

Synthetic Development of New 3-(4-Arylmethylamino)butyl-5-arylidene-rhodanines under Microwave Irradiation and Their Effects on Tumor Cell Lines and against Protein Kinases

Camille Déliko Dago, Christelle Ambeu, Wacothon-Karime Coulibaly, Yves-Alain Békro, Janat Mamyrbékova, Audrey Defontaine, Blandine Baratte, Stéphane Bach, Sandrine Ruchaud, Rémy Le Guével, et al.

▶ **To cite this version:**

Camille Déliko Dago, Christelle Ambeu, Wacothon-Karime Coulibaly, Yves-Alain Békro, Janat Mamyrbékova, et al.. Synthetic Development of New 3-(4-Arylmethylamino)butyl-5-arylidene-rhodanines under Microwave Irradiation and Their Effects on Tumor Cell Lines and against Protein Kinases. Molecules, 2015, 20 (7), pp.12412-12435. 10.3390/molecules200712412 . hal-01187427

HAL Id: hal-01187427

https://univ-rennes.hal.science/hal-01187427

Submitted on 15 Feb 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Molecules **2015**, *20*, 12412-12435; doi:10.3390/molecules200712412

OPEN ACCESS

molecules

ISSN 1420-3049

www.mdpi.com/journal/molecules

Article

Synthetic Development of New 3-(4-Arylmethylamino)butyl-5-arylidene-rhodanines under Microwave Irradiation and Their Effects on Tumor Cell Lines and against Protein Kinases

Camille Déliko Dago [1,2], Christelle N´ta Ambeu [1,2], Wacothon-Karime Coulibaly [3], Yves-Alain Békro [2], Janat Mamyrbékova [2], Audrey Defontaine [4], Blandine Baratte [4], Stéphane Bach [4], Sandrine Ruchaud [4], Rémy Le Guével [5], Myriam Ravache [5], Anne Corlu [5] and Jean-Pierre Bazureau [1,*]

1 Université de Rennes 1, Institut des Sciences Chimiques de Rennes (ISCR), UMR CNRS 6226, Groupe ICMV, Bât. 10A, Campus de Beaulieu, 263 Avenue du Général Leclerc, CS 74205, 35042 Rennes Cedex, France; E-Mails: deliko.dago@univ-rennes1.fr (C.D.D.); christelle.ambeu@univ-rennes1.fr (C.N.A.)

2 Laboratoire de Chimie Bio-Organique et de Substances Naturelles (LCBOSN), Université Nangui Abrogoua, Abidjan 02, BP 802, Cote d'Ivoire; E-Mails: bekro2001@yahoo.fr (Y.-A.B.); kojanova1926@hotmail.fr (J.M.)

3 UFR des Sciences Biologiques, Université de Péléforo Gon Coulibaly, Korhogo, BP 1328, Cote d'Ivoire; E-Mail: wacothon@yahoo.fr

4 Station Biologique de Roscoff, USR 3151, CNRS-UPMC, Kinase Inhibitory Specialized Screening facility, KISSf, Place George Teissier, BP 74, 29682 Roscoff, France; E-Mails: audrey.defontaine@sb-roscoff.fr (A.D.); blandine.baratte@sb-roscoff.fr (B.B.); stephane.bach@sb-roscoff.fr (S.B.); sandrine.ruchaud@sb-roscoff.fr (S.R.)

5 Université de Rennes 1, ImPACcell, SFR Biosit, Bât. 8, 2 Avenue du Professeur Léon Bernard, CS 34317, 35043 Rennes Cedex, France; E-Mails: remy.leguevel@univ-rennes1.fr (R.L.G.); myriam.ravache@gmail.com (M.R.); anne.corlu@univ-rennes1.fr (A.C.)

* Author to whom correspondence should be addressed; E-Mail: jean-pierre.bazureau@univ-rennes1.fr; Tel.: +33-223-236-603; Fax: +33-223-236-374.

Academic Editor: Marilena Radoiu

Received: 27 May 2015 / Accepted: 30 June 2015 / Published: 8 July 2015

Abstract: A new route to 3-(4-arylmethylamino)butyl-5-arylidene-2-thioxo-1,3-thiazolidine-4-one **9** was developed in six steps from commercial 1,4-diaminobutane **1** as starting material. The key step of this multi-step synthesis involved a solution phase "one-pot

two-steps" approach assisted by microwave dielectric from *N*-(arylmethyl)butane-1,4-diamine hydrochloride **6a**–**f (**as source of the first point diversity) and commercial bis-(carboxymethyl)-trithiocarbonate reagent **7** for construction of the rhodanine platform. This platform was immediately functionalized by Knoevenagel condensation under microwave irradiation with a series of aromatic aldehydes **3** as second point of diversity. These new compounds were prepared in moderate to good yields and the fourteen synthetic products **9a**–**n** have been obtained with a *Z*-geometry about their exocyclic double bond. These new 5-arylidene rhodanines derivatives **9a**–**n** were tested for their kinase inhibitory potencies against four protein kinases: Human cyclin-dependent kinase 5-p25, *Hs*CDK5-p25; porcine Glycogen Synthase Kinase-3, GSK-3α/β; porcine Casein Kinase 1, *Ss*CK1 and human *Hs*Haspin. They have also been evaluated for their *in vitro* inhibition of cell proliferation (HuH7 D12, Caco 2, MDA-MB 231, HCT 116, PC3, NCI-H727, HaCat and fibroblasts). Among of all these compounds, **9j** presented selective micromolar inhibition activity on *Ss*CK1 and **9i** exhibited antitumor activities in the HuH7 D12, MDA-MBD231 cell lines.

Keywords: one-pot two-steps; Knoevenagel condensation; 5-arylidene rhodanine; protein kinase; inhibitor; *Ss*CK1; *Hs*CDK5-p25; cell lines; Alzheimer's disease; cancer

1. Introduction

During the last decades, the five-membered heterocycle rings (FMHRs) are considered "privileged scaffolds" in medicinal chemistry [1]. Among the five-membered heterocycle rings, the 5-arylidene-2-thioxo-1,3-thiazolidine-4-ones or 5-arylidene rhodanine derivatives represented particularly privileged moieties in drug discovery because they have an inherent tendency for biological activity [2], such as DDX3 inhibitor for HIV replication [3], as potent and selective inhibitors of the "atypical" dual-specificity phosphatase (DSP) family member-JNK-stimulating phosphatase-1 (JSP-1) [4], as pancreatic cholesterol esterase (CEase) inhibitor [5] with IC_{50} values ranging from 1.44 to 85 μM. To discover chemical probes to further understand the function of human DNA polymerase λ in cancer, an inhibitor high-throughput screening (HTS) using SYBR® Green-base assay [6] revealed that three 5-arylidene-2-thioxo-1,3-thiazolidine-4-ones were identified as strong inhibitors. A new series of D-glutamic acid-base *Escherichia coli* MurD inhibitors incorporating the 5-arylidene rhodanine scaffold have been designed, synthesized and evaluated [7]. Substituted 5-arylidene-2-thioxo-1,3-thiazolidine-4-ones were able to inhibit HIV replication in MT-4 cells at low micromolar concentration with an appreciable selectivity index and are good scaffolds for the development of novel HIV-1 integrase inhibitors [8]. Recently, a family of 5-arylalkylidene rhodanine derivatives presented antiviral activity against chikungunya virus (LR2006_OPY1) in Vero cell culture by cytopathic effect CPE reduction assay [9]. For Alzheimer's disease, the FMHRs derived from 5-arylidene-2-thioxo-1,3-thiazolidine-4-ones have been described for amyloid polypeptide fibril formation [10], regulation of Cathepsin D immuno-reactivity in the senile plaques [11] and inhibition of tau aggregation [12].

Protein kinases represent an important class of enzymes that play an important role in the regulation of various processes. These enzymes catalyze protein-phosphorylation on serine, threonine and tyrosine

residues, which are frequently deregulated in human diseases. Only the 518 human kinases have been investigated as potential therapeutic targets [13]. Consequently, the search of protein-kinase inhibitors represented interesting targets in the pharmaceutical industry for new therapeutic agents. Over the past decade, our research group have investigated the chemical development of five-membered heterocycle rings derived from marine alkaloid as low-molecular weight-inhibitors of dual specificity, tyrosine phosphorylation-regulated kinases (DYRKs) and CLKs (cdc2-like kinases) [14–16], two families of kinases involved in various diseases including Alzheimer's disease (AD) [17], and also cancer [18–20].

Continuing in the effort to identify new DYRK inhibitors, particularly DYRK1A, we continued to explore successively the synthesis of *N*,*N*′-bis-(5-arylidene-4-oxo-3,5-dihydro-4*H*-imidazol-2-yl) diamines [21], *N*,*N*′-bis-(5-arylidene-4-oxo-4,5-dihydrothiazolidine-2-yl)aminopropylpiperazines [22] and finally unsymmetrical linked bis-5-arylidene-rhodanine derivatives linked in *N*-3 position [23] as potential kinases inhibitors (Figure 1). Among the three series of symmetrical or unsymmetrical *N*,*N*′-diamines bearing various platforms (imidazolidine-4-one or 2-thioxo-1,3-thiazolidine-4-one moieties), only one of these compounds has shown nanomolar inhibition potency (IC_{50} 40 nM) towards DYRK1A. Owing to the fact that none of the unsymmetrical linked bis-5-arylidene rhodanines presented a significant activity against representative tumoral cell lines, we decided to change one of the two heterocyclic platforms by various (arylmethyl)aminobutyl moieties grafted on *N*-3 position of 5-arylidene-rhodanine derivatives in order to obtain significant potential biological activities on tumoral cell lines and effects on some protein kinases (*Hs*CDK5-p25, *Ss*GSK3α/β, *Ss*CK1 and *Hs*Haspin). Herein, we present the building of this 3-(4-arylmethylamino)butyl-5-arylidene-rhodanine library in which the 5-arylidene-2-thioxo-1,3-thiazolidine-4-one moiety was mostly carried out under microwave irradiation [24] and the biological activities of these compounds.

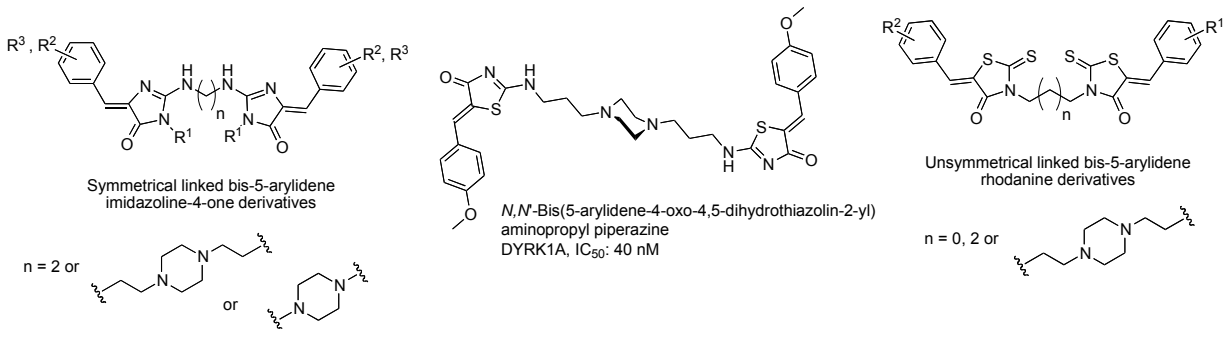


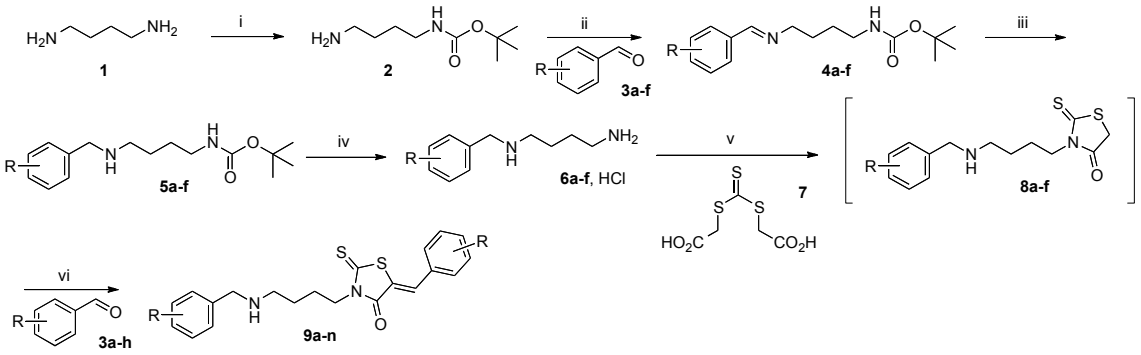
Figure 1. Symmetrical linked bis-5-arylidene imidazoline-4-ones, symmetrical linked bis-5-arylidene-1,3-thiazoline-4-ones and unsymmetrical linked bis-5-arylidene rhodanines derivatives (as inhibitors of the proteine kinase DYRK1A or against cell proliferation on cell tumor lines) identified and developed in our group.

