s, 1H, $H_7$), 8.50 (d, 1H, *J* 8.8 Hz, $H_5$), 9.23 (s, 1H, $H_2$); $^{13}$C-NMR δ (100 MHz, $CDCl_3$) 49.85, 116.57, 126.10, 128.06, 128.50, 129.13, 129.46, 135.20, 146.03, 146.93, 149.60, 153.15, 157.76, 159.78.

## *Pharmacology*

### *CDK and GSK-3 kinase activity assays*

*Biochemical Reagents*. Sodium *ortho*-vanadate, EGTA, EDTA, 3-*N*-morpholinopropanesulfonic acid (Mops), ß-glycerophosphate, phenyl phosphate, sodium fluoride, dithiothreitol (DTT), glutathione-agarose, glutathione, bovine serum albumin (BSA), nitrophenyl phosphate, leupeptin, aprotinin, pepstatin, soybean trypsin inhibitor, benzamidine and histone H1 (type III-S) were obtained from Sigma Chemicals. [γ-$^{32}$P]-ATP (PB 168) was obtained from Amersham.

The GS-1 peptide (YRRAAVPPSPSLSRHSS-PHQSpEDEEE) was synthesised by the Peptide Synthesis Unit, Institute of Biomolecular Sciences, University of Southampton, Southampton SO16 7PX, U.K.

*Buffers*. The buffers were prepared as following: Homogenization buffer: 60 mM ß-glycerophosphate, 15 mM *p*-nitrophenyl phosphate, 25 mM Mops (pH 7.2), 15 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM NaF, 1 mM phenyl phosphate, 10 μg leupeptin/mL, 10 μg aprotinin/mL, 10 μg soybean trypsin inhibitor/mL and 100 μM benzamidine.

Buffer A: 10 μM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl pH 7.5, 50 μg heparin/mL.

Buffer C: homogenization buffer but 5 mM EGTA, no NaF and no protease inhibitors.

*Kinase preparations and assays*. CDKs and GSK-3 were assayed in the presence of 10 μM of each thiazoloquinazolinone. For molecules showing inhibitory activity at 10 μM, dose-response curves were performed to calculate the $IC_{50}$ value.

Kinases activities were assayed in buffer A or C (unless otherwise stated), at 30°C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities calculated as pmoles of phosphate incorporated for a 10 min incubation. The activities are usually expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of dimethylsulfoxide.

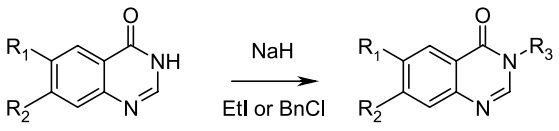
GSK-3α/β was purified from porcine brain by affinity chromatography on immobilised axin [12]. It was assayed, following a 1/100 dilution in 1 mg BSA/mL 10 mM DTT, with 5 μL 40 μM GS-1 peptide as a substrate, in buffer A, in the presence of 15 μM [γ-$^{33}$P] ATP (3,000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 30 min incubation at 30°C, 25 μL aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and, 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid/litre of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid.

CDK1/cyclin B was extracted in homogenisation buffer from M phase starfish (*Marthasterias glacialis*) oocytes and purified by affinity chromatography on $p9^{CKShs1}$-sepharose beads, from which it was eluted by free $p9^{CKShs1}$ as previously described [13]. The kinase activity was assayed in buffer C, with 1 mg histone H1 /mL, in the presence of 15 μM [γ-$^{33}$P] ATP (3,000 Ci/mmol; 1 mCi/mL) in a final volume of 30 μL. After 10 min incubation at 30°C, 25 μL aliquots of supernatant were spotted onto P81 phosphocellulose papers and treated as described above.

CDK5/p25 was reconstituted by mixing equal amounts of recombinant mammalian CDK5 and p25 expressed in *E. coli* as GST (Glutathione-S-transferase) fusion proteins and purified by affinity chromatography on glutathione-agarose (vectors kindly provided by Dr. J.H. Wang) (p25 is a truncated version of p35, the 35 kDa CDK5 activator). Its activity was assayed in buffer C as described for CDK1/cyclin B.

