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Non-ATP competitive glycogen synthase kinase 3β (GSK-3β) inhibitors: Study of structural requirements for thiadiazolidinone derivatives

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Abstract—The 2,4-disubstituted thiadiazolidinones (TDZD) were described as the first non-ATP competitive GSK-3β inhibitors. New modifications in this heterocyclic ring are here reported to study the influence on the biological activity. The basic skeleton of 1,2,4-thiadiazole and also one of the carbonyl groups are kept, while different modifications are introduced in positions 3 and 5, respectively. The GSK-3β activity of the new thiadiazole derivatives here synthesized showed IC_{50} values for some of the compounds in the micromolar range. Additionally, ATP competition studies have been carried out, showing that as well as the first generation of TDZD, these new compounds act in a non-competitive manner. With this study, additional requirements for the biological activity of the TDZD family have been delineated.

1. Introduction

Alzheimer disease (AD) is the most common type of dementia,[1,2] being among the 10 principal causes of mortality in the developed countries.[3] It is a neurological disorder characterized by a slow, progressive decline in cognitive function and behavior. The neuropathologic hallmarks of the disease include amyloid-rich senile plaques, neurofibrillary tangles (intracellular filamentous aggregates of hyperphosphorylated tau protein), and neuronal degeneration.[4]

The relationship between the senile plaques and neurofibrillary tangles formation is still not clear. It has been reported by some research groups that either β-amyloid precursor protein (APP) or β-amyloid peptide (Aβ) influences the formation of the tangles.[5] The role that a dysregulation of tau phosphorylation may play in the neuronal dysfunction in Alzheimer's disease,[6] as well as the study of the physiology and pathophysiology of this protein, has moved to consider tau as an important therapeutic target for the treatment of the AD and other associated pathological processes (taupathies).[7] Besides, it has been well established that the binding of tau to the microtubules as well as to the cellular membrane is regulated by phosphorylation processes. A great number of kinase proteins are involved in the regulation of the phosphorylation process of tau,[8] being GSK-3β, a multi-tasking enzyme that is implied on the regulation of multiple cellular processes,[9–11] responsible of the microtubule stabilization.[12,13] This fact has conducted to consider GSK-3β as a very interesting pharmacological target for drug discovery,[14,15] being considered also as a drug target in Alzheimer disease.[16,17] Recent data pointed out that GSK-3β could be the link between senile plaques and tangles.

In 2002, the family of 1,2,4-thiadiazole-3,5-dione (TDZD) was reported by our group as the first non-

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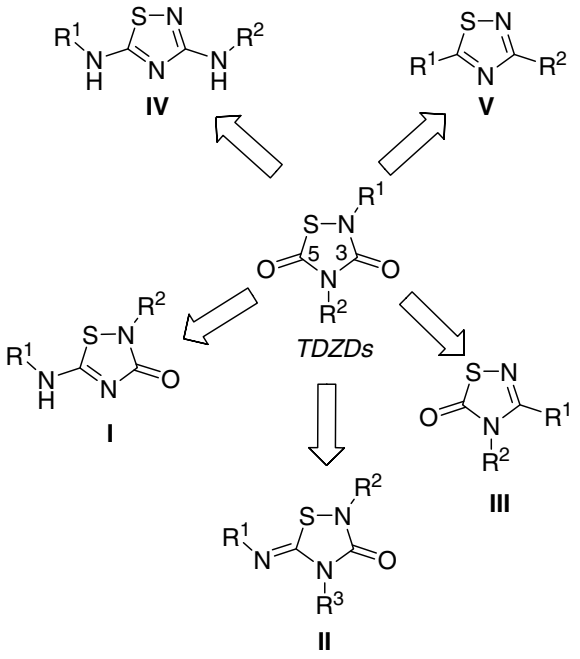
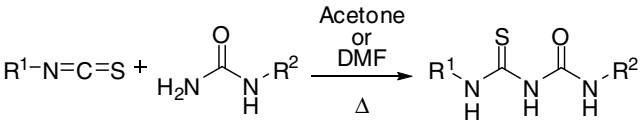


Figure 1. Proposed substitutions in 3 and 5 positions of 1,2,4-thiadiazole ring.