2. Results and Discussion

2.1. Chemistry

Access to the planned 3-(4-arylphenylamino)butyl-5-arylidene-2-thioxo-1,3-thiazolidine-4-ones **9** is outlined in Scheme 1. For this study, we selected a diamino linker **1** with a butyl chain in order to obtain a good molecular flexibility between the 4-arylmethyl moiety and the 2-thioxo-1,3-thiazolidine-4-one

platform. A molecule with the lowest number of carbons for the diamino linker is more conformationally restrained [25]. The 1,4-diamino-butane linker **1** was treated with di-*tert*-butyldicarbonate (Boc_2O) in 1,4-dioxane at room temperature to afford mainly the mono-*N*-Boc protected amine **2** in good yield (84%) [26]. To obtain a sufficient number of compounds suitable for a preliminary biological screening, we privileged the transformation of *N*-Boc-1,4-diamino-butane **2** into mono protected *N*-arylmethyl diamine **5** by reductive amination in two steps. The (4-arylmethylamino)butyl chain appended on the *N*-3 position of the 2-thioxo-1,3-thiazolidine-4-one platform represent the first point of diversity for the desired target compounds **9**. Preparation of **4** was easily realized by reaction between of appropriate arylaldehyde **3a**–**f** with *N*-Boc diamine **2** in the presence of molecular sieves 3Å and, the condensation was conducted in a solution of diethyl ether Et_2O at room temperature during 24 h. Then, transformation of arylaldimines **4** into mono protected *N*-arylmethyl diamines **5** could be readily accomplished in good yields (85% to 98%) using $NaBH_4$ (5 equiv.) in MeOH at 50 °C during 24 h. For the cleavage of *N*-Boc group, the use of trifluoroacetic acid in dichloromethane is often efficient, but in our case, we observed the formation of impurities resulting from uncontrolled degradation of **5** in this strong acidic media. We thus preferred to use a more classical and practical approach by using a solution of 6M HCl. The deprotection was conducted in 1,4-dioxane at room temperature after 4 h of reaction time. As can be seen from the data presented in Table 1, the *N*-(arylmethyl)butane-1,4-diamine hydrochloride **6a**–**f** were efficiently prepared with electron-rich and electron-poor aldehydes **3a**–**f** in yields ranging from 72% to 98%.

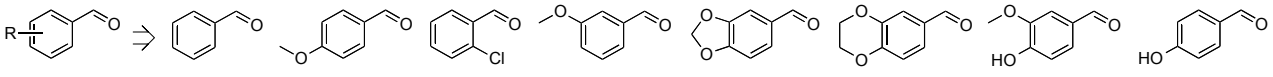


Scheme 1. Route used for the preparation of 3-(4-arylphenylamino)butyl-5-arylidene-2-thioxo-1,3-thiazolidine-4-ones 9 via the "one-pot two-steps" reaction under microwave. *Reagents and reaction conditions:* (i) $(t\text{-}BuO_2C)_2O$, 1,4-dioxane, 25 °C, 24 h. (ii) **3** 1 equiv., molecular sieve 3Å, Et_2O, 25 °C, 16 h. (iii) $NaBH_4$ 5 equiv., MeOH, 50 °C, 24 h. (iv) HCl 6 M, 1,4-dioxane, 25 °C, 4 h. (v) **6** 1 equiv., **7** 1 equiv., Et_3N 2 or 3 equiv., DME, MWI, 90 °C, 15 or 30 min. (vi) **3** 1 equiv., MWI, 110 °C, 15 or 30 min.

With the desired salts **6** in hand, we wished to examine the construction of the 2-thioxo-1,3-thiazolidine-4-one platform followed by Knoevenagel condensation using arylaldehyde **3** under microwave irradiation for installation of the 5-arylidene moiety. The choice of arylaldehydes **3** in Knoevenagel condensation represent, in fact, the second point of diversity in the final structure of the targeted compounds **9**. In a previous work issued from our laboratory [23], we have developed a "one-pot two-steps" method under microwave irradiation, which was applied for the synthesis of unsymmetrical linked bis-arylidene rhodanines from symmetric diamines.

Table 1. Results for the preparation of compounds **2**, **4**, **5**, **6** and **9**.

Compound	Starting Product	Reaction Conditions under MWI [a]				Yield [b] (%)
		Starting Product 6	Number of equiv. for Et_3N	Reaction Time for 8 [c] (min)	Reaction Time for Condensation [d] (min)	
2	-	-	-	-	-	84
4a	**3a**	-	-	-	-	97
4b	**3b**	-	-	-	-	99
4c	**3c**	-	-	-	-	98
4d	**3d**	-	-	-	-	98
4e	**3e**	-	-	-	-	98
4f	**3f**	-	-	-	-	99
5a	**4a**	-	-	-	-	93
5b	**4b**	-	-	-	-	99
5c	**4c**	-	-	-	-	85
5d	**4c**	-	-	-	-	90
5e	**4e**	-	-	-	-	97
5f	**4f**	-	-	-	-	98
6a	**5a**	-	-	-	-	84
6b	**5b**	-	-	-	-	97
6c	**5c**	-	-	-	-	98
6d	**5d**	-	-	-	-	98
6e	**5e**	-	-	-	-	72
6f	**5f**	-	-	-	-	92
9a	**3e**	**6b**	3	30	30	10
9b	**3b**	**6b**	3	30	30	6
9c	**3f**	**6b**	3	30	30	5
9d	**3g**	**6b**	3	30	30	30
9e	**3e**	**6d**	3	15	15	21
9f	**3f**	**6d**	3	15	15	9
9g	**3d**	**6d**	3	30	30	5
9h	**3e**	**6e**	2	15	15	59
9i	**3f**	**6e**	2	15	15	59
9j	**3h**	**6e**	2	15	15	34
9k	**3g**	**6e**	3	30	30	46
9l	**3e**	**6f**	3	15	15	15
9m	**3f**	**6f**	3	15	15	7
9n	**3g**	**6f**	3	30	30	34



3 **3a** **3b** **3c** **3d** **3e** **3f** **3g** **3h**

Notes: [a] Reaction realized in a tube (sealed with a snap cap) under microwave irradiation (μω) with the Monowave® 300 Anton-Paar reactor. [b] Isolated yield. [c] Reaction temperature: 90 °C for the preparation of **8** (1st period of microwave irradiation). [d] Reaction temperature: 110 °C for condensation reaction after addition of **3** (2nd period of microwave irradiation).

Application of this methodology, based on a "modified Holmberg method", first involved, in our case, the reaction of the commercial bis-(carboxymethyl)-trithiocarbonate reagent **7** with the synthesized

N-(arylmethyl)butane-1,4-diamine hydrochloride **6** to afford the intermediate **8** and secondly, Knoevenagel condensation to produce the desired 5-arylidene rhodanines **9**. This "one-pot two-steps" methodology was realized under microwave dielectric heating because the use of commercial scientific laboratory microwave apparatus favoured higher product yields and significant rate enhancements compared to reactions which run with conventional heating (*i.e.*, in oil bath) [27]. After several experiments, optimal reaction conditions for this "one-pot two-steps" synthesis involved reaction of a stoichiometric mixture of *N*-(arylmethyl)butane-1,4-diamine hydrochloride **6** and commercial reagent **7** solubilized in dimethoxyethane with one or two equivalents of triethylamine. This starting reaction mixture was placed in a commercial glass tube closed with a snap cap and was irradiated with appropriate reaction time (15 or 30 min.) at 90 °C. After this first period of microwave irradiation followed by a cooling down to room temperature, aromatic aldehyde **3** was directly added to the crude suspension and was submitted again to microwave dielectric heating at 110 °C during 15 or 30 min for condensation.

The desired compound **9** was obtained as precipitate after addition of methanol in the solventless crude reaction mixture (after elimination of the volatile compounds *in vacuo*) and triturated, followed by successive washings with deionized water, ethanol, ether and finally was recrystallized from absolute EtOH to increase the quality of the precipitated product **9**. A set of 14 pure compounds **9a**–**n** was prepared in 5%–59% yield (Table 1) and all the products **9** were characterized by ^{1}H-, ^{13}C-NMR and HRMS before entering the biological tests. For the exocyclic double bond (CH=C) in C-5 position of all the 3-(4-arylmethylamino)butyl-2-thioxo-1,3-thiazolidine-4-one **9a**–**n**, it's possible in theory to observe *E*- and/or *Z*-geometrical isomers. Examination of their ^{1}H-NMR spectra in DMSO-d_6 showed only one signal for the methylene proton (CH=) in the range 7.54–7.74 ppm at lower field values than those expected for the *E*-isomers, which indicates that all the compounds **9a**–**n** have the *Z*-configuration due to the high degree of thermodynamic stability of this isomer [28,29]. In ^{13}C-NMR, the signal of C-5 (C=) appears in the range 120.0–127.5 ppm, and we also observed only one signal for the exocyclic methylene proton ($134.6 < \delta_{CH=} < 135.9$ ppm) that confirmed the presence of only the *Z*-geometrical isomer for the targeted compounds **9a**–**n**.

2.2. Biology

As an initial effort to investigate their *in vitro* bioactivity, the new synthesized 3-(4-arylmethylamino) butyl-2-thioxo-1,3-thiazolidine-4-one **9a**–**n** and also their precursors **6a**–**f** were evaluated for their *in vitro* inhibition of cell proliferation. For this study, we used a panel of seven representative tumoral cell lines, namely HuH7 D12 (differential hepato cellular carcinoma), Caco 2 (differentiating colorectal adenocarcinoma), MDA-MBD231 (prostate carcinoma), HCT 116 (actively proliferating colorectal adenocarcinoma), PC3 (prostate carcinoma), NCI-H727 (lung carcinoma), HaCat keratinocyte and, diploid skin fibroblasts as normal cell lines for control. Roscovitine, Doxorubicine and Taxol were also used as positive controls and their IC_{50} values are compared with those obtained for compounds **6** and **9**. Results of the *in vitro* antiproliferative data activity are reported in Table 2. None of the *N*-(arylmethyl) butane-1,4-diamines hydrochloride **6a**–**f** presented a significant activity against the seven representative tumoral cell lines. For the other compounds **9**, the most active compound was clearly **9i** (Figure 2) and exhibited antitumor activities in the HuH7 D12, MDA-MBD231 and HaCat cell lines with IC_{50} values lower than 10 μM (HuH7 D12 and MDA-MBD231: IC_{50} 9 μM) and did not inhibit the growth of normal

fibroblasts (IC_{50} > 25 μM). In addition, compounds **9d**, **9h**, **9j** and **9n** presented moderate antitumor activities (IC_{50} 10–17 μM) in all cell lines of the panel but without selectivity.

Table 2. Antiproliferative activity of compounds **6**(**a**–**e**) and **9**(**a**–**n**) on six representative tumor cell lines.

Compound	% of Survival [a] and IC_{50} (μM) of Selected Compounds [b]							
	Huh7 D12	**Caco 2**	**MDA-MB231**	**HCT 116**	**PC3**	**NCI-H727**	**HaCat**	**Fibroblasts**
6a	102	114	104	112	100	97	97	93
6b	107	114	100	112	100	86	106	100
6c	82	93	102	98	96	100	80	84
6d	107	111	99	111	98	92	96	93
6e	91	106	102	102	96	98	96	93
6f	88	109	99	96	97	92	94	105
9a	85	109	100	109	92	104	103	104
9b	105	108	97	102	99	90	104	167
9c	88	93	92	97	97	95	101	102
9d	7 (10)	23 (15)	15 (11)	1 (10)	41 (17)	11 (10)	2 (10)	29 (>25)
9e	74	97	97	99	85	102	86	122
9f	102	110	98	113	98	96	100	134
9g	111	117	104	118	98	95	110	100
9h	7 (10)	42 (14)	49 (10)	65 (13)	51 (13)	43 (>25)	10 (10)	80 (>25)
9i	5 (9)	17 (13)	17 (9)	8 (14)	39 (14)	9 (11)	6 (8)	54 (>25)
9j	22 (11)	41 (27)	42 (19)	40 (22)	38 (19)	33 (19)	21 (13)	77 (>25)
9k	72	98	92	98	83	96	80	84
9l	90	105	98	106	93	96	95	128
9m	97	107	99	103	96	98	94	149
9n	41 (15)	28 (16)	55 (15)	52 (21)	61 (19)	63 (17)	48 (15)	86 (>25)
Roscovitine	21 (15)	3 (15)	21 (12	10 (9)	24 (13)	30 (43)	6 (11)	53 (125)
Doxorubicine	63 (0.03)	43 (0.03)	82 (0.01)	22 (0.03)	34	65	88 (0.02)	74 (>0.25)
Taxol	36 (0.003)	29 (0.04)	41 (0.04)	9 (<0.001)	35 (<0.001)	59	11 (0.001)	76 (>25)
DMSO	100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)	100 (>25)

Notes: [a] Percentage of survival measured at 25 μM (after 48 h using a single dose, triplicate). [b] IC_{50} values in brackets are expressed in μM and are the average of three assays, standard error ± 0.5 μM.

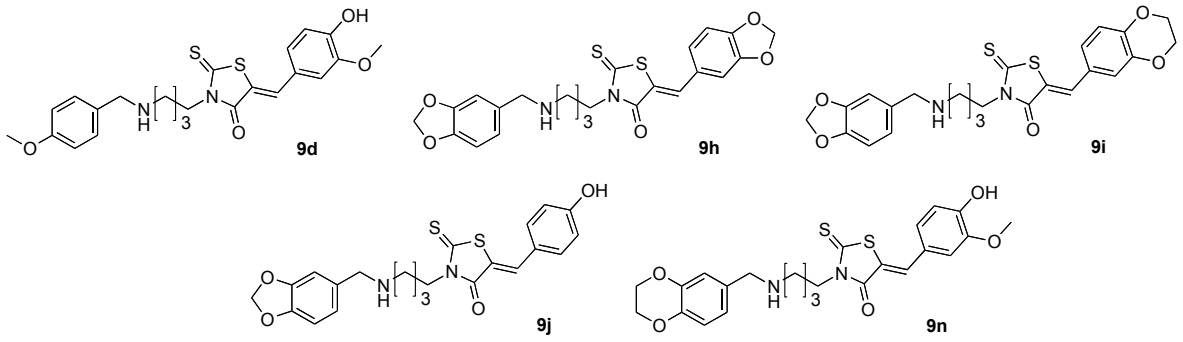


Figure 2. Structure of compounds **9d**, **9**(**h**–**j**) and **9n**, which are active against protein kinases and/or tumor cell lines.