### *Antiproliferation and cytotoxicity assays*

*Cell culture*. One human breast carcinoma cell line, MDA-MB-231, kindly provided by Dr. M. Mareel (Laboratoire de cancérologie expérimentale, Hôpital Universitaire, Ghent, Belgique) was used in the present study. MDA-MB-231 is classified both as a hormone-independent and a highly invasive breast cancer cell line [14]. MDA-MB-231 cells were




$R_1 = NO_2$, $R_2 = H$ **1**
$R_1 = H$, $R_2 = NO_2$ **2**

**3-6**

| Compound | $R_1$ | $R_2$ | $R_3$ | Yield % |
|---|---|---|---|---|
| **3** | $NO_2$ | H | Et | 98 |
| **4** | $NO_2$ | H | Bz | 85 |
| **5** | H | $NO_2$ | Et | 50 |
| **6** | H | $NO_2$ | Bz | 41 |

Scheme 1. Alkylation of nitroquinazolinones **1** and **2**. Reaction conditions: NaH (60% dispersion in mineral oil), DMF, 140°C, μw.

cultured at 37°C in a 5% $CO_2$/95% air humidified atmosphere, in DMEM-HAM's F12 medium (1:1, v/v, Gibco), supplemented with 10% heat inactivated fetal calf serum (v/v, Dutscher) supplemented with penicillin 100 U mL$^{-1}$ and streptomycin 100 μg mL$^{-1}$. *In vitro* drug sensitivity was measured with the CellTiter 96® non-radioactive cell proliferation assay (Promega) which allows the determination of the fraction of viable cells remaining after drug treatment [15]. The test compounds were dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich) to give $10^{-3}$ M stock solutions from which further dilutions were made in culture medium.

*Selection of quinazolinones and doses tested on MDA-MB-231*. Among all the synthesized thiazoloquinazolinones, eight were selected for cytotoxicity and antiproliferative activity evaluation on the MDA-MB-231 cell line. Compounds **3**, **7** and **25** were selected as *N*-ethylated quinazolinones selectively active on GSK-3, whilst **19** and **23** were selected as *N*-ethylated thiazoloquinazolinones active on CDK1, CDK5 and GSK-3. Compounds **21, 22** and **30** were selected as control thiazoloquinazolinones, since they displayed no inhibitory activity on any of the three kinases. In order to identify the compounds exerting the highest activity in cell-based assays, drugs were tested at the two pharmacological doses of $10^{-6}$ M and $10^{-9}$ M.

*Cytotoxicity of thiazoquinazolinones on MDA-MB-231 breast cancer cell line*. Cells were preincubated in 96-well microplates ($2.2 \times 10^5$ cells per well, 90 μL) for 24 h at 37°C and 5% $CO_2$ to allow stabilization prior to addition of drugs. 10 μL of $10^{-8}$ or $10^{-5}$ dilutions of thiazoloquinazolinones were then added to each well, to reach final concentrations of $10^{-9}$ or $10^{-6}$ M respectively, and cells were incubated in the presence of thiazoloquinazolinones for 24 h. A solution of MTT tetrazolium salt (15 μL) was then added. The plates were further incubated for 4 h to allow for MTT metabolism to formazan by the succinate-tetrazolium reductase system active only in viable cells. A solubilization/stop solution (100 μL) was added to stop the MTT assay and the optical densities were determined on a plate reader (VERSAmax, Molecular Devices) at 570 nm. The data were then analyzed to calculate the % of cytotoxicity determined by the equation:

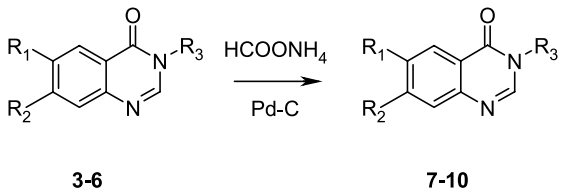
$$\%\ \text{cytotoxicity} = 100 - \left(\frac{\text{OD test}}{\text{OD control}} \times 100\right)$$

where OD is the optical density at 570 nm recorded for the experimental sample and OD control is the optical density at 570 nm recorded in absence of drug. Antiproliferative activity of thiazoloquinazolinones on MDA-MB-231 breast cancer cell line The antiproliferative effect of thiazoloquinazolinones was tested with cells seeded at a density of 5000 cells/well in 96-well culture plates. On day 0, a 50 μL aliquot of medium containing $2.10^{-9}$ or $2.10^{-6}$ M of thiazoloquinazolinone was added to each well of 96-well plates. After equilibration at 37°C in a humidified 5% $CO_2$ atmosphere, 50 μL of the cell suspension (5000 cells) were dispensed into all wells of the pre-equilibrated 96-well plate. After incubation at 37°C for 72 h in a humidified 5% $CO_2$ atmosphere, cell growth inhibition was measured with the CellTiter 96® non-radioactive cell proliferation assay. The data were then analyzed to determine the % of growth inhibition through a comparison of samples with untreated cells (control, 0% inhibition).