R^1=Bz	R^2=H	**1**
R^1=Bz	R^2=Me	**2**
R^1=Bz	R^2=Bn	**3**
R^1=Bn	R^2=H	**4**
R^1=Bn	R^2=Me	**5**
R^1=Bn	R^2=Bn	**6**
R^1=Me	R^2=Me	**7**
R^1=*p*-BrPh	R^2=Me	**8**
R^1=Ph	R^2=Me	**9**
R^1=$(CH_2)_2$Ph	R^2=Me	**10**
R^1=CH_2CO_2Et	R^2=Me	**11**
R^1=CO_2Et	R^2=Me	**12**

Scheme 1. Synthesis of 2,4-thiobiurets.

ATP competitive GSK-3β inhibitors.[18] After that, further studies were performed over this skeleton (keeping the 3,5-dione moiety) in order to study the influence of structural modifications on the biological activity. After an extensive SAR study among different heterocyclic families, TDZDs were revealed as a privileged scaffold for the selective inhibition of GSK-3β.[19]

In the present work, new modifications on the TDZD family are reported. On one hand, the basic skeleton of 1,2,4-thiadiazole and also one carbonyl group are kept, while different modifications are introduced in positions 3 and 5, respectively (Fig. 1). Thus, a set of 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (**I**), 5-imino-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (**II**), and 5-oxo-4,5-dihydro-1,2,4-thiadiazole (**III**) are prepared. On the other hand, the substitution of the two carbonyl groups of the TDZD ring is also taken into account and amino and alkyl or aryl groups are placed in positions 3 and 5 of the 1,2,4-thiadiazole ring (Fig. 1) to obtain 3,5-diamino-1,2,4-thiadiazole (**IV**) and 3,5-diaryl/dialkyl-1,2,4-thiadiazole (**V**) derivatives. Thus a large set of thiadiazoles and thiadiazolidines is here presented and its synthesis and biological evaluation described.

2. Chemistry

5-Amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole derivatives **I** were obtained in a two-step process from ring closure of thiobiurets (**1**–**12**), which were synthesized by condensation of monosubstituted ureas and isothiocyanates (Scheme 1).[20]

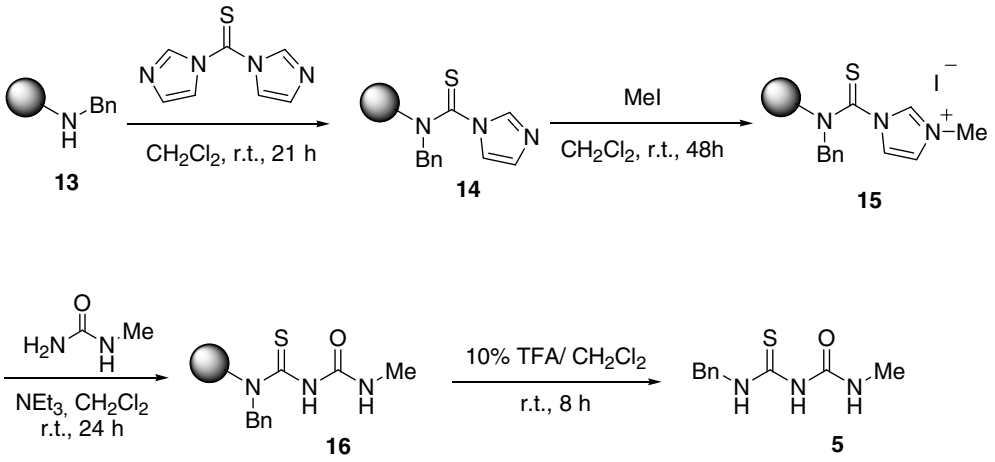
Taking into account that the solid-phase synthesis of thiobiurets has not been reported, we describe here an approach to it. The goal of this approximation was to try to automatize the synthesis of these intermediates in order to accelerate the synthetic pathway to the desired thiadiazoles. The first step of the strategy to obtain the thiobiuret **5** was the immobilization of benzylamine on solid supports by reductive-amination of the resin-bound aldehyde *N*-polystyrylmethyl-5-(4-formyl-3-methoxyphenoxy)-pentanamide.[21]

The subsequent treatment of the supported amine **13** with fivefold excess of thiocarbonyldiimidazole (TCDI) yielded thiourea **14**, whose thiocarbamoylimidazolium salts **15** were synthesized in order to enhance its reactivity toward nucleophilic attack.[22] These salts were obtained by methylation with iodomethane. Finally, reaction with excess of methylurea yielded the thiobiuret **5** in 10% yield after the acidic cleavage (TFA 10% in CH_2Cl_2 at room temperature for 10 min) of **16** (Scheme 2).