Then, we evaluated the intermediates salts **6a**–**f** and fourteen final compounds **9a**–**n** on four protein kinases: *Hs*CDK5-p25 (cyclin-dependant kinase 5-p25), *Ss*GSK-3α/β (glycogen synthase kinase-3α/β) *Ss*CK1δ/ε (Caseine kinase 1) and *Hs*Haspin as an essential mitotic kinase [30]. All assays were run in the presence of 15 μM of ATP and appropriate substrates. IC_{50} values were determined from dose-response curves. Concerning the library of the fourteen (5*Z*) 3-(4-arylmethylamino)butyl-5-arylidene-2-thioxo-1,3-thiazolidine-4-ones **9a**–**n** and also the six diamines salts **6a**–**f**, the results are given in Table 3. Among all the products, the main part of their inhibition activity was focused on two proteins kinases (*Ss*CK1 and *Hs*CDK5-p25) for four products, which exhibited inhibitory activity in the micromolar range (*Ss*CK1: 1.4 μM < IC_{50} < 6.6 μM; *Hs*CDK5-p25: IC_{50} 1.2 μM). For the compound **9j** and **9n**, we observed micromolar inhibition activity on *Ss*CK1 (IC_{50} values for **9j**: 1.4 μM and **9n**: 2 μM) with good selectivity. It is interesting to note that these two compounds are respectively substituted with an hydroxyl function on *para*-position of the 5-arylidene moiety and also with a similar bulky group (1,3-benzodioxol-5-yl for **9j** and 2,3-dihydro-benzo[1,4]dioxin-6-yl for **9n**) on the arylmethylamino moiety grafted on the *N*-3 butyl chain. On the contrary, the presence of 1,3-benzodioxol-5-yl (for **9h**) or 2,3-dihydro-benzo[1,4]dioxin-6-yl moiety (for **9i**) in the 5-arylidene group induced a moderate loss of inhibition activity for *Ss*CK1 (IC_{50} for **9h**: 6.6 μM and **9i**: 5.4 μM) associated with micromolar affinity for *Hs*CDK5-p25 (IC_{50} for **9h**: 1.1 μM and **9i**: 1.3 μM). Compound **9i** presented also inhibition proliferation activity on Huh7 D12, MDA-MBD231 cell lines and is inactive against normal fibroblasts.

Table 3. Effects of the compounds **6a**–**f** and **9a**–**n** on the catalytic activity of four purified protein kinases [a].

Compound	*Hs*CDK5/p25	*Ss*GSK-3α/β	*Ss*CK1δ/ε	*Hs*Haspin
6a–f	>10	>10	>10	>10
9a–g	>10	>10	>10	>10
9h	1.1	>10	6.6	>10
9i	1.3	>10	5.1	>10
9j	>10	>10	1.4	>10
9k–m	>10	>10	>10	>10
9n	10	>10	2	>10

Note: [a] Compounds were tested at various concentrations on each kinase as described in Experimental Section. IC_{50} values, calculated from the dose-response curves, are reported in μM > 10, inhibitory but IC_{50} > 10 μM.

3. Experimental Section

3.1. Chemistry Section

3.1.1. General Section

Melting points were determined on a Kofler melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was accomplished on 0.2-mm precoated plates of silica gel 60 F-254 (Merck, Fontenay-sous-Bois, France). Visualization was made with ultraviolet light (254 and 365 nm) or with a fluorescence indicator. ^{1}H-NMR spectra were recorded on BRUKER AC 300 P (300 MHz) spectrometer, ^{13}C-NMR spectra on a BRUKER AC 300 P (75 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Data are given in the

following order: δ value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), number of protons, coupling constants *J* is given in Hertz. The mass spectra (HRMS) were taken respectively on a MS/MS ZABSpec Tof Micromass (EBE TOF geometry) at an ionizing potential of 8 eV and on a VARIAN MAT 311 at an ionizing potential of 70 eV in the Centre Régional de Mesures Physiques de l'Ouest (CRMPO, Rennes, France). Reactions under microwave irradiations were realized in the Anton Paar Monowave 300® microwave reactor (Anton-Paar, Courtaboeuf, France) using borosilicate glass vials of 10 mL equipped with snap caps (at the end of the irradiation, cooling reaction was realized by compressed air). The microwave instrument consists of a continuous focused microwave power output from 0 to 800 W for this Monowave 300® apparatus. All the experiments in the microwave reactor were performed using a stirring option. The target temperature was reached with a ramp of 5 min and the chosen microwave power stayed constant to hold the mixture at this temperature. The reaction temperature is monitored using calibrated infrared sensor and the reaction time included the ramp period. The microwave irradiation parameters (power and temperature) were monitored by the Monowave software package of the Monowave 300® microwave reactor. Solvents were evaporated with a BUCHI rotary evaporator. All reagents and solvents were purchased from Acros, Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France), TCI France and Fluka France and were used without further purification.

tert-Butyl (4-aminobutyl)carbamate (**2**). In a 250 mL two-necked round-bottomed flask, provided with magnetic stirrer and condenser, commercial 1,4-diaminobutane **1** (14.3 mL, 12.5 g, 0.14 mol) was solubilized in 69 mL of 1,4-dioxane at room temperature. To this mixture was added dropwise a solution of commercial di-*tert*-butyldicarbonate (6.5 g, 30 mmol) in 1,4-dioxane (85 mL) over a period of 3 h. After vigorous stirring at 25 °C during a 12 h period, the volatile compounds of the reaction mixture were eliminated *in vacuo* and into the crude reaction mixture was poured 150 mL of deionized water. The mixture was extracted with methylene chloride (5 × 50 mL), organic phases were collected and dried over magnesium sulfate. The filtrate was concentrated in a rotary evaporator under reduced pressure and was dried under high vacuum (10^{-2} Torr) at 25 °C for 10 min. The desired carbamate **2** (1.58 g) was obtained as a colourless mobile oil in 84% yield and was further used without purification. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (s, 9H, Me_3CO); 1.40 (m, 4H, CH_2); 2.61 (t, 2H, J = 6.7 Hz, CH_2); 3.02 (m, 2H, CH_2NH); 4.93 (br s, 2H, NH_2); 5.71 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 27.2 (CH_2); 28.4 ($OC(\underline{C}H_3)_3$); 29.5 (CH_2); 40.2 (CH_2); 41.1 (CH_2); 78.9 ($O\underline{C}(CH_3)_3$); 156.1 (C=O). HRMS, *m*/*z*: 189.1600 found (calculated for $C_9H_{21}N_2O_2$ $[M + H]^+$ requires 189.1603).

3.1.2. Standard Procedure for the Preparation of Aldimines **4a**–**f** from *tert*-Butyl (4-aminobutyl) Carbamate **2** and Aromatic Aldehyde **3a**–**f**

In a 100 mL two-necked round-bottomed flask, provided with magnetic stirrer and condenser, containing a solution of *tert*-butyl (4-aminobutyl)carbamate **2** (1.97 g, 10.5 mmol) in anhydrous ether (50 mL) was added dropwise during 30 min, a suspension of commercial aldehyde (10 mmol) in anhydrous ether (30 mL) and molecular sieves (2 g, 3 Å, 8–12 mesh). The crude reaction mixture is stirred vigorously for 16 h at room temperature until the disappearance of aromatic aldehyde **3** controlled by thin-layer chromatography on 0.2-mm precoated plates of silica gel 60 F-254 (Merck). The crude reaction mixture

was filtered on filter paper and then concentrated in a rotary evaporator under reduced pressure. The crude residue was dried under high vacuum (10^{-2} Torr) at 25 °C for 10 min. The desired aldimine **4** was obtained as yellowish viscous oil and was further used without purification.

[4-(Benzylidene-amino)-butyl]-carbamic acid tert-butyl ester (**4a**). Compound **4a** was prepared in 97% yield (2.68 g) from benzaldehyde **3a** (1.06 g, 10 mmol) according to the standard procedure as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.40–1.48 (m, 2H, CH_2, H-2); 1.54–1.62 (m, 2H, CH_2, H-3); 2.96 (q, 2H, J = 6.8 Hz, CH_2NH, H-1); 3.55 (t, 2H, J = 6.8 Hz, CH_2N=, H-4); 6.79–6.83 (br s, 1H, NH); 7.41–7.46 (m, 3H, H-3′, H-4′, H-5′, Ar); 7.71–7.75 (m, 2H, H-2′, H-6′, Ar); 8.32 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6) δ: 27.3 (C-2); 27.8 (C-3); 28.2 (OC($\underline{C}H_3)_3$); 39.6 (C-1); 60.1 (C-4); 77.2 (O$\underline{C}$($CH_3)_3$); 127.7 (C-2′, C-6′, Ar); 128.5 (C-3′, C-5′, Ar); 130.4 (C-4′, Ar); 136.1 (C-1′, Ar); 155.5 ($\underline{C}$=O); 160.5 (N=$\underline{C}$H).

{4-[(4-Methoxy-benzylidene)-amino]-butyl}-carbamic acid tert-butyl ester (**4b**). Compound **4b** was prepared in 99% yield (3.03 g) from 4-methoxybenzaldehyde **3b** (1.36 g, 10 mmol) according to the standard procedure as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.32–1.46 (m, 2H, CH_2, H-2); 1.52–1.61 (m, 2H, CH_2, H-3); 2.94 (q, 2H, J = 6.9 Hz, CH_2NH, H-1); 3.50 (t, 2H, J = 6.8 Hz, CH_2N=, H-4); 3.80 (s, 3H, O$\underline{C}H_3$); 6.77–6.82 (br s, 1H, NH); 6.98 (d, 2H, J = 8.8 Hz, H-2′, H-6′, Ar); 7.66 (d, 2H, J = 8.8 Hz, H-3′, H-5′, Ar); 8.24 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6) δ: 27.4 (C-2); 28.0 (C-3); 28.2 (OC($\underline{C}H_3)_3$); 39.6 (C-1); 55.2 (O$\underline{C}H_3$); 60.1 (C-4); 77.3 (O$\underline{C}$($CH_3)_3$); 114.0 (C-2′, C-6′, Ar); 129.0 (C-1′, Ar); 129.3 (C-3′, C-5′, Ar); 155.5 ($\underline{C}$=O); 159.7 (N=$\underline{C}$H); 161.0 (C-4′, Ar).

{4-[(2-Chloro-benzylidene)-amino]-butyl}-carbamic acid tert-butyl ester (**4c**). Compound **4c** was prepared in 98% yield (3.05 g) from 2-chlorobenzaldehyde **3c** (1.41 g, 10 mmol) according to the standard procedure as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.34–1.48 (m, 2H, CH_2, H-2); 1.56–1.63 (m, 2H, CH_2, H-3); 2.96 (q, 2H, J = 6.7 Hz, CH_2NH, H-1); 3.61 (t, 2H, J = 6.8 Hz, CH_2N=, H-4); 6.78–6.82 (br s, 1H, NH); 7.36–7.52 (m, 3H, H-4′, H-5′, H-6′, Ar); 7.94–7.97 (m, 1H, H-3′, Ar); 8.65 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6) δ: 27.3 (C-2); 27.7 (C-3); 28.2 (OC($\underline{C}H_3)_3$); 39.6 (C-1); 60.4 (C-4); 77.2 (O$\underline{C}$($CH_3)_3$); 127.4 (C-5′, Ar); 128.0 (C-4′, Ar); 129.8 (C-6′, Ar); 132.0 (C-3′, Ar); 132.7 (C-1′, C-2′, Ar); 133.8 (C-1′, C-2′, Ar); 155.5 ($\underline{C}$=O); 156.6 (N=$\underline{C}$H).

{4-[(3-Methoxy-benzylidene)-amino]-butyl}-carbamic acid tert-butyl ester (**4d**). Compound **4d** was prepared in 98% yield (3.03 g) from 3-methoxybenzaldehyde **3d** (1.36 g, 10 mmol) according to the standard procedure as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.36–1.45 (m, 2H, CH_2, H-2); 1.54–1.64 (m, 2H, CH_2, H-3); 2.96 (q, 2H, J = 6.7 Hz, CH_2NH, H-1); 3.54 (t, 2H, J = 6.7 Hz, CH_2N=, H-4); 3.78 (s, 3H, OCH_3), 6.76–6.82 (br s, 1H, NH); 6.99–7.03 (m, 1H, H-5′, Ar); 7.27–7.38 (m, 3H, H-2′, H-4′, H-6′, Ar); 8.29 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6) δ: 27.3 (C-2); 27.8 (C-3); 28.2 (OC($\underline{C}H_3)_3$); 39.6 (C-1); 55.0 (O$\underline{C}H_3$); 60.1 (C-4); 77.2 (O$\underline{C}$($CH_3)_3$); 111.9 (C-5′, Ar); 116.6 (C-6′, Ar); 120.6 (C-4′, Ar); 129.6 (C-2′, Ar); 137.6 (C-1′, Ar); 155.5 ($\underline{C}$=O); 159.4 (C-3′, Ar); 160.4 ($\underline{C}$=N).