## Results and discussion

### *Chemistry*

The pharmaceutical interest of the unsubstituted molecules **VI** and **VII** (Figure 2) has been limited, so we decided to investigate the effect of various pharmacomodulations on their biological activity, especially on the capacity of these molecules to inhibit CDKs and GSK-3. Following this strategy, we performed the *N*-alkylation of the quinazolinone **1** and **2** and studied the possible modifications of the




**3-6** **7-10**

| Compound | R | R | R | Yield % |
|---|---|---|---|---|
| **7** | $NH_2$ | H | Et | 95 |
| **8** | $NH_2$ | H | Bz | 98 |
| **9** | H | $NH_2$ | Et | 85 |
| **10** | H | $NH_2$ | Bz | 98 |

Scheme 2. Reduction of quinazolinones **3**–**6**. Reaction conditions: ammonium formate, Pd-C, ethanol, 140°C, μw.

carbon substituent present on position 2, between the nitrogen and the sulphur atom of the thiazole moiety of the thiazoloquinazolinone ring.

The chemistry of *N*-arylimino-1,2,3-dithiazoles is one of the major axes of our research. Synthesis of rare 2,8-substituted thiazolo[5,4-*f*]quinazolin-9-one **IV** and 2,7-substituted thiazolo[4,5-*h*]quinazolin-6-one **V** rings (Figure 2) was performed in six steps *via* the known 6- or 7-nitroquinazolinones **1** and **2** respectively, which were prepared from the starting commercially available nitroantranilic acids. In connection with our recent work on the use of microwaves in organic chemistry, we investigated whether it was possible to achieve better yields and cleaner reactions by performing all the reactions under microwave irradiation in sealed tubes rather than using the purely thermal process. In all cases, besides resulting in good to excellent yields, our method offers much faster reactions compared to earlier published procedures at atmospheric pressure.

We previously reported the synthesis of the unsubstituted thiazoloquinazolinone-2-carbonitriles **VI, VII** [9]. Whatever the experimental conditions and the nature of the base used, their alkylation led to complicated mixtures. We decided to alkylate the quinazolinone skeleton before forming the thiazole ring. Selective *N*-alkylation in position 3 of the quinazolinone ring was performed in various yields (41–98%) by treatment of the nitro quinazolinones **1** and **2** with sodium hydride and ethyl iodide or benzyl chloride as alkylating agents (Scheme 1). Contrary to classical heating, no trace of *O*-alkylation was observed.

Using ammonium formate for catalytic transfer hydrogenation in ethanol, the reduction of the nitroquinazolinones led to the 3-amino derivatives in good yields (Scheme 2).

*N*-Arylimino-1,2,3-dithiazoles are highly versatile intermediates in heterocyclic synthesis. It is well known that reaction of 4,5-dichoro-1,2,3-dithiazolium chloride with primary aromatic amines, in dichloromethane at room temperature, allows access to stable the Z-isomer of *N*-arylimino-4-chloro-5*H*-1,2,3-dithiazoles. In order to obtain regioselectively the angular thiazolo isomers **IV** and **V** a mild procedure, which consists in heating *ortho* bromoimines in the presence of cuprous iodide in pyridine at reflux, was applied (Figure 3).

Thus, the aminoquinazolinones **7**–**10** were firstly brominated in the presence of bromine in acetic acid. The *ortho* brominated imines **11**–**14** obtained were condensed with 4,5-dichloro-1,2,3-dithiazolium chloride in dichloromethane at room temperature, followed by addition of pyridine, to give the desired imino-1,2,3-dithiazoloquinazolinones **15**–**18** in good yields (Scheme 3).