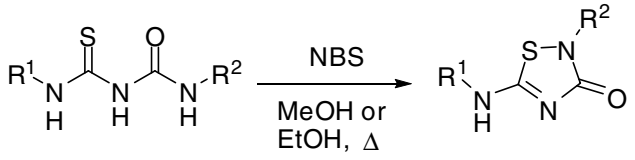
Although we can consider this solid-phase approach as a new methodology for the synthesis of thiobiurets, unfortunately, the yield was lower than the corresponding one after the solution procedure.

Finally, the desired 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole derivatives **17**–**26** were achieved by oxidative cyclization of the thiobiurets (**1**–**10**) via N–S bond formation with *N*-bromosuccinimide in methanol.[23] The ring closure of thiobiurets **11** and **12** was accomplished in ethanol in order to avoid transesterification reactions, and heterocycles **27** and **28** were obtained (Scheme 3).

In order to increase the diversity of the synthesized thiadiazoles, attempts to debenzoylate **17** and **18**, together with ester reduction or acidic hydrolysis of **27**, were carried out. Neither reaction was successful due to the instability of the heterocycles to the reaction conditions. Instead of performing the transformations on the closed ring, the reactions were carried out on the



Scheme 2. Solid-phase synthesis of thiobiuret **5**.



1	R^1=Bz	R^2=H	**17**
2	R^1=Bz	R^2=Me	**18**
3	R^1=Bz	R^2=Bn	**19**
4	R^1=Bn	R^2=H	**20**
5	R^1=Bn	R^2=Me	**21**
6	R^1=Bn	R^2=Bn	**22**
7	R^1=Me	R^2=Me	**23**
8	R^1=*p*-BrPh	R^2=Me	**24**
9	R^1=Ph	R^2=Me	**25**
10	R^1=$(CH_2)_2$Ph	R^2=Me	**26**
11	R^1=CH_2CO_2Et	R^2=Me	**27**
12	R^1=CO_2Et	R^2=Me	**28**

Scheme 3. Oxidative cyclization of thiobiurets.

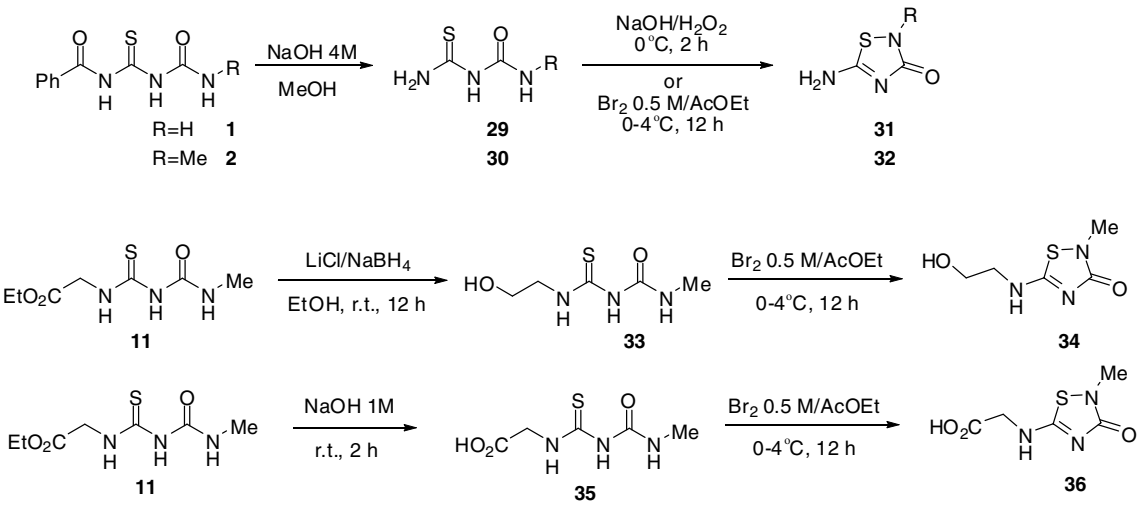
precursors thiobiurets **1**, **2**, and **11** (Scheme 4). Debenzoylation of **1** and **2** was successfully achieved after treatment with NaOH 4 M in methanol and subsequent acidification, yielding **29** and **30**. The thiadiazoles **31** and **32** were obtained by oxidative cyclization of these thiobiurets with H_2O_2 or Br_2 0.5 M, respectively. On the other hand, the ester group of **11** was reduced to the alcohol derivative **33** with LiCl/$NaBH_4$[24] and its basic hydrolysis to the corresponding acid **35** was obtained with NaOH 1 M in ethanol. The new thiobiurets **33** and **35** allowed the cyclization to thiadiazoles **34** and **36** with alcoholic and carboxylic groups.