{4-[(Benzo[1,3]dioxol-5-ylmethylene)-amino]-butyl}-carbamic acid tert-butyl ester (**4e**). Compound **4e** was prepared in 98% yield (3.13 g) from 3,4-methylenedioxybenzaldehyde **3e** (1.51 g, 10 mmol)

according to the standard procedure as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.30–1.46 (m, 2H, CH_2, H-2); 1.51–1.61 (m, 2H, CH_2, H-3); 2.94 (q, 2H, J = 6.9 Hz, CH_2NH, H-1); 3.49 (t, 2H, J = 6.7 Hz, CH_2N=, H-4); 6.07 (s, 2H, H-2′, Ar), 6.77–6.82 (br s, 1H, NH); 6.97 (d, 1H, J = 8 Hz, H-6′, H-7′, Ar); 7.16–7.20 (m, 1H, H-6′, H-7′, Ar); 7.27–7.28 (m, 1H, H-4′, Ar), 8.20 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6) δ: 27.3 (C-2); 27.9 (C-3); 28.2 (OC($\underline{C}H_3$)$_3$); 39.6 (C-1); 59.8 (C-4); 77.4 (O$\underline{C}$(CH_3)$_3$); 101.4 (C-2′, Ar); 105.8 (C-6′, Ar); 108.1 (C-7′, Ar); 124.0 (C-4′, Ar); 130.9 (C-5′, Ar); 147.8 (C-3a′, C-7a′, Ar); 149.3 (C-3a′, C-7a′, Ar); 155.5 ($\underline{C}$=O); 160.0 ($\underline{C}$=N).

{4-[(2,3-Dihydro-benzo[1,4]dioxin-6-ylmethylene)-amino]-butyl}-carbamic acid tert-butylester (**4f**). Compound **4f** was prepared in 99% yield (3.31 g) from 2,3-dihydro-benzo[1,4]dioxin-6-carbaldehyde **3f** (1.50 g, 10 mmol) according to the standard procedure as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.34–1.45 (m, 2H, CH_2, H-2); 1.51–1.60 (m, 2H, CH_2, H-3); 2.94 (q, 2H, J = 6.8 Hz, CH_2NH, H-1); 3.50 (t, 2H, J = 6.7 Hz, CH_2N=, H-4); 4.23–4.30 (m, 4H, H-2′, H-3′, Ar), 6.77–6.81 (br s, 1H, NH); 6.88–6.91 (m, 1H, H-7′, Ar); 7.18–7.21 (m, 2H, H-5′, H-8′, Ar); 8.17 (s, 1H, N=CH). ^{13}C-NMR (DMSO-d_6) δ: 27.3 (C-2); 27.9 (C-3); 28.2 (OC($\underline{C}H_3$)$_3$); 39.6 (C-1); 60.0 (C-4); 63.9 (C-2′, Ar), 64.2 (C-3′, Ar), 77.2 (O$\underline{C}$(CH_3)$_3$); 116.0 (C-7′, Ar); 117.1 (C-8′, Ar); 121.2 (C-5′, Ar); 129.8 (C-6′, Ar); 143.4 (C-4a′, C-8a′, Ar); 145.4 (C-4a′, C-8a′, Ar); 155.5 (C=O); 159.6 (C=N).

3.1.3. Standard Procedure for Reduction of Aldimines **4a**–**f** into *N*-Boc Monoprotected Diamines **5a**–**f**

In a 50 mL two-necked round-bottomed flask, provided with a magnetic stirrer and reflux condenser, compound **4** (5 mmol) was dissolved in methanol p.a. (30 mL) under vigorous stirring and cooled at 0 °C. To this solution was added by small portions commercial sodium borohydride $NaBH_4$ (0.95 g, 25 mmol) over a period of 20 min. The resulting suspension was stirred at 50 °C for 24 h (monitored by TLC on 0.2-mm precoated plates of silica gel 60 F-254, Merck). After cooling down to room temperature, the volatile compounds were removed in a rotary evaporator under reduced pressure, then deionized water (40 mL) was added in one portion to the crude residue. The mixture was transferred to a separating funnel and was extracted with dichloromethane (3 × 50 mL). The combined organic phases were dried over magnesium sulphate $MgSO_4$, filtered on filter paper and the solvent was eliminated *in vacuo*. The crude residue was dried under high vacuum (10^{-2} Torr) at 25 °C for 2 h. The desired compound **5** was obtained as yellowish viscous oil and was further used without purification.

(4-Phenylmethylamino-butyl)-carbamic acid tert-butyl ester (**5a**). Compound **5a** was prepared in 93% yield (1.25 g) from [4-(benzylidene-amino)-butyl]-carbamic acid *tert*-butyl ester **4a** (1.38 g, 5 mmol) according to the standard procedure that gave **5a** as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.34–1.42 (m, 4H, CH_2, H-2, H-3); 2.45 (t, 2H, J = 6.2 Hz, CH_2NH, H-4); 2.89–2.91 (m, 2H, CH_2NH, H-1); 3.66 (s, 2H, Ar$\underline{C}H_2$NH); 6.78–6.82 (br s, 1H, NHCO); 7.18–7.13 (m, 1H, H-4′, Ar); 7.26–7.33 (m, 4H, H-2′, H-3′, H-5′, H-6′, Ar). ^{13}C-NMR (DMSO-d_6) δ: 27.4 (C-3); 26.8 (C-2); 39.8 (C-1); 48.4 (C-4); 53.0 (Ar$\underline{C}H_2$NH); 77.2 (O$\underline{C}$(CH_3)$_3$); 126.3 (C-4′, Ar); 127.8 (C-2′, C-6′, Ar); 128.0 (C-3′, C-5′, Ar); 141,0 (C-1′, Ar); 155,5 (C=O).

[4-(4-Methoxy-phenylmethylamino)-butyl]-carbamic acid tert-butyl ester (**5b**). Compound **5b** was prepared in 99% yield (1.52 g) from {4-[(4-methoxy-benzylidene)-amino]-butyl}-carbamic acid

tert-butyl ester **4b** (1.53 g, 5 mmol) according to the standard procedure that gave **5b** as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.33–1.41 (m, 4H, H-2, H-3); 2.45 (t, 2H, J = 6.5 Hz, $C\underline{H}_2NH$, H-4); 2.88–2.90 (m, 2H, $C\underline{H}_2NHCO$, H-1); 3.60 (s, 2H, $ArC\underline{H}_2NH$); 3.72 (s, 3H, $OC\underline{H}_3$); 6.76–6.80 (br s, 1H, $N\underline{H}CO$); 6.85 (d, 2H, J = 8.5 Hz, H-2′, H-6′, Ar); 7.22 (d, 2H, J = 8.6 Hz, H-3′, H-5′, Ar). ^{13}C-NMR (DMSO-d_6) δ: 26.6 (C-2); 27.4 (C-3); 28.2 ($OC(\underline{C}H_3)_3$); 39.8 (C-4); 48.2 (C-1); 52.3 ($Ar\underline{C}H_2NH$); 54.9 ($O\underline{C}H_3$); 77.2 ($O\underline{C}(CH_3)_3$); 113.4 (C-2′, C-6′, Ar); 129.0 (C-3′, C-5′, Ar); 132.6 (C-1′, Ar); 155.5 (C=O); 158.0 (C-4′, Ar).

[4-(2-Chloro-phenylmethylamino)-butyl]-carbamic acid tert-butyl ester (**5c**). Compound **5c** was prepared in 85% yield (1.33 g) from {4-[(2-chloro-benzylidene)-amino]-butyl}-carbamic acid *tert*-butyl ester **4c** (1.55 g, 5 mmol) according to the standard procedure that gave **5c** as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.34–1.43 (m, 4H, $C\underline{H}_2$, H-2, H-3, 4H); 2.47–2.52 (m, 2H, $ArCH_2NH$, H-4); 2.88–2.92 (m, 2H, $C\underline{H}_2NHCO$, H-1); 3.75 (s, 2H, $ArC\underline{H}_2NH$); 6.77–6.80 (br s, 1H, $N\underline{H}CO$); 7.21–7.33 (m, 2H, H-4′, H-5′, Ar); 7.37–7.4 (m, 1H, H-6′, Ar); 7.5–7.53 (m, 1H, H-3′, Ar). ^{13}C-NMR (DMSO-d_6) δ: 26.7 (C-2); 27.4 (C-3); 28.2 ($OC(\underline{C}H_3)_3$); 39.8 (C-4); 48.5 (C-1); 50.0 ($Ar\underline{C}H_2NH$); 77.2 ($O\underline{C}(CH_3)_3$); 126.9 (C-5′, Ar); 128.0 (C-4′, Ar); 128.9 (C-6′, Ar); 129.6 (C-3′, Ar); 132.5 (C-1′, Ar); 138.2 (C-2′, Ar); 155.5 (C=O).

[4-(3-Methoxy-phenylmethylamino)-butyl]-carbamic acid tert-butyl ester (**5d**). Compound **5d** was prepared in 90% yield (1.39 g) from {4-[(3-methoxy-benzylidene)-amino]-butyl}-carbamic acid *tert*-butyl ester **4d** (1.53 g, 5 mmol) according to the standard procedure that gave **5d** as pale pink viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.34–1.46 (m, 4H, H-2, H-3); 2.45 (t, 2H, J = 6.4 Hz, CH_2NH, H-4); 2.89–2.91 (m, 2H, $C\underline{H}_2NHCO$, H-1); 3.64 (s, 2H, $ArC\underline{H}_2NH$); 3.74 (s, 3H, OCH_3); 6.75–6.80 (br s, 1H, NHCO); 6.75–6.91 (m, 3H, H-4′, H-5′, H-6′, Ar); 7.17–7.22 (m, 1H, H-2′, Ar). ^{13}C-NMR (DMSO-d_6) δ: 26.8 (C-2); 27.4 (C-3); 28,2 ($OC(\underline{C}H_3)_3$); 39.8 (C-4); 48.4 (C-1); 52.9 ($Ar\underline{C}H_2NH$); 54.8 ($O\underline{C}H_3$); 77.2 ($O\underline{C}(CH_3)_3$); 111.8 (C-5′, Ar); 113.2 (C-6′, Ar); 120.0 (C-4′, Ar); 128.9 (C-2′, Ar); 142.8 (C-1′, Ar); 155.5 (C=O); 159.2 (C-3′, Ar).

{4-[(Benzo[1,3]dioxol-5-ylmethyl)-amino]-butyl}-carbamic acid tert-butyl ester (**5e**). Compound **5e** was prepared in 97% yield (1.56 g) from {4-[(benzo[1,3]dioxol-5-ylmethylene)-amino]-butyl}-carbamic acid *tert*-butyl ester **4e** (1.60 g, 5 mmol) according to the standard procedure that gave **5e** as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.23–1.40 (m, 4H, H-2, H-3); 2.43 (t, 2H, J = 6.5 Hz, CH_2NH, H-4); 2.89–2.91 (m, 2H, CH_2NHCO, H-1); 3.64 (s, 2H, $ArC\underline{H}_2NH$); 5.96 (s, 2H, H-2′, Ar); 6.73–6.82 (br s, 1H, NHCO); 6.73–6.90 (m, 3H, H-4′, H-6′, H-7′, Ar). ^{13}C-NMR (DMSO-d_6) δ: 26.8 (C-2); 27.4 (C-3); 28.2 ($OC(\underline{C}H_3)_3$); 39.8 (C-4); 48.1 (C-1); 52.7 ($Ar\underline{C}H_2NH$); 77.2 ($O\underline{C}(CH_3)_3$); 100.6 (C-2′, Ar); 107.7 (C-6′, Ar); 108.2 (C-7′, Ar); 120.8 (C-4′, Ar); 135.0 (C-5′, Ar); 145.7 (C-3a′, C-7a′, Ar); 147.1 (C-3a′, C-7a′, Ar); 155.5 (C=O).

{4-[(2,3-Dihydro-benzo[1,4]dioxin-6-ylmethyl)-amino]-butyl}-carbamic acid tert-butyl ester (**5f**). Compound **5f** was prepared in 88% yield (1.48 g) from {4-[(2,3-dihydro-benzo[1,4]dioxin-6-ylmethylene)-amino]-butyl}-carbamic acid *tert*-butylester **4f** (1.67 g, 5 mmol) according to the standard procedure that gave **5f** as yellowish viscous oil. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (s, 9H, Me_3C); 1.33–1.43 (m, 4H, H-2, H-3); 2.42 (t, 2H, J = 6.4 Hz, CH_2NH, H-4); 2.88–2.90 (m, 2H, CH_2NHCO, H-1); 3.53 (s, 2H,

ArCH$_2$NH); 4.20 (s, 4H, H-2′, H-3′, Ar); 6.75–6.80 (br s, 1H, NHCO); 6.75–6.80 (m, 3H, H-5′, H-7′, H-8′, Ar). ^{13}C-NMR (DMSO-d_6) δ: 26.8 (C-2); 27.4 (C-3); 28.2 (OC(<u>C</u>H$_3$)$_3$); 39.8 (C-4); 48.2 (C-1); 52.3 (Ar<u>C</u>H$_2$NH); 63.9 (C-2′, C-3′, Ar); 64.0 (C-2′, C-3′, Ar); 77.2 (O<u>C</u>(CH$_3$)$_3$); 116.4 (C-7′, Ar); 116.5 (C-8′, Ar); 120.6 (C-5′, Ar); 134.0 (C-6′, Ar); 141.9 (C-4a′, C-8a′, Ar); 143.0 (C-4a′, C-8a′, Ar); 155.5 (C=O).

3.1.4. Standard Procedure for the Preparation of Hydrochloride Salts **6a**–**f** after Deprotection of *N*-Boc Monoprotected Diamines **5a**–**f**

In a 100 mL two-necked round-bottomed flask provided with a magnetic stirrer and condenser, *N*-Boc monoprotected diamine **5** (12.1 mmol, 1 equiv.) was solubilized in 1,4-dioxane (80 mL) at room temperature under vigorous stirring for 10 min. To this homogeneous solution was added dropwise for 1 h a solution of 6 M HCl (20 mL). The reaction mixture was stirred during 4 h at 25 °C and was concentrated in a rotary evaporator under reduced pressure for elimination of volatile compounds. To the crystallized crude reaction mixture was added 60 mL of anhydrous Et$_2$O and after triturating, the insoluble salt **6** was collected by filtration, on a Büchner funnel (porosity N°4) then washed with 6 × 10 mL of Et$_2$O. The desired salt **6** was dried under high vacuum (10^{-2} Torr) at 25 °C for 1 h that gave a yellowish or white powder and was further used without purification.