The thermolysis procedure consisted in heating the imines **15**–**18**, at 160°C, in the presence of cuprous iodide in pyridine under microwave irradiation. The expected compounds **19**–**22** were obtained in yields superior to 60% (Schemes 4 and 5). Preliminary cytotoxicity evaluation of thiazoloquinazolinone-2-carbonitriles **19**–**22** showed better activities for compounds **19**, **20** compared to compounds **21**, **22**. Our best candidates **19** and **20** which exhibit a good cytotoxicity were modified in very good yields (Scheme 6).

It is known that the cyano group in position 2 of the thiazolocarbonitriles ring is very reactive and that its transformation into imidate, imidazoline, amidine and

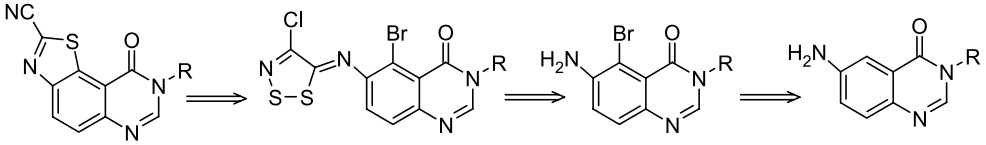
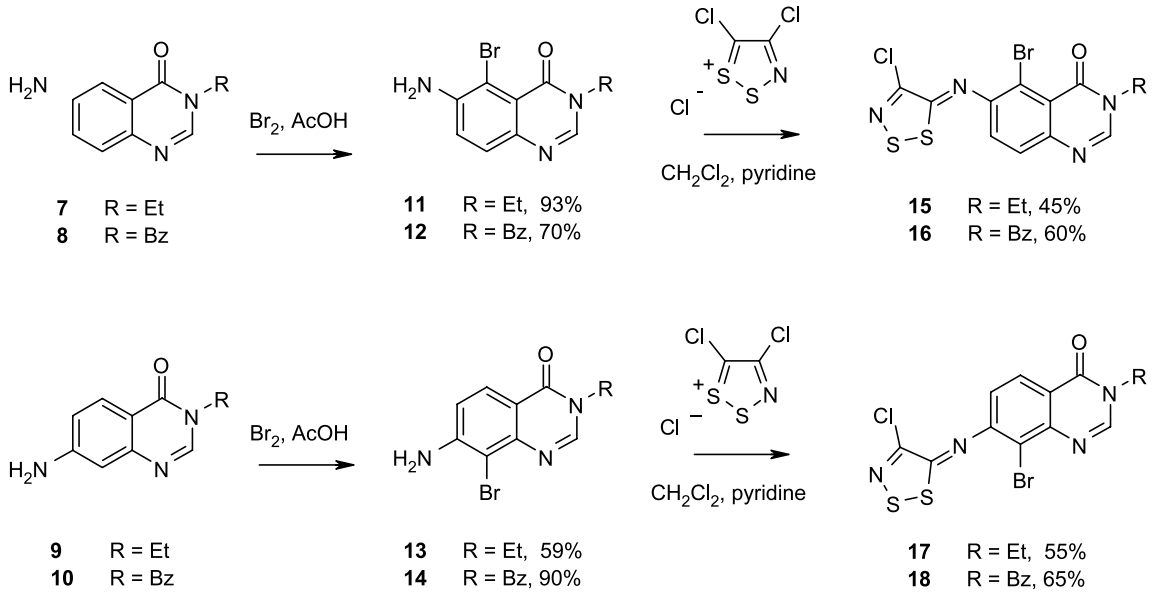


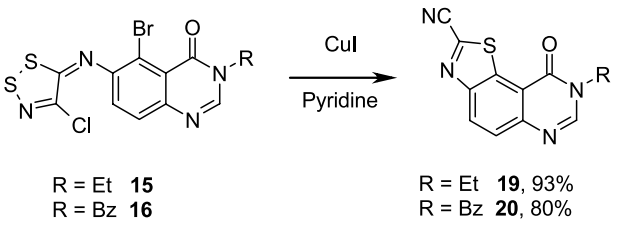

Figure 3. Retrosynthesis of 8*H*-thiazolo[5,4-*f*]quinazolin-9-ones **IV.**




Scheme 3. Synthesis of bromo-iminodithiazoles **15**–**18**.

decyanated derivative can be easily realized. Amidates **23** and **24** were respectively obtained in good yields from derivatives **19** and **20** by refluxing in alcohol in the presence of 1 equivalent of NaOH 2.5 N. The condensation of thiazoloquinazolinones-2-carbonitriles **19** and **20** with the commercially available appropriate amines in various solvents (e.g. ethanol, THF) was studied to give the desired substituted thiazoloquinazolinones **25**–**28** (Scheme 6). Treatment, under microwave irradiation, of compounds **19** and **20** with ethylene diamine or *N,N*-dimethylethylenediamine afforded, respectively in modest yields, imidazolines **25** (70%) and **26** (40%), and *N*-amidines **27** (50%) and **28** (70%). For some of prepared compounds, we expected that the basic side chain might provide cationic molecules leading to better water solubility and impacting on their biological properties (e.g. for DNA binding ability).