Alkylation of 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole derivatives **17**, **18**, **19**, **21**, and **24** allowed us to obtain the second family of heterocycles: the 5-imino-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole derivatives **II**. Starting from **17**, a mixture of substituted products, **37**–**38** and **39**–**40**, was obtained after treatment with the corresponding alkylating agent in NaH/DMF. Otherwise, starting from disubstituted thiadiazoles **18**, **19**, **21**, and **24**, the three substituted derivatives **41**–**48** were obtained. An additional modification of these compounds was done by hydrolysis of the ester functionality of derivatives **43** and **45** in NaOH 1 M to the corresponding carboxylic acids **49** and **50** (Scheme 5).

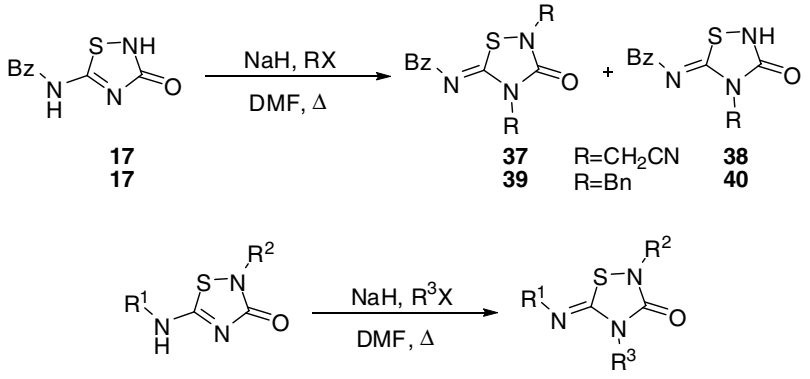
Two different approaches were used to obtain the 5-oxo-4,5-dihydro-1,2,4-thiadiazole derivatives **III**. Treatment of sulphone **51** with potassium *tert*-butoxide in *tert*-butyl alcohol[25] produced the thiadiazole **52** which was converted to the 3,4-disubstituted thiadiazole **53** by alkylation with ethyl 2-bromoacetate. On the other hand, according to a previously described procedure, condensation of 2-methylbenzonitrile with hydroxylamine followed by substitution/rearrangement reaction with TCDI in the presence of boron trifluoride etherate gave thiadiazole **55**[26] (Scheme 6).

3,5-Diamino-1,2,4-thiadiazole (**IV**) has been obtained by using [bis(acyloxy)iodo]arenes as a more specific oxidant reagent than the traditional ones (Scheme 7).[27] In our case, aliphatic monosubstituted thioureas and diacetoxyiodobenzene (DIB) were used to get heterocycles **56**–**59**.

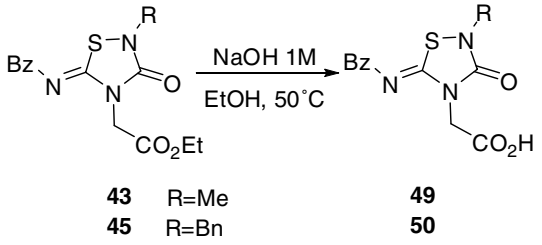
To obtain 3,5-diaryl/dialkyl-1,2,4-thiadiazole (**V**), different synthetic procedures have been described.[28–32] Diphenyl thiadiazol **60**[29] was prepared after condensation/oxidation reaction of two equivalents of phenylthioamide (Scheme 8). Also the reaction of phenylthioamide with dimethylacetal allowed the formation of *N*′-(thioaroyl)-*N*,*N*-dimethylamidines (**61** and **62**) that after amination cyclization with hydroxyl-amine-*O*-sulfonic acid (HSA) provided **63** and **64**[30] in excellent yields. Other well-known procedure for the preparation of this kind of heterocycles is the thermolysis of oxathiazolone **65**–**72** that yielded nitrile sulfides which reacted with cyano acti-



Scheme 4. Derivatization of the thiobiurets **1**, **2**, and **11** and following cyclization.