N-1-Phenylmethyl-butane-1,4-diamine hydrochloride (**6a**). Compound **6a** was prepared in 84% yield (2.18 g) from (4-phenylmethylamino-butyl)-carbamic acid *tert*-butyl ester **5a** (3.37 g, 12.1 mmol) according to the standard procedure as yellowish powder. Mp > 260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.57–1.67 (m, 2H, CH$_2$, H-3); 1.70–1.81 (m, 2H, CH$_2$, H-2); 2.79 (t, 2H, J = 7.3 Hz, CH$_2$NH, H-4); 2.89 (t, 2H, J = 7.5 Hz, CH$_2$NH$_2$, H-1); 4.10 (s, 2H, CH$_2$NH, H-4); 7.4–7.45 (m, 3H, H-3′, H-4′, H-5′, Ar); 7.58–7.62 (m, 2H, H-2′, H-6′, Ar); 8.19 (br s, 2H, NH$_2$); 9.57 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (C-3); 24.0 (C-2); 38.0 (C-4); 45.5 (C-1); 49.7 (Ar<u>C</u>H$_2$NH); 128.5 (C-3′, C-5′, Ar); 128.8 (C-4′, Ar); 130.1 (C-2′, C-6′, Ar); 132.0 (C-1′, Ar).

N-1-(4-Methoxy-phenylmethyl)-butane1,4-diamine hydrochloride (**6b**). Compound **6b** was prepared in 97% yield (2.87 g) from (4-methoxyphenylmethylamino-butyl)-carbamic acid *tert*-butyl ester **5b** (3.73 g, 12.1 mmol) according to the standard procedure as pale brownish powder. Mp > 260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.57–1.66 (m, 2H, H-3); 1.69–1.79 (m, 2H, H-2); 2.77–2.84 (m, 4H, H-1, H-4); 3.76 (s, 3H, OC<u>H</u>$_3$); 4.03 (s, 2H, ArC<u>H</u>$_2$NH); 6.97 (d, 2H, J = 8.7 Hz, H-2′, H-6′, Ar); 7.51 (d, 2H, J = 8.7 Hz, H-3′, H-5′, Ar), 8.15 (br s, 2H, N<u>H</u>$_2$); 9.41 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (C-3); 24.0 (C-2); 38.0 (C-4); 45.3 (C-1); 49.2 (Ar<u>C</u>H$_2$NH); 55.2 (O<u>C</u>H$_3$); 113.9 (C-2′, C-6′, Ar); 123.7 (C-1′, Ar); 131.6 (C-3′, C-5′, Ar); 159.6 (C-4′, Ar).

N-1-(2-Chloro-phenylmethyl)-butane1,4-diamine hydrochloride (**6c**). Compound **6c** was prepared in 98% yield (2.95 g) from (2-chlorophenylmethylamino-butyl)-carbamic acid *tert*-butyl ester **5c** (3.79 g, 12.1 mmol) according to the standard procedure as pale brownish powder. Mp = 206–208 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.60–1.69 (m, 2H, H-3); 1.74–1.84 (m, 2H, H-2); 2.73–2.84 (m, 2H, CH$_2$NH, H-4); 2.93–3.02 (m, 2H, CH$_2$NH$_2$, H-1); 4.23 (t, 2H, J = 5.9 Hz, ArC<u>H</u>$_2$NH); 7.39–7.48 (m, 2H, H-4′, H-5′, Ar); 7.52–7.56 (m, 1H, H-6′, Ar); 7.81–7.85 (m, 1H, H-3′, Ar); 8.18 (br s, 2H, NH$_2$, 2H); 9.68

(br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (C-3); 24.0 (C-2); 38.0 (C-4); 46.1 (C-1); 46.7 (Ar<u>C</u>H$_2$NH); 127.5 (C-5′, Ar); 129.5 (C-4′, Ar); 129.9 (C-1′, Ar); 130.7 (C-6′, Ar); 131.9 (C-3′, Ar); 133.5 (C-2′, Ar).

N-1-(3-Methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride (**6d**). Compound **6d** was prepared in 98% yield (2.98 g) from [4-(3-methoxy-phenylmethylamino)-butyl]-carbamic acid *tert*-butyl ester **5d** (3.73 g, 12.1 mmol) according to the standard procedure as pale brownish powder. Mp = 190–192 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.58–1.67 (m, 2H, H-3); 1.71–1.81 (m, 2H, H-2); 2.78 (t, 2H, J = 7.3 Hz, CH$_2$NH, H-4); 2.87 (t, 2H, J = 7.6 Hz, CH$_2$NH$_2$, H-1); 3.77 (s, 3H, OCH$_3$); 4.08 (t, 2H, J = 5.6 Hz, ArCH$_2$NH); 6.94–6.97 (m, 1H, H-5′, Ar); 7.12–7.14 (m, 1H, H-6′, Ar); 7.29–7.35 (m, 2H, H-2′, H-4′, Ar); 8.18 (br s, 2H, NH$_2$); 9.58 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (C-3); 24.0 (C-2); 38.0 (C-4); 45.5 (C-1); 49.7 (Ar<u>C</u>H$_2$NH); 55.2 (OCH$_3$); 114.5 (C-5′, Ar); 115.5 (C-6′, Ar); 122.0 (C-4′, Ar); 129.6 (C-2′, Ar); 133.4 (C-1′, Ar); 159.2 (C-3′, Ar).

N-1-Benzo[1,3]dioxol-5-ylmethyl-butane-1,4-diamine hydrochloride (**6e**). Compound **6e** was prepared in 72% yield (2.25 g) from {4-[(benzo[1,3]dioxol-5-ylmethyl)-amino]-butyl}-carbamic acid *tert*-butyl ester **5e** (3.90 g, 12.1 mmol) according to the standard procedure as pale brownish powder. Mp > 260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.58–1.67 (m, 2H, H-3); 1.71–1.81 (m, 2H, H-2); 2.78 (t, 2H, J = 7.3 Hz, CH$_2$NH, H-4); 2.87 (t, 2H, J = 7.6 Hz, CH$_2$NH$_2$, H-1); 3.77 (s, 3H, OCH$_3$); 4.08 (t, 2H, J = 5.6 Hz, ArC<u>H</u>$_2$NH); 6.94–6.97 (m, 1H, H-5′, Ar); 7.12–7.14 (m, 1H, H-6′, Ar); 7.29–7.35 (m, 2H, H-2′, H-4′, Ar); 8.18 (br s, 2H, NH$_2$); 9.58 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (C-3); 24.0 (C-2); 38.0 (C-4); 45.2 (C-1); 49.4 (Ar<u>C</u>H$_2$NH); 101.2 (C-2′, Ar); 108.2 (C-6′, Ar); 110.4 (C-7′, Ar); 124.1 (C-4′, Ar); 125.4 (C-5′, Ar); 147.2 (C-3a′, C-7a′, Ar); 147.6 (C-3a′, C-7a′, Ar).

N-1-(2,3-Dihydro-benzo[1,4]dioxin-6-ylmethyl)-butane-1,4-diamine hydrochloride (**6f**). Compound **6e** was prepared in 92% yield (3.04 g) from {4-[(2,3-dihydro-benzo[1,4]dioxin-6-ylmethyl)-amino]-butyl}-carbamic acid *tert*-butyl ester **5f** (4.07 g, 12.1 mmol) according to the standard procedure as pale brownish powder. Mp = 239–241 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.57–1.66 (m, 2H, H-3); 1.68–1.78 (m, 2H, H-2); 2.73–2.82 (m, 4H, H-1, H-4); 3.97 (t, 2H, J = 5.4 Hz, ArC<u>H</u>$_2$NH); 4.24 (s, 4H, OCH$_2$CH$_2$O); 6.87 (d, 1H, J = 8.2 Hz, H-7′, Ar); 7.01–7.04 (m, 1H, H-8′, Ar); 7.15 (m, 1H, H-5′, Ar); 8.18 (br s, 2H, NH$_2$); 9.43 (br s, 1H, NH). ^{13}C-NMR (DMSO-d_6) δ: 22.3 (C-3); 24.0 (C-2); 38.0 (C-4); 45.2 (C-1); 49.1 (ArCH$_2$NH); 64.0 (C-2′, Ar); 64.1 (C-3′, Ar); 117.0 (C-7′, Ar); 119.0 (C-8′, Ar); 123.2 (C-5′, Ar); 124.7 (C-6′, Ar); 143.1 (C-4a′, C-8a′, Ar); 143.8 (C-4a′, C-8a′, Ar).

3.1.5. Standard Procedure for the Preparation of *N*-Substituted 5-Arylidene Rhodanine Derivatives **9a**–**f** under Microwave Irradiation Using "One-Pot Two-steps" Protocol

In a 10 mL glass tube were placed successively bis-(carboxymethyl)trithiocarbonate **7** (0.11 g, 0.50 mol, 1 equiv.), dimethoxyethane (2 mL), triethylamine (135 mL, 101 mg, 1 mmol, 2 equiv. or 202 mL, 152 mg, 1.5 mmol, 3 equiv.) and hydrochloride salt **6** (0.5 mmol, 1 equiv.). The glass tube was sealed with a snap cap and placed in the Monowave® 300 Anton Paar microwave cavity (p = 800 Watt). The reaction mixture was irradiated at 90 °C for 15–60 min. under vigorous magnetic stirring. After microwave dielectric heating, the crude reaction mixture was allowed to cool down at room temperature, aldehyde **3** (0.5 mol, 1 equiv.) was added to the cooled reaction mixture which was immediately

submitted to microwave irradiation at 110 °C for 5–30 min. After cooling down to room temperature, the volatile compounds of the reaction mixture were removed in a rotary evaporator under reduced pressure. To the crude reaction mixture was added 4 mL of MeOH and after triturating, the insoluble product **9** was collected by filtration on a Büchner funnel (porosity N°4), washed with deionized water (5 mL), triturated and mixing with ethanol (3 × 5 mL) during 3 h, washed successively with ethanol (5 mL) and anhydrous ether (5 × 2 mL), then dried under high vacuum (10^{-2} Torr) at 25 °C for 1 h. The desired compound **9** was eventually purified by recrystallization in EtOH after control by ^{1}H-NMR in solution of $CDCl_3$/TFA (98:2).

(5Z)3-[4-(4-Methoxyphenylmethylamino)butyl]-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9a**). According to the standard procedure, compound **9a** was prepared in 10% yield (23 mg) from *N-1*-(4-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6b** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 µL, 152 mg, 1.5 mmol, 3 equiv.) and piperonaldehyde **3e** (75.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min. at 110 °C (for condensation step with **3e**), which gave **9a** as a yellowish powder. Mp = 170–229 °C (decomposition). ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.61–1.69 (m, 4H, CH_2, H-2″, H-3″); 3.05–3.11 (m, 2H, ArC$\underline{H}_2$NH); 3.73 (s, 3H, OC$\underline{H}_3$); 4.00–4.08 (m, 4H, CH_2, H-1″, H-4″), 5.97 (s, 2H, CH_2, H-2′); 6.80–7.16 (m, 7H, H-2‴, H-3‴, H-5‴, H-6‴, H-4′, H-6′, H-7′, Ar); 7.55 (br s, 1H, NH); 7.61 (s, 1H, $\underline{H}$C=C). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″, 3″); 23.8 (C-3″, 2″); 44.2 (C-4″, C-1″); 47.0 (Ar$\underline{C}H_2$NH); 52.1 (C-1″, C-4″); 55.5 (O$\underline{C}H_3$); 102.2 (C-2′); 109.4 (C-2‴, C-6‴); 109.5 (C-3‴, C-5‴); 115.0 (C-6′); 120.0 (C-1‴); 127.5 ($\underline{C}$=CH); 128.2 (C-7′); 131.2 (C-4′); 135.0 (C=$\underline{C}$H); 148.8 (C-5′); 148.9 (C-4‴); 150.6 (C-3′a); 150.9 (C-7′a); 169.4 ($\underline{C}$=O, C-4); 192.9 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 457.1256 found (calculated for $C_{23}H_{25}N_2O_4S_2$ $[M + H]^+$ requires 457.1253).

(5Z)3-[4-(4-Methoxyphenylmethylamino)butyl]-5-(4-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (**9b**). According to the standard procedure, compound **9b** was prepared in 6% yield (13.3 mg) from *N-1*-(4-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6b** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 µL, 152 mg, 1.5 mmol, 3 equiv.) and 4-methoxybenzaldehyde **3b** (68.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min. at 110 °C (for condensation step with **3b**), which gave **9b** as a yellowish powder. Mp = 253–255 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.67–1.73 (m, 4H, CH_2, H-2″, H-3″); 3.07–3.13 (m, 2H, ArC$\underline{H}_2$NH); 3.81 (s, 6H, ArOC$\underline{H}_3$); 4.04–4.11 (s, 4H, CH_2, H-1″, H-4″), 6.94 (dd, 4H, *J* = 8.8 Hz, H-2′, H-6′, H-2‴, H-6‴, Ar); 7.40 (d, 4H, *J* = 8.8 Hz, H-3′, H-5′, H-3‴, H-5‴, Ar); 7.60 (br s, 1H, NH); 7.67 (s, 1H, C$\underline{H}$=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 24.3 (C-2″, C-3″); 44.0 (C-1″, C-4″); 46.8 (Ar$\underline{C}H_2$NH); 55.6 (C-4′, O$\underline{C}H_3$, C-4‴, O$\underline{C}H_3$); 115.1 (C-2′, C-6′, C-2‴, C-6‴); 119.6 (C-1′, C-1‴); 126.0 ($\underline{C}$=); 133.1 (C-3′, C-5′, C-3‴, C-5‴); 130.5 (C-5′, C-5‴); 134.6 ($\underline{C}$H=); 162.0 (C-4′, C-4‴); 169.0 ($\underline{C}$=O, C-4); 193.2 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 443.1463 found (calculated for $C_{23}H_{27}N_2O_3S_2$ $[M + H]^+$ requires 443.1460).