Thus, employing microwave assisted organic synthesis allowed us to establish efficient conditions for the preparation of *N*-substituted thiazoloquinazolinones.

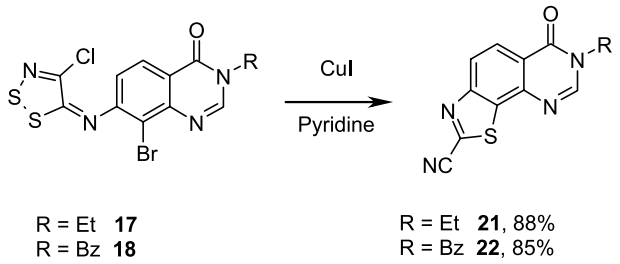



Scheme 4. Synthetic route to 9-oxo-thiazolo[5,4-*f*]quinazoline-2-carbonitriles. Reaction conditions: CuI, pyridine, 160°C, μw.

*Pharmacology*

*Inhibition of CDKs and GSK-3 by the novel synthesized thiazoloquinazolinones*. The effects of the new thiazoloquinazolinones on CDK1, CDK5 and GSK-3 are summarised in Table I. Most synthesised quinazolinones exhibited a moderate to potent GSK-3 inhibitory activity with $IC_{50}$ ranging from 1.3 to 60 μM. As expected, several inhibitors of GSK-3 also targeted CDK1, and CDK5, suggesting that the global cell growth inhibition observed with these compounds is probably associated with inhibition of several other kinases. N-Substitution by an ethyl group on a quinazolinone or thiazoloquinazolinone ring was generally speaking, associated with good GSK-3 inhibitory activity (compounds **3**, **7**, **19**, **23**, **25**, **27** and **29**). However, the two thiazoloquinazolinone isomers (**21** or **22**) were devoid of inhibitory activity on the studied kinases, suggesting that the most promising compounds are those containing a thiazole motif located near the carbonyl function. **22** was not tested against CDK1.The *N*-ethyl-thiazoloquinazolinone substituted with a carbonitrile group (compound **19**) exerted a significant inhibitory activity on the three kinases CDK1, CDK5 and GSK-3.On the other hand, two compounds, **24** and **25**, bearing, at C-2 of the thiazoloquinazolinone, an iminoether function or an amidine (incorporated into an imidazoline ring) exerted a selective inhibition towards GSK-3

*Cytotoxicity and growth inhibition of the novel synthesized thiazoloquinazolinones on MDA-MB-231 breast cancer cells*. We chose a hormone-independent cell line (MDA-MB231, invasive) known to be very



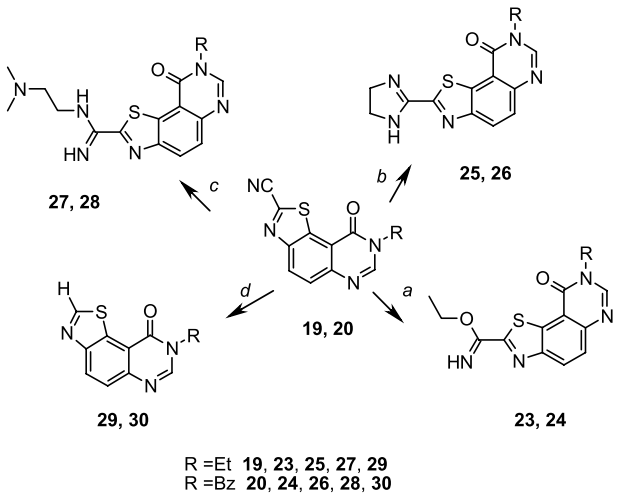

Scheme 5. Synthetic route to 6-oxo-thiazolo[4,5-*h*]quinazoline-2-carbonitriles. Reaction conditions: CuI, pyridine, 160°C, μw.