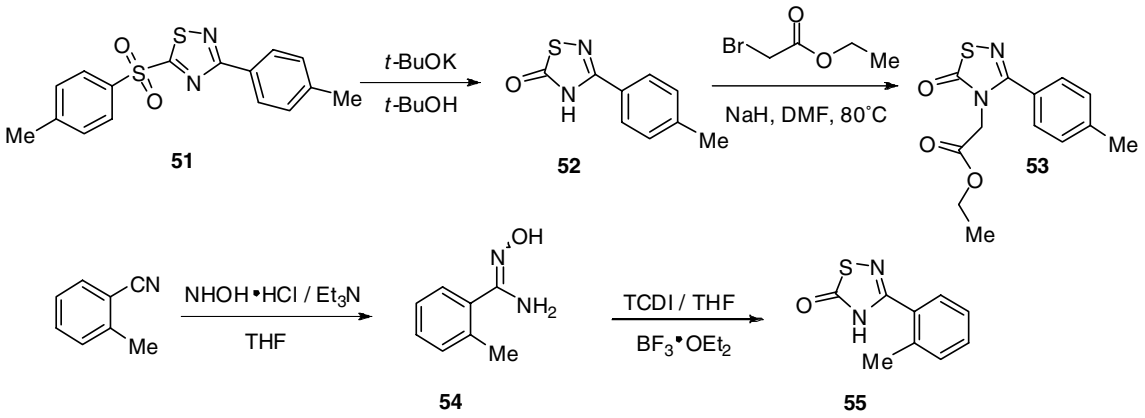
18	R^1=Bz	R^2=Me	R^3=Bn	**41**
18	R^1=Bz	R^2=Me	R^3=Me	**42**
18	R^1=Bz	R^2=Me	R^3=CH_2CO_2Et	**43**
18	R^1=Bz	R^2=Me	R^3=CH_2CN	**44**
19	R^1=Bz	R^2=Bn	R^3=CH_2CO_2Et	**45**
21	R^1=Bn	R^2=Me	R^3=Bn	**46**
21	R^1=Bn	R^2=Me	R^3=CH_2CO_2Et	**47**
24	R^1=*p*-BrPh	R^2=Me	R^3=Me	**48**



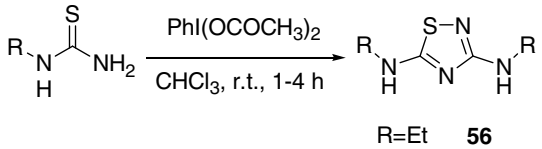
Scheme 5. Synthesis of 5-imino-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (**II**).

vated derivatives and allowed us to obtain thiadiazoles **73**–**80**[31,32] through 1,3-dipolar cycloaddition reaction (Scheme 8).

The structure of all new compounds was elucidated from their analytical and spectroscopic data (^{1}H and ^{13}C NMR) which are collected in Section 5. Unequivocal



Scheme 6. Synthesis of 5-oxo-4,5-dihydro-1,2,4-thiadiazole (**III**).



R=Et **56**
R=Ac **57**
R=Ph **58**
R=Py **59**

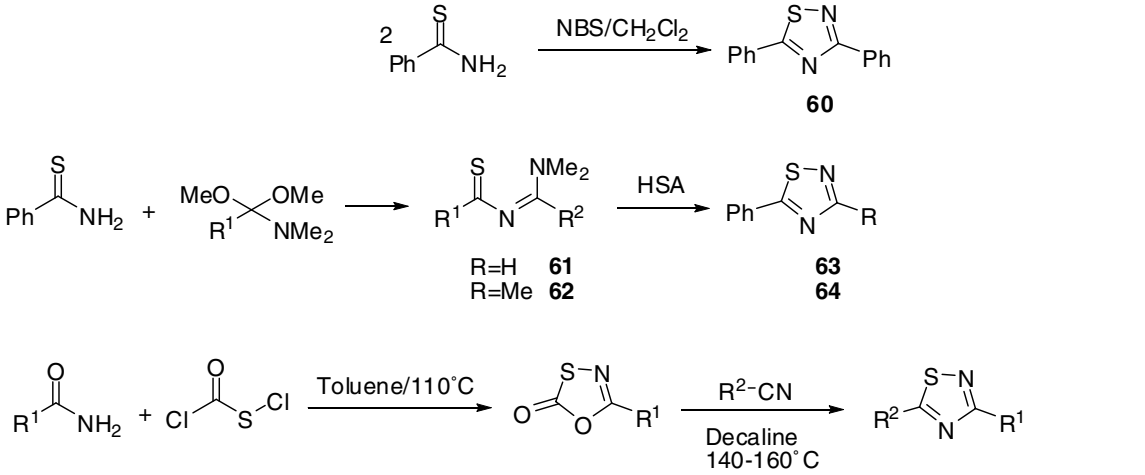
Scheme 7. Synthesis of 3,5-diamino-1,2,4-thiadiazole (**IV**).

assignment of all chemical shifts (^{1}H and ^{13}C NMR) and the site of substitution were determined using two-dimensional experiments such as HMQC or HMBC.