(5Z)3-[4-(4-Methoxyphenylmethylamino)butyl]-5-(2,3-dihydro-1,4-benzodioxin-6-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9c**). According to the standard procedure, compound **9c** was prepared in 5% yield (11.8 mg) from *N-1*-(4-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6b** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 µL, 152 mg, 1.5 mmol, 3 equiv.) and 2,3-dihydro-1,4-benzodioxin-

6-carboxaldehyde **3f** (82.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min. at 110 °C (for condensation step with **3f**), which gave **9c** as a yellowish powder. Mp = 255–261 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.68 (s, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.03–3.08 (m, 2H, ArC$\underline{H}_2$NH); 3.73 (s, 3H, OC$\underline{H}_3$); 4.07 (s, 4H, CH_2, H-1″, H-4″), 4.20–4.25 (m, 2H, C$\underline{H}_2$, H-2′, H-3′); 6.84–6.97 (m, 7H, H-5′, H-7′, H-8′, H-2‴, H-3‴, H-5‴, H-6‴, Ar); 7.58 (s, 1H, C$\underline{H}$=); 7.60 (br s, 1H, NH). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 24.2 (C-2″, C-3″); 44.1 (C-4″, C-1″); 47.1 (Ar$\underline{C}H_2$NH); 55.0 (O$\underline{C}H_3$); 64.2 (C-2′); 64.8 (C-3′); 118.4 (C-5′, C-7′, C-8′); 119.7 (C-2‴, C-6‴); 120.3 ($\underline{C}$=); 125.7 (C-3‴, C-5‴); 126.8 (C-6′); 135.0 ($\underline{C}$H=); 143.9 (C-8′a); 146.7 (C-4′a); 169.5 ($\underline{C}$=O, C-4); 193.2 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 471.1412 found (calculated for $C_{24}H_{27}N_2O_4S_2$ $[M + H]^+$ requires 471.1415).

(5Z)3-[4-(4-Methoxyphenylmethylamino)butyl]-5-(4-hydroxy-3-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (**9d**). According to the standard procedure, compound **9d** was prepared in 30% yield (68.8 mg) from *N-1*-(4-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6b** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and 4-hydroxy-3-methoxybenzaldehyde **3g** (76.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min. at 110 °C (for condensation step with **3g**), which gave **9d** as a yellowish powder. Mp = 191–193 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.69 (m, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.06–3.07 (m, 2H, ArC$\underline{H}_2$NH); 3.72 (s, 3H, C-4‴, OC$\underline{H}_3$); 3.86 (s, 3H, C-3′, OC$\underline{H}_3$); 4.04–4.08 (m, 4H, CH_2, H-1″, H-4″), 6.81–7.17 (m, 7H, H-2′, H-5′, H-6′, H-2‴, H-3‴, H-5‴, H-6‴, Ar); 7.55 (br s, 1H, NH); 7.60 (s, 1H, C$\underline{H}$=C). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″, C-3″); 23.9 (C-2″, C-3″); 43.1 (C-1″, C-4″); 47.0 (Ar$\underline{C}H_2$NH); 55.5 (ArO$\underline{C}H_3$); 56.1 (ArO$\underline{C}H_3$); 112.6 (C-6′); 115.0 (C-2‴, C-6‴); 115.6 (C-2′); 118.9 (C-1‴); 121.2 ($\underline{C}$=); 125.9 (C-1′); 126.8 (C-5′); 131.2 (C-3‴, C-5‴); 135.9 ($\underline{C}$H=); 147.2 (C-4‴); 148.9 (C-3′); 160.8 (C-4′); 169.4 ($\underline{C}$=O, C-4); 193.1 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 459.1412 found (calculated for $C_{23}H_{27}N_2O_4S_2$ $[M + H]^+$ requires 459.1416).

(5Z)3-[4-(3-Methoxyphenylmethylamino)butyl]-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9e**). According to the standard procedure, compound **9e** was prepared in 21% yield (48.0 mg) from *N-1*-(3-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6d** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and piperonaldehyde **3e** (75.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3e**), which gave **9e** as a yellowish powder. Mp = 178–246 °C (decomposition). ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.82 (s, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.19–3.22 (m, 2H, ArC$\underline{H}_2$NH); 3.83 (s, 3H, OC$\underline{H}_3$); 4.13-4.23 (m, 4H, CH_2, H-1″, H-4″), 6.10 (s, 2H, C$\underline{H}_2$, H-2′); 6.92–7.28 (m, 6H, H-2‴, H-4‴, H-6‴, H-4′, H-6′, H-7′, Ar), 7.33–7.38 (m, 1H, H-5‴, Ar), 7.55 (br s, 1H, NH); 7.70 (s, 1H, C$\underline{H}$=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″, C-3″); 23.9 (C-3″, C-2″); 42.9 (C-4″, C-1″); 46.8 (Ar$\underline{C}H_2$NH); 52.1 (C-1″, C-4″); 55.3 (O$\underline{C}H_3$); 102.1 (C-2′); 109.3 (C-6‴); 109.4 (C-5‴); 114.9 (C-4‴); 115.9 (C-2‴); 119.8 (C-1‴); 121.8 (C-6′); 127.4 ($\underline{C}$=); 127.6 (C-5′); 127.9 (C-7′); 130.7 (C-4′); 134.5 ($\underline{C}$H=); 148.8 (C-7′a); 150.5 (C-3′a); 160.3 (C-3‴); 168.5 ($\underline{C}$=O, C-4); 193.1 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 457.1259 found (calculated for $C_{23}H_{25}N_2O_4S_2$ $[M + H]^+$ requires 457.1257).

(5Z)3-[4-(3-Methoxyphenylmethylamino)butyl]-5-(2,3-dihydro-1,4-benzodioxin-6-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9f**). According to the standard procedure, compound **9c** was prepared in 9%

yield (21.2 mg) from *N-1*-(3-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6d** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and 2,3-dihydro-1,4-benzodioxin-6-carboxaldehyde **3f** (82.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3f**), which gave **9f** as a yellowish powder. Mp = 247–251 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.84 (s, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.04–3.12 (m, 2H, ArC$\underline{H}_2$NH); 3.95 (s, 3H, OC$\underline{H}_3$); 4.23 (s, 4H, CH_2, H-1″, H-4″); 4.37–4.40 (m, 4H, H-2′, H-3′); 6.99–7.28 (m, 6H, H-2‴, H-4‴, H-5‴, H-6‴, H-5′, H-7′, H-8′, Ar); 7.60 (br s, 1H, NH); 7.74 (s, 1H, C$\underline{H}$=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 24.1 (C-2″, C-3″); 44.1 (C-1″, C-4″); 47.3 (Ar$\underline{C}H_2$NH); 64.2 (C-2′); 64.8 (C-3′); 118.5 (C-5′, C-7′, C-8′); 119.8 (C-4‴, C-6‴); 125.9 (C-2‴, C-5‴); 126.8 ($\underline{C}$=); 135.3 ($\underline{C}$H=); 143.8 (C-8′a); 146.6 (C-4′a); 168.5 ($\underline{C}$=O, C-4); 193.3 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 471.1412 found (calculated for $C_{24}H_{27}N_2O_4S_2$ $[M + H]^+$ requires 471.1415).

(5Z)3-[4-(3-Methoxyphenylmethylamino)butyl]-5-(3-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (**9g**). According to the standard procedure, compound **9g** was prepared in 5% yield (11.1 mg) from *N-1*-(3-methoxy-phenylmethyl)-butane-1,4-diamine hydrochloride **6d** (122.3 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and 3-methoxybenzaldehyde **3d** (68.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min at 110 °C (for condensation step with **3d**), which gave **9g** as a yellowish powder. Mp = 225–227 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.73 (s, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.06–3.10 (m, 2H, ArC$\underline{H}_2$NH); 3.80 (s, 6H, ArOC$\underline{H}_3$); 4.11 (s, 4H, C$\underline{H}_2$, H-1″, H-4″), 6.93–6.96 (m, 4H, H-2′, H-6′, H-2‴, H-6‴, Ar); 7.04 (d, 2H, *J* = 7.7 Hz, H-4′, H-4‴, Ar), 7.32 (m, 2H, H-5′, H-5‴, Ar), 7.55 (br s, 1H, NH); 7.66 (s, 1H, C$\underline{H}$=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 24.3 (C-2″, C-3″); 44.0 (C-1″, C-4″); 47.1 (Ar$\underline{C}H_2$NH); 55.5 (ArO$\underline{C}H_3$); 115.3 (C-6′, C-6‴); 117.3 (C-2′, C-2‴); 123.1 ($\underline{C}$=); 123.6 (C-4′, C-4‴); 130.5 (C-5′, C-5‴); 134.0 ($\underline{C}$H=); 134.5 (C-1′, C-1‴); 160.0 (C-3′, C-3‴); 168.5 ($\underline{C}$=O, C-4); 193.3 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 443.1463 found (calculated for $C_{23}H_{27}N_2O_3S_2$ $[M + H]^+$ requires 443.1465).

(5Z)3-[4-(1,3-Benzodioxol-5-ylmethylamino)butyl]-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9h**). According to the standard procedure, compound **9h** was prepared in 59% yield (139 mg) from *N-1*-benzo[1,3]dioxol-5-ylmethyl-butane-1,4-diamine hydrochloride **6e** (129.4 mg, 0.5 mmol, 1 equiv.), triethylamine (135 μL, 101 mg., 1 mmol, 2 equiv.) and piperonaldehyde **3e** (75.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3e**), which gave **9h** as a yellowish powder. Mp = 245–256 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.74 (s, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.04–3.05 (m, 2H, ArC$\underline{H}_2$NH); 4.05–4.07 (m, 4H, C$\underline{H}_2$, H-1″, H-4″); 5.91 (s, 2H, C$\underline{H}_2$, H-2‴); 6.00 (s, 2H, C$\underline{H}_2$, H-2′); 6.72–7.01 (m, 6H, H-4′, H-6′, H-7′, H-4‴, H-6‴, H-7‴, Ar); 7.58 (s, 1H, C$\underline{H}$=); 7.65 (br s, 1H, NH);. ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″, C-3″); 24.0 (C-3″); 43.1 (C-1″, C-4″); 46.5 (Ar$\underline{C}H_2$NH); 51.9 (C-4″, C-1″); 101.8 (C-2′, C-2‴); 102.2 (C-2‴, C-2′); 109.0 (C-6‴); 109.3 (C-6′); 109.4 (C-7‴); 109.8 (C-7′); 119.8 (C-5‴); 122.6 ($\underline{C}$=); 124.3 (C-4‴); 127.4 (C-4′); 128.1 (C-5′); 134.9 ($\underline{C}$H=); 148.6 (C-7′a); 148.9 (C-3′a); 149.2 (C-7‴a); 150.6 (C-3‴a); 168.9 ($\underline{C}$=O, C-4); 193.0 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 471.1048 found (calculated for $C_{23}H_{23}N_2O_5S_2$ $[M + H]^+$ requires 471.1049).

(5Z)3-[4-(1,3-Benzodioxol-5-ylmethylamino)butyl]-5-(2,3-dihydro-1,4-benzodioxin-6-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9i**). According to the standard procedure, compound **9i** was prepared in 59% yield (143 mg) from *N-1*-benzo[1,3]dioxol-5-ylmethyl-butane-1,4-diamine hydrochloride **6e** (129.4 mg, 0.5 mmol, 1 equiv.), triethylamine (135 µL, 101 mg., 1 mmol, 2 equiv.) and 2,3-dihydro-1,4-benzodioxin-6-carboxaldehyde **3f** (82.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3f**), which gave **9i** as a yellowish powder. Mp = 253–257 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.68 (s, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.05–3.06 (m, 2H, ArC$\underline{H}_2$NH); 4.03–4.04 (m, 4H, C$\underline{H}_2$, H-1″, H-4″); 4.20–4.25 (m, 4H, C$\underline{H}_2$, H-2′, H-3′); 5.89 (s, 2H, C$\underline{H}_2$, H-2‴); 6.70 (s, 3H, H-4‴, H-6‴, H-7‴, Ar); 6.85–6.96 (m, 3H, H-5′, H-7′, H-8′, Ar); 7.50 (br s, 1H, NH); 7.55 (s, 1H, C$\underline{H}$=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″, C-3″); 23.8 (C-3″, C-2″); 43.1 (C-1″, C-4″); 47.0 (Ar$\underline{C}H_2$NH); 52.4 (C4″, C-1″); 64.2 (C-2′, C-3′); 64.8 (C-3′, C-2′); 101.8 (C-2‴); 109.1 (C-6‴); 109.5 (C-7‴); 118.5 (C-7′); 119.7 (C-5‴); 119.8 (C-8′); 122.3 ($\underline{C}$=); 124.1 (C-4‴); 125.9 (C-5′); 126.6 (C-6′); 135.5 ($\underline{C}$H=); 144.0 (C-7‴a); 146.9 (C-3‴a); 148.6 (C-8′a); 149.4 (C-4′a); 169.5 ($\underline{C}$=O, C-4); 193.2 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 485.1205 found (calculated for $C_{24}H_{25}N_2O_5S_2$ $[M + H]^+$ requires 485.1203).