aggressive and resistant to drugs [14,15]. It appears that, after 24 hours of thiazoloquinazolinone treatment at $10^{-6}$ M, no cytotoxic effect on MDA-MB-231 cells was observed, suggesting that cytotoxicity $IC_{50}$ values were very superior to 1 μM. This analysis is in line with antiproliferative results which revealed that cell growth, at 72 hours, was poorly affected by a drug dose inferior to $10^{-6}$ M except for **3** and **30** with 21% and 28% inhibition, respectively (Figure 4). Antiproliferative activity of compound **3** is probably related to its moderate GSK-3 inhibitory activity ($IC_{50} = 42$ μM). Several compounds active *in vitro* on isolated kinases did not induce any cell growth inhibition when tested at 1 μM (e.g. **19** and **23**). One possibility to explain this lack of cellular effects is the difference in ATP concentration existing between living cells (in the millimolar range) and in *in vitro* assays (15 μM). For this reason, much higher concentrations of protein kinase inhibitors could be needed to inhibit the activity of protein kinases in MDA-MB-231 cells.

Table I. Effects of (thiazolo)quinazolinones on CDK1, CDK5 and GSK-3 activity.

| Compound | CDK1 $IC_{50}$ (μM) | CDK5 $IC_{50}$ (μM) | GSK-3 $IC_{50}$ (μM) |
|---|---|---|---|
| 3 | >100 | >100 | 42 |
| 4 | ND | >10 | >10 |
| 5 | ND | >10 | >10 |
| 7 | >100 | >100 | 60 |
| 8 | ND | >10 | >10 |
| 9 | ND | >10 | >10 |
| 19 | 12 | 27 | 6.2 |
| 20 | ND | >10 | >10 |
| 21 | >10 | >10 | >10 |
| 22 | ND | >10 | >10 |
| 23 | 50 | ND | 2.1 |
| 24 | >100 | >100 | 6.2 |
| 25 | >100 | >100 | 4.2 |
| 26 | ND | >10 | >10 |
| 27 | 17 | ND | 1.3 |
| 28 | ND | >10 | >10 |
| 29 | >12 | ND | 2.3 |
| 30 | ND | > 10 | >10 |

ND: not determined




Scheme 6. Variations in position 2 of thiazoloquinazolinones **19** and **20**. Reaction conditions and yields: (*a*) NaOH, ethanol, rt, 15 min., **23** ($C_2H_5$, 64%), **24** ($CH_2C_6H_5$, 60%); (*b*) $NH_2CH_2CH_2NH_2$, THF, 4 min., 130°C, μw, **25** ($C_2H_5$, 70%), **26** ($CH_2C_6H_5$, 40%); (c) *N*,*N*-Dimethylethylenediamine, THF, 30 min., 80°C, μw, **27** ($C_2H_5$, 50%), **28** ($CH_2C_6H_5$, 70%); (*d*) HBr 48%, 30 min., 115°C, 60 W, μw, **29** ($C_2H_5$, 74%), **30** ($CH_2C_6H_5$, 93%).

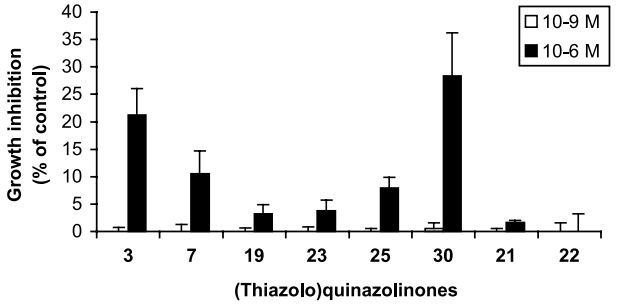



Figure 4. Antiproliferative activity of (thiazolo)quinazolinones.

In conclusion, this work has uncovered a family of 2,8-substituted thiazoloquinazolinones some of whose congeners inhibit GSK-3 in the micromolar range. We believe that this family constitutes a scaffold from which more potent inhibitors could be designed. It has been previously observed that many CDK inhibitors are also potent inhibitors of GSK-3 [16,17]. In the present case, although two compounds (**24** and **25**) were inefficient towards CDK1, moderate inhibitory activity was detected on CDK1 with the most GSK-3 active compounds.

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