3. Biological results and SAR studies

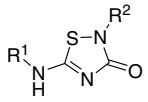
The GSK-3β activity of the new thiadiazol derivatives here synthesized was determined following a method described in the experimental part. Briefly, GSK-3β enzyme (Sigma) was incubated with ATP and GS-1 as substrate[33] in the presence and in the absence of the corresponding test compound. Finally, the GSK-3β activity was expressed in picomoles of phosphate incorporated per 20 min of incubation or in the percentage of maximal activity.

A wide range of the 5-amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (**I**) inhibits GSK-3β with IC_{50} values in the micromolar range (Table 1). The members of this family



Scheme 8. Synthesis of 3,5-diaryl/dialkyl-1,2,4-thiadiazole (**V**).

Table 1. GSK-3β inhibition of the 5-amino-1,2,4-thiadiazole (**I**)



Compound	R^1	R^2	IC_{50}[a] (μM)
17	Bz	H	>100
18	Bz	Me	>100
19	Bz	Bn	>100
20	Bn	H	6.5
21	Bn	Me	20.0
22	Bn	Bn	>100
23	Me	Me	18.0
24	*p*-BrC_6H_4	Me	15.0
25	Ph	Me	12.5
26	$(CH_2)_2Ph$	Me	15.0
27	CH_2CO_2Et	Me	20.0
28	CO_2Et	Me	>100
31	H	H	25.0
32	H	Me	40.0
34	$(CH_2)_2OH$	Me	>100
36	CH_2CO_2H	Me	75.0

[a] IC_{50}: 50% inhibitory concentration of glycogen synthase kinase-3β activity (μM). Assays were performed in triplicate.

showed activities between 6.5 and 40 μM, except the benzoylic derivatives (**17**–**19**), where a complete loss of activity was observed. For the rest of compounds of this family it can be observed that a decrease in the volume of the substituent in position 2 leads to a significant increase of the inhibitory activity (**22** vs **21** and vs **20**). It seems to be important also the presence of hydrophobic group in position 5 due to the lack of activity of derivatives with polar substituent in this position (**20**, **24**, **25**, **26** vs **27** or **28**, **34**, **36**).

To verify the ATP non-competitive mechanism of action of the compounds synthesized in this study, a kinetic analysis was performed. The experiments were carried out using combinations of six ATP concentrations (from 6.5 to 100 μM) and two inhibitor concentrations for our compound **21** and the known ATP competitive inhibitor Ro 31-8220.[34] We have measured the kinase activity increasing ATP up to 100 μM where enzyme saturation could be ensured. Double-reciprocal plotting of the data (Fig. 2), in which each point is mean of two different experiments, suggests that compound **21** acts as a non-competitive inhibitor of ATP binding while the ATP competitive binding mode of Ro 31-8220 is shown. Based on these results and due to the similarity with the TDZD families previously reported,[18,19] we could assume a similar binding mode to the enzyme.

However, none of the 3,5-diamino-1,2,4-thiadiazoles **56**–**59**, 3,5-disubstituted-1,2,4-thiadiazole (**60**, **63**, **64**, **73**–**80**) derivatives, the 5-imino-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole derivatives **37**–**50**, and 5-oxo-4,5-dihydro-1,2,4-thiadiazole derivatives **52**, **53**, and **55** assayed resulted to be good inhibitors of GSK-3β at the studied doses (IC_{50} > 100 μM). This fact reveals the importance of carbonyl moiety between both nitrogen atoms and points out to the subtle substitution allowed in position 5, where some amino substituents but no imino ones are tolerated.