(5Z)3-[4-(1,3-Benzodioxol-5-ylmethylamino)butyl]-5-(4-hydroxy-benzylidene)-2-thioxo-1,3-thiazolidin-4-one (**9j**). According to the standard procedure, compound **9j** was prepared in 34% yield (75.2 mg) from *N-1*-benzo[1,3]dioxol-5-ylmethyl-butane-1,4-diamine hydrochloride **6e** (129.4 mg, 0.5 mmol, 1 equiv.), triethylamine (135 µL, 101 mg, 1 mmol, 2 equiv.) and 4-hydroxybenzaldehyde **3h** (61.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3h**), which gave **9i** as a orange powder. Mp = 93–124 °C (decomposition). ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.59–1.73 (m, 4H, C$\underline{H}_2$, H-2″, H-3″); 3.01–3.13 (m, 2H, ArC$\underline{H}_2$NH); 4.00–4.05 (m, 4H, CH_2, H-1″, H-4″); 5.88 (s, 2H, C$\underline{H}_2$, H-2‴); 6.66–6.69 (m, H-4‴, H-6‴, H-7‴, Ar); 6.86 (dd, 2H, *J* = 8.7 Hz, H-2′, H-6′, Ar); 7.32 (dd, 2H, *J* = 8.7 Hz, H-3′, H-5′, Ar); 7.55 (br s, 1H, NH); 7.60 (s, 1H, C$\underline{H}$=, 1H). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 22.5 (C-2″, C-3″); 23.2 (C-3″, C-2″); 42.5 (C-1″, C-4″); 46.4 (Ar$\underline{C}H_2$NH); 51.9 (C-4″, C-1″); 101.2 (C-2‴); 108.5 (C-6‴); 108.8 (C-7‴); 116.1 (C-2′, C-6′); 118.6 (C-5‴); 121.7 ($\underline{C}$=); 123.4 (C-4‴); 125.6 (C-1′); 132.9 (C-3′, C-5′); 134.8 ($\underline{C}$H=); 148.0 (C-7‴a); 148.8 (C-3‴a); 157.6 (C-4′); 169.0 ($\underline{C}$=O, C-4); 192.6 ($\underline{C}$=S, C-2). HRMS, *m*/*z*: 443.1099 found (calculated for $C_{22}H_{23}N_2O_4S_2$ $[M + H]^+$ requires 443.1095).

(5Z) 3-[4-(1,3-Benzodioxol-5-ylmethylamino)butyl]-5-(4-hydroxy-3-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (**9k**). According to the standard procedure, compound **9k** was prepared in 46% yield (109 mg) from *N-1*-benzo[1,3]dioxol-5-ylmethyl-butane-1,4-diamine hydrochloride **6e** (129.4 mg, 0.5 mmol, 1 equiv.), triethylamine (202 µL, 152 mg, 1.5 mmol, 3 equiv.) and 4-hydroxy-3-methoxybenzaldehyde **3g** (76.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min. at 110 °C (for condensation step with **3g**), which gave **9k** as a red powder. Mp = 200–203 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.71 (s, 4H, CH_2, H-2″, H-3″); 3.05–3.06 (m, 2H, ArC$\underline{H}_2$NH); 3.88 (s, 3H, OC$\underline{H}_3$); 4.04 (s, 4H, C$\underline{H}_2$, H-1″, H-4″); 5.91 (s, 2H, C$\underline{H}_2$, H-2‴); 6.74 (s, 3H, H-4‴, H-6‴, H-7‴, Ar); 6.87–7.02 (m, 3H, H-2′, H-5′, H-6′, Ar); 7.58 (s, 1H, C$\underline{H}$=); 7.65 (br s, 1H, NH). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″, C-3″); 23.9 (C-3″, C-2″); 42.9 (C-1″, C-4″); 46.6 (Ar$\underline{C}H_2$NH); 52.1 (C-4″, C-1″); 56.1 (O$\underline{C}H_3$); 101.8 (C-2‴); 109.0 (C-6‴); 109.6 (C-7‴); 112.3

(C-6′); 115.5 (C-2′); 119.1 (C-5‴); 122.7 (C=); 124.0 (C-5′); 125.8 (C-1′); 126.5 (C-4‴); 134.9 (CH=); 147.1 (C-3′); 148.6 (C-7‴a); 148.8 (C-4′); 149.2 (C-3‴a); 168.5 (C=O, C-4); 193.2 (C=S, C-2). HRMS, *m*/*z*: 473.1205 found (calculated for $C_{23}H_{25}N_2O_5S_2$ $[M + H]^+$ requires 473.1206).

(5Z)3-[4-(2,3-Dihydro-benzo[1,4]dioxin-6-ylmethylamino)butyl]-5-(1,3-benzodioxol-5-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9l**). According to the standard procedure, compound **9l** was prepared in 15% yield (36.3 mg) from *N-1*-(2,3-dihydro-benzo[1,4]dioxin-6-ylmethyl)-butane-1,4-diamine hydrochloride **6f** (136.4 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and piperonaldehyde **3e** (75.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3e**), which gave **9l** as a yellowish powder. Mp = 172–233 °C (decomposition). ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.66–1.69 (m, 4H, CH_2, H-2″, H-3″); 3.03–3.10 (m, 2H, ArCH_2NH); 4.03–4.07 (m, 4H, CH_2, H-1″, H-4″); 4.16–4.19 (m, 4H, CH_2, H-2‴, H-3‴); 5.97 (s, 2H, CH_2, H-2′); 6.66–7.00 (m, 6H, H-4′, H-6′, H-7′, H-5‴, H-7‴, H-8‴, Ar); 7.55 (br s, 1H, NH); 7.59 (s, 1H, CH=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.8 (C-2″, C-3″); 24.2 (C-3″); 43.1 (C-1″, C-4″); 47.0 (ArCH_2NH); 52.1 (C-4″, C-1″); 64.2 (C-2‴); 64.4 (C-3‴); 102.2 (C-2′); 109.4 (C-7‴); 109.5 (C-8‴); 118.5 (C-5‴); 119.5 (C-6‴); 122.0 (C=); 122.9 (C-6′); 127.3 (C-5′); 128.2 (C-7′); 135.0 (C-4′); 135.5 (CH=); 143.9 (C-4‴a); 145.1 (C-8‴a); 150.6 (C-3′a); 150.8 (C-7′a); 168.5 (C=O, C-4); 193.0 (C=S, C-2). HRMS, *m*/*z*: 485.1205 found (calculated for $C_{24}H_{25}N_2O_5S_2$ $[M + H]^+$ requires 485.1206).

(5Z)3-[4-(2,3-Dihydro-benzo[1,4]dioxin-6-ylmethylamino)butyl]-5-(2,3-dihydro-benzo[1,4]dioxin-6-ylmethylene)-2-thioxo-1,3-thiazolidin-4-one (**9m**). According to the standard procedure, compound **9m** was prepared in 7% yield (17.5 mg) from *N-1*-(2,3-dihydro-benzo[1,4]dioxin-6-ylmethyl)-butane-1,4-diamine hydrochloride **6f** (136.4 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and 2,3-dihydro-1,4-benzodioxin-6-carboxaldehyde **3f** (82.1 mg, 0.5 mmol, 1 equiv.) after 15 min. at 90 °C, followed by a second reaction time of 15 min. at 110 °C (for condensation step with **3f**), which gave **9m** as a yellowish powder. Mp = 170–243 °C (decomposition). ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.64–1.69 (m, 4H, CH_2, H-2″, H-3″); 3.04–3.10 (m, 2H, ArCH_2NH); 4.03–4.08 (m, 4H, CH_2, H-1″, H-4″); 4.16–4.23 (m, 8H, CH_2, H-2′, H-3′, H-2‴, H-3‴); 6.75–7.57 (m, 6H, H-5′, H-7′, H-8′, H-5‴, H-7‴, H-8‴, Ar); 7.60 (br s, 1H, NH); 8.77 (s, 1H, CH=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 24.2 (C-2″, C-3″); 44.0 (C-1″, C-4″); 64.2 (C-2′, C-2‴); 64.8 (C-3′, C-3‴); 118.4 (C-7′, C-7‴); 119.6 (C-8′, C-8‴); 120.3 (C=); 125.6 (C-5′, C-5‴); 126.8 (C-6′, C-6‴); 134.5 (CH=); 144.0 (C-8′a, C-8‴a); 146.6 (C-4′a, C-4‴a); 166.6 (C=O, C-4); 182.5 (C=S, C-2). HRMS, *m*/*z*: 499.1364 found (calculated for $C_{25}H_{27}N_2O_5S_2$ $[M + H]^+$ requires 499.1366).

(5Z)3-[4-(2,3-Dihydro-benzo[1,4]dioxin-6-ylmethylamino)butyl]-5-(4-hydroxy-3-methoxybenzylidene)-2-thioxo-1,3-thiazolidin-4-one (**9n**). According to the standard procedure, compound **9n** was prepared in 34% yield (82.7 mg) from *N-1*-(2,3-dihydro-benzo[1,4]dioxin-6-ylmethyl)-butane-1,4-diamine hydrochloride **6f** (136.4 mg, 0.5 mmol, 1 equiv.), triethylamine (202 μL, 152 mg, 1.5 mmol, 3 equiv.) and 4-hydroxy-3-methoxybenzaldehyde **3g** (76.1 mg, 0.5 mmol, 1 equiv.) after 30 min. at 90 °C, followed by a second reaction time of 30 min. at 110 °C (for condensation step with **3g**), which gave **9n** as a red powder. Mp = 202–204 °C. ^{1}H-NMR ($CDCl_3$/TFA 98:2) δ: 1.69 (s, 4H, CH_2, H-2″, H-3″); 3.06 (s, 2H, ArCH_2NH);

3.86 (s, 3H, OC<u>H</u>$_3$); 4.03 (s, 4H, C<u>H</u>$_2$, H-1″, H-4″); 4.16 (s, 4H, C<u>H</u>$_2$, H-2‴, H-3‴); 6.67–7.03 (m, 6H, H-2′, H-5′, H-6′, H-5‴, H-7‴, H-8‴, Ar); 7.60 (s, 1H, C<u>H</u>=). ^{13}C-NMR ($CDCl_3$/TFA 98:2) δ: 23.1 (C-2″ or 3″); 23.8 (C-3″ ou C-2″); 43.1 (C-1″ ou 4″); 47.0 (Ar<u>C</u>H$_2$NH); 52.1 (C-4″, C-1″); 56.1 (O<u>C</u>H$_3$); 64.4 (C-2‴, C-3‴); 64.4 (C-3‴, C-2‴); 112.6 (C-6′); 115.6 (C-2′); 118.4 (C-7‴); 118.6 (C-8‴); 119.0 (C-6‴); 122.0 (<u>C</u>=); 122.9 (C-5‴); 125.9 (C-1′); 126.8 (C-5′); 135.9 (<u>C</u>H=); 143.9 (C-8‴a); 145.1 (C-3′a); 147.2 (C-4‴a); 148.9 (C-4′);169.4 (<u>C</u>=O, C-4); 193.1 (<u>C</u>=S, C-2). HRMS, *m*/*z*: 487.1361 found (calculated for $C_{24}H_{27}N_2O_5S_2$ $[M + H]^+$ requires 487.1359).

3.2. Biochemistry Section

3.2.1. Protein Kinase Assay Buffers

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

Buffer B: 60 mM β-glycerophosphate, 15 mM *p*-nitrophenyl-phosphate, 25 mM Mops (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenylphosphate.

3.2.2. Kinase Preparations and Assays

Kinase activities for each enzyme were assayed in buffer A (25 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 50 µg/mL Heparin, BSA 0.15 mg/mL) or B (60 mM β-glycerophosphate, 30 mM *p*-Nitrophenylphosphate, 25 mM MOPS, 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 0.1 mM Na vanadate), with their corresponding substrates, in the presence of 15 µM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 µL. After 30 min incubation at 30 °C, the reaction was stopped by harvesting, using a FilterMate harvester (Packard, Meriden, CT, USA), onto P81 phosphocellulose papers (GE Healthcare, Velizy-Villacoublay, France) which were washed in 1% phosphoric acid. Scintillation fluid was added and the radioactivity measured in a Packard counter. Blank values were subtracted and activities calculated as pmoles of phosphate incorporated during the 30 min incubation. The activities were expressed in a percentage of the maximal activity, *i.e.*, in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. *CK1* and *Haspin* peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

CDK5/p25 (human, recombinant) was prepared as previously described [31]. Its kinase activity was assayed in buffer B, with 1 mg histone H1/mL.

Casein kinase 1 (CK1δ/ε) (porcine brain, native) was assayed with 0.67 µg of CKS peptide (RRKHAAIGpSAYSITA) [32].

*GSK-3*α/β (porcine brain, native) was assayed, as described for CDK5/p25 but in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine) [33]. GS-1 was synthesized by Millegen (Labege, France).

Haspin kinase domain (*HsHaspin*-kd aa 470 to 798) encoding cDNA, obtain by RT-PCR, was cloned into pGex-6P-3. The fusion protein was expressed in *Escherichia coli* strain BL21-KRX (Promega, Madison, WI, USA) and purified by affinity chromatography on glutathione-agarose beads (Sigma). Haspin-kd activity was assayed with 3 µM Histone H3 (1-21) peptide, a specific Haspin substrate, (ARTKQTARKSTGGKAPRKQLA), in Buffer H (MOPS 25 mM pH 7.5; 10 mM $MgCl_2$).