4. Conclusions

In order to study the requirements for the biological activity of the TDZD family as GSK-3β inhibitors, new modifications are reported. The basic skeleton of 1,2,4-thiadiazole and also one of the carbonyl group are kept, while different modifications are introduced in positions

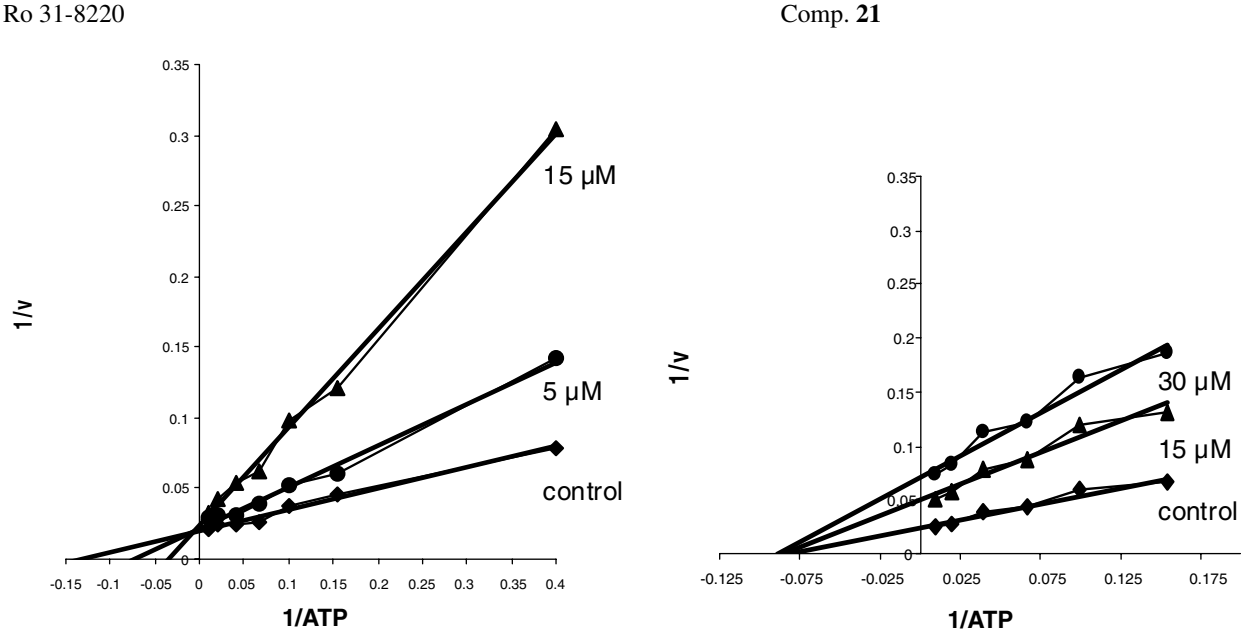


Figure 2. Double-reciprocal plot of kinetic data from assays of GSK-3β protein kinase activity at different concentrations of inhibitors. ATP concentrations in the reaction mixture varied from 6.5 to 100 μM, and inhibitor concentrations are 15 and 30 μM for compound **21** and 5 and 15 μM for Ro 31-8220. The concentration of GS-1 was kept constant in both experiments at 15 μM. *V* is picomoles of phosphate/20 min.

3 and 5, respectively. The GSK-3β activity of the new thiadiazole derivatives here synthesized showed IC_{50} values for some of the compounds in the micromolar range. Additionally, ATP competition studies have been carried out, showing that as well as the first generation of TDZD, these new compounds act in a non-competitive manner. With this study, additional requirements for the biological activity of the TDZD family have been deliniated, showing that just one carbonyl group can be enough to keep the biological effect. Carbonyl 3 of TDZD framework is indispensable for activity while some alkyl/aryl or amino fragments are tolerated in position 5. These results open new roads for further optimization in this interesting series of compounds.

5. Experimental

5.1. Chemical procedures

Substrates were either purchased from commercial sources or used without further purification. *N*-Polystyrylmethyl-5-(4-formyl-3-methoxyphenoxy)-pentanamide (70–90 mesh) batch: A31603, 1% DVB, substitution: 0.88 mmol/g was purchased from NovaBiochem. Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Flash column chromatography was carried out at medium pressure using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–240 mesh ASTM) and preparative centrifugal circular thin-layer chromatography (CCTLC) on a circular plate coated with a 1-mm layer of Kieselgel 60 PF254, Merk, by using a Chromatotron with the indicated solvent as eluent. Compounds were detected with UV light (254 nm). ^{1}HNMR spectra were obtained on Varian XL-300 and Gemini-200 spectrometers working at 300 and 200 MHz, respectively. Typical spectral parameters: spectral width 10 ppm, pulse width 9 μs (57°), data size 32 K. ^{13}C NMR experiments were carried out on the Varian Gemini-200 spectrometer operating at 50 MHz. The acquisition parameters: spectral width 16 kHz, acquisition time 0.99 s, pulse width 9 μs (57°), data size 32 K. Chemical shifts are reported in values (ppm) relative to internal Me_4Si and *J* values are reported in Hertz. IR (infrared-spectroscopy): the resins were measured as KBr pellets on a Perkin-Elmer Spectrum One Spectrometer. ps = polystyrene, MS (mass spectroscopy): EI (electronic ionization mass spectroscopy), MSD 5973 Hewlett–Packard and ESI (electrospray ionization mass spectroscopy), LC/MSD-Serie 100 Hewlett–Packard. Elemental analyses were performed by the analytical department at CENQUIOR (CSIC), and the results obtained were within ±0.4% of the theoretical values. In order to get the molecular mass of the resin and to calculate the elemental analysis, the following calculation has to be performed:

Formula 1. Formula for the calculation of the molar mass of a derivatized resin. The molar $mass_{Sub}$ is the molecular mass of the fragment being substituted (e.g., Cl in case of Merrifield resin), while molar $mass_{Add}$ is the molecular mass of the fragment being added.

$$\text{molar mass}_{new} = \frac{1000}{\text{Loading}_{old}} - (\text{molar mass}_{Sub} - \text{molar mass}_{Add})$$

5.1.1. General procedure for the synthesis of 1-benzoyl-5-amino-2-thiobiurets (1–3). One millimole of benzoylisothiocyanate was added over a solution of 1 mmol of the corresponding urea in 2.5 ml of acetone pa. The mixture was refluxed during 10 h. After that, the reaction mixture was allowed to room temperature and the suspension obtained was purified as indicated in each case.

5.1.1.1. 1-Benzoyl-2-thiobiuret (1).[20] Reagents: urea (48 mg, 1 mmol), benzoylisothiocyanate (0.13 ml, 1 mmol). Isolation: filtration of the precipitate. Purification: recrystallization from acetone. Yield: 183 mg (82%). Mp 170–172 °C (lit.[20] 174–175 °C). ^{1}H NMR (DMSO-d_6) δ 11.50 (br s, 1H, BzN*H*CS); 6.21 (br s, 1H, CSN*H*CO); 5.41 (br s, 2H, $CONH_2$); 7.91 (dd, 2Ho, *J* = 7.5 and 1.5 Hz); 7.72 (dd, 1Hp, *J* = 7.8 and 1.5 Hz); 7.65 (dd, 2Hm, *J* = 7.8 and 7.5 Hz). ^{13}C NMR (DMSO-d_6) δ 179.5 (C2); 154.8 (C4); 165.8 (*C*OPh); 133.6 (Ci); 131.5 (Cp); 129.4 (2Cm); 128.5 (2Co). MS (EI): *m*/*z* 223 (M^+, 25).

5.1.1.2. 1-Benzoyl-5-methyl-2-thiobiuret (2).[35] Reagents: methylurea (74 mg, 1 mmol), benzoylisothiocyanate (0.13 ml, 1 mmol). Isolation: filtration of the precipitate. Purification: silica gel column chromatography using hexane/AcOEt (4:1) as eluent. Yield: 202 mg (85%). Mp 172–174 °C (lit.[35] 170–172 °C). ^{1}H NMR (DMSO-d_6) δ 13.15 (br s, 1H, BzN*H*CS); 11.15 (br s, 1H, CSN*H*CO); 8.00 (bm, 1H, CON*H*CH_3); 7.87 (dd, 2Ho, *J* = 6.9 and 1.4 Hz); 7.29 (dd, 1Hp, *J* = 7.4 and 1.4 Hz); 7.25 (dd, 2Hm, *J* = 7.4 and 6.9 Hz); 2.73 (d, 3H, *J* = 4,7 Hz, C*H*$_3$NH). ^{13}C NMR (DMSO-d_6) δ 179.0 (C2); 158.2 (C4); 170.0 (*C*OPh); 136.3 (Ci); 134.0 (Cp); 131.4 (2Cm); 130.0 (2Co); 26.0 (CH_3). MS (EI): *m*/*z* 238 (M^+, 25).

5.1.1.3. 1-Benzoyl-5-benzyl-2-thiobiuret (3). Reagents: benzylurea (150 mg, 1 mmol), benzoylisothiocyanate (0.13 ml, 1 mmol). Isolation: filtration of the precipitate. Purification: recrystallization from acetone. Yield: 250 mg (80%). Mp 