3.3. Cell Culture and Survival Assays

Skin diploid fibroblastic cells were provided by BIOPREDIC International Company (Rennes, France). Caco2 (Ref ECACC: 86010202), Huh-7D12 (Ref ECACC: 01042712), MDA-MB-231 (Ref ECACC: 92020424), HCT-116 (Ref ECACC: 91091005), PC3 (Ref ECACC: 90112714), NCI-H727 (Ref ECACC: 94060303) cell lines were obtained from the ECACC collection and HaCaT (from Cell Lines Service, Eppelheim, Germany). Cells were grown according to ECACC recommendations [34]. The toxicity test of the compounds on these cells was as follows: 2×10^3 cells for HCT-116 cells or 4×10^3 for the other cells were seeded in 96 multiwell plates in triplicate and left for 24 h for attachment, spreading and growing. Then, cells were exposed for 48 h to increasing concentrations of the compounds, ranging from 0.1 to 25 μM in a final volume of 120 μL of culture medium. Cells were fixed in cooled solution of 90% ethanol/5% acetic acid, nuclei were stained with Hoechst 3342 (Sigma) and counted using automated imaging analysis (Cellomics Arrayscan VTI/HCS Reader, Thermo/Scientific, Waltham, MA, USA). The IC_{50} were graphically determined.

4. Conclusions

In summary, we have developed, in this preliminary project, a new route to (5*Z*) 3-(4-arylmethylamino) butyl-5-arylidene-2-thioxo-1,3-thiazolidine-4-ones **9**. Starting from commercial butane-1,4-diamine, the process involved six steps and the key step is a solution phase "one-pot two-steps" approach for the construction of the 2-thioxo-1,3-thiazolidine-4-one platform under microwave dielectric heating followed by Knoevenagel condensation for installation of the 5-arylidene moiety as second point of diversity. This methodology offered the possibility of preparing a library of fourteen new compounds in moderate to good yields and, the targeted compounds **9a**–**n** have been built with a *Z*-geometry. The *in vitro* inhibition of cell proliferation was carried out on a panel of seven representative tumoral cell lines and the compounds **9a**–**n** were also evaluated against four protein kinases. Among all of these compounds, the compound **9j** turned out to be interesting because it presented selective micromolar inhibition activity on *Ss*CK1 (IC_{50} 1.4 μM). Molecules **9h** and **9i** were also bioactive on *Ss*CK1 and *Hs*CDK5-p25. The current results are the starting point of a new larger program within our group to investigate intensively the biological properties of these new inhibitors with potential application in Alzheimer's disease or in cancer.

Acknowledgments

Two of us (C.D.D. and C.N.A.) wish to thank the "Benian International Fondation", "ElecBTP" and the "Ministère de l'Enseignement Supérieur et de la Recherche de la Côte d'Ivoire" for their respective grants. Financial support of this program carried out under the French National Cancer Institute "Cancéropôle Grand Ouest" by contract "Ion Channel-Network CGO 2012", is gratefully acknowledged.

Author Contributions

J.-P.B. was the manager of the project. J.-P.B., Y.-A.B. and J.M. conceived and designed the experiments. C.D.D., C.N.A. and W.-K.C. performed the chemical experiments. A.D. and B.B. performed the kinase assays. S.B. and S.R. analyzed the data of kinase assays. M.R. and R.L.G. performed the

antiproliferative activity assays. R.L.G. and A.C. analyzed the data of antiproliferative assays and J.-P.B. wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Mentgen, T.; Steuer, C.; Klein, C.D. Privileged scaffolds or promiscuous binders: A comparative study on rhodanines and related heterocycles in medicinal chemistry. *J. Med. Chem.* **2012**, *55*, 743–753.
2. Masic, L.P.; Tomasic, T. Rhodanine as a privileged scaffold in drug discovery. *Curr. Med. Chem.* **2009**, *16*, 1596–1629.
3. Maga, G.; Falchi, F.; Garbelli, A.; Belfiore, A.; Witvrow, M.; Manetti, F.; Botta, M. Pharmacophore modeling and molecular docking led to the discovery of inhibitors of human immunodeficiency virus-1. Replication targeting the human cellular aspartic acid-glutamic acid-alanine-aspartic acid box polypeptide 3. *J. Med. Chem.* **2008**, *51*, 6635–6638.
4. Cutshall, N.S.; O'Day, C.; Prezhdo, M. Rhodanine derivatives as inhibitors of JSP-1. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3374–3382.
5. El-Kabbani, M.; Ruiz, F.; Darmanim, C.; Chung, R.P.T. Aldose reductase structures: Implications for mechanism and inhibition, *Cell. Mol. Life Sci.* **2004**, *61*, 750–752.
6. Summerer, D.; Rudinger, N.Z.; Ilka Detmer, I.; Marx, A. Enhanced Fidelity in Mismatch Extension by DNA Polymerase through Directed Combinatorial Enzyme Design. *Angew. Chem. Int. Ed.* **2005**, *44*, 4712–4715.
7. Tomašić, T.; Zidar, N.; Šink, R.; Kovač, A.; Blanot, D.; Contreras-Martel, C.; Dessen, A.; Müller-Premru, M.; Zega, A.; Gobec, S.; *et al.* Structure-Based Design of a New Series of D-Glutamic Acid Based Inhibitors of Bacterial UDP-*N*-acetylmuramoyl-l-alanine: D-Glutamate Ligase (MurD). *J. Med. Chem.* **2011**, *54*, 4600–4610.
8. Rinaldi, M.; Tintori, C.; Franchi, L.; Vignaroli, G.; Innitzer, A.; Massa, S.; Esté, J.A.; Gonzalo, E.; Christ, F.; Debyser, Z.; *et al.* A Versatile and Practical Synthesis toward the Development of Novel HIV-1 Integrase Inhibitors. *Chem. Med. Chem.* **2011**, *6*, 343–352.
9. Jadav, S.S.; Sinha, B.N.; Hilgenfeld, R.; Pastorino, B.; de Lamballerie, X.; Jayaprakash, V. Thiazolidone derivatives as inhibitors of chikungunya virus. *Eur. J. Med. Chem.* **2015**, *89*, 172–178.
10. Bulic, B.; Pickhardt, M.; Schmidt, B.; Mandelkow, E.M.; Waldmann, H.; Mandelkow, E. Development of Tau Aggregation Inhibitors for Alzheimer's Disease. *Angew. Chem. Int. Ed.* **2009**, *48*, 1740–1752.
11. Bulic, B.; Pickhardt, M.; Schmidt, B.; Mandelkow, E.M. Tau protein and tau aggregation inhibitors. *Neuropharmacology* **2010**, *59*, 276–289.
12. Strittmatter, T.; Bareth, B.; Immel, T.A.; Huhn, T.; Mayer, T.U.; Marx, A. Small Molecule Inhibitors of Human DNA Polymerase λ. *Chem. Biol.* **2011**, *6*, 314–319.
13. Fedorov, O.; Müller, S.; Knapp, S. The (un)targeted cancer kinome. *Nat. Chem. Biol.* **2010**, *6*, 166–169.

14. Bazureau, J.P.; Carreaux, F.; Renault, S.; Meijer, L.; Lozach, O. Imidazolone derivatives, preparation method thereof and biological use of same. Patent WO 2009/05032 A2, 23 April 2009.
15. Debdab, M.; Carreaux, F.; Renault, S.; Soundararajan, M.; Fedorov, O.; Filippakopoulos, P.; Lozach, O.; Babault, L.; Tahtouh, T.; Baratte, B.; *et al*. Design, synthesis and biological evaluation of leucettines, a class of potent CLK and DYRK kinases inhibitors derived from the marine sponge leucettamine B. Modulation of alternative RNA splicing. *J. Med. Chem.* **2011**, *54*, 4172–4186.
16. Tahtouh, T.; Elkins, J.M.; Filippakopoulos, P.; Soundararajan, M.; Burgy, G.; Durieu, E.; Cochet, C.; Schmid, R.S.; Lo, D.C.; Delhommel, F.; *et al.* Selectivity, cocrystal structures, and neuroprotective properties of Leucettines, a family of protein kinase inhibitors derived from the marine sponge alkaloid Leucettamine B. *J. Med. Chem.* **2012**, *55*, 9312–9330.
17. Smith, B.; Medda, F.; Gokhale, V.; Dunckley, T.; Hulme, C. Recent Advances in the Design, Synthesis, and Biological Evaluation of Selective DYRK1A Inhibitors: A New Avenue for a Disease Modifying Treatment of Alzheimer's? *ACS Chem. Neurosci.* **2012**, *11*, 857–872.
18. Ionescu, A.; Dufrasne, F.; Gelbcke, M.; Jabin, I.; Kiss, R.; Lamoral-Theys, D. DYRK1A Kinase inhibitors with emphasis on cancer. *Mini-Rev. Med. Chem.* **2012**, *12*, 1315–1329.
19. Xiao, Y.A.; Wang, Z.Q.; Wang, X.M.; Hui, Y.; Ling, Y.; Wang, X.Y.; He, L.Q. Synthesis and *in-vitro* biological evaluation of novel 2-aminoimidazolinone derivatives as anti-tumor agents. *Chin. Chem. Lett.* **2013**, *24*, 727–730.
20. Ling, Y.; Wang, Z.Q.; Xiao, Y.A.; Zhu, C.; Shen, L.; Wang, X.M.; Hui, Y.; Wang, X.Y. Benzylidene 2-aminoimidazolones derivatives: Synthesis and *in vitro* evaluation of anti-tumor carcinoma activity. *Chem. Pharm. Bull.* **2013**, *61*, 1081–1084.
21. Coulibaly, W.K.; Paquin, L.; Bénie, A.; Bekro, Y.A.; Durieu, E.; Meijer, L.; Bazureau, J.P. Synthesis of *N*,*N*′-bis(5-arylidene-4-oxo-3,5-dihydro-4*H*-imidazol-2-yl)diamines bearing various linkers and biological evaluation as potential inhibitors of kinases. *Eur. J. Med. Chem.* **2012**, *58*, 581–590.
22. Coulibaly, W.K.; Paquin, L.; Bénie, A.; Bekro, Y.A.; Durieux, E.; Rucheau, S.; Meijer, L.; le Guével, R.; Corlu, A.; Bazureau, J.P. Synthesis of new *N*,*N*′-bis(5-arylidene-4-oxo-4,5-dihydro-thiazolidine-2-yl)piperazine derivatives under microwave irradiation and preliminary biological evaluation. *Sci. Pharm.* **2012**, *80*, 825–836.
23. Coulibaly, W.K.; Paquin, L.; Bénie, A.; Bekro, Y.A.; le Guével, R.; Ravache, M.; Corlu, A.; Bazureau, J.P. Prospective directed to the synthesis of unsymmetrical linked bis-5-arylidene rhodanine derivatives via "one-pot two steps" reactions under microwave irradiation with their antitumor activity. *Med. Chem. Res.* **2015**, *24*, 1653–1661.
24. Bazureau, J.P.; Paquin, L.; L'Helgoual'ch, J.M.; Carrie, D.; Limanton, E.; Guihéneuf, S.; Burgy, G.; Coulibaly, W.K.; Komaty, S. Microwaves in Heterocyclic Chemistry. In *Microwaves in Organic Synthesis*, 3rd ed.; Loupy, A., de la Hoz, A., Eds.; Wiley-VCH: Weinheim, Germany, 2012; Chapter 14, pp 673–766.
25. Raj, R.; Mehra, V.; Gut, J.; Rosenthal, P.J.; Wicht, K.J.; Egan, T.J.; Hopper, M.; Wrischnik, L.A.; Land, K.M.; Kumar, V. Discovery of highly selective 7-chloroquinoline-thiohydantoins with potent antimalarial activity. *Eur. J. Med. Chem.* **2014**, *84*, 425–432.
26. Bonger, K.M.; Hoogendoorn, S.; van Koppen, C.J.; Timmers, C.M.; Overkleeft, H.S.; van der Marel, G.A. Synthesis and pharmacological evaluation of dimeric follicle-stimulating hormone receptor antagonists. *ChemMedChem* **2009**, *4*, 2098–2102.

27. Krstenansky, J.L.; Cotteril, I. Recent advances in microwave-assisted organic syntheses. *Curr. Opin. Drug. Discov. Dev.* **2000**, *3*, 454–461.
28. Guihéneuf, S.; Paquin, L.; Carreaux, F.; Durieu, É.; Bénédetti, H.; Le Guével, R.; Corlu, A.; Meijer, L.; Bazureau, J.P. Microwave Assisted Organic Synthesis (Maos) of New Dispacamide a Derivatives Bearing a Thiazolinone Platform, Biological Assays on Inhibition of Protein Kinases and Cell Effects. *Curr. Microw. Chem.* **2014**, *1*, 33–40.
29. Xia, Z.; Knaak, C.; Ma, J.; Beharry, Z.M.; Mc Innes, C.; Wang, W.; Kraft, A.S.; Smith, C.D. Synthesis and evaluation of novel inhibitors of Pim-1 and Pim-2 protein kinases. *J. Med. Chem.* **2009**, *52*, 74–86.
30. Dai, J.; Sultan, S.; Taylor, S.S.; Higgins, J.M. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. *Genes Dev.* **2005**, *19*, 472–488.
31. Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J.A.; Snyder, G.L.; Greengard, P.; Biernat, J.; Mandelkow, E.M.; Eisenbrand, G.; *et al.* Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J. Biol. Chem.* **2001**, *276*, 251–260.
32. Reinhardt, J.; Ferandin, Y.; Meijer, L. Purification CK1 by affinity chromatography on immobilised axin. *Protein Expr. Purif.* **2007**, *54*, 101–109.
33. Primot, A.; Baratte, B.; Gompel, M.; Borgne, A.; Liabeuf, S.; Romette, J.L.; Costantini, F.; Meijer, L. Purification of GSK-3 by affinity chromatography on immobilised axin. *Protein Exp. Purif.* **2000**, *20*, 394–404.
34. Nakabayashi, H.; Taketssa, K.; Miyano, K.; Yamane, T.; Sato, J. Growth of human hepatoma cell lines with differentiated functions in chemically defined medium. *Cancer Res.* **1982**, *42*, 3858–3863.

Sample Availability: Samples of the compounds **9a**–**n** are available from the authors.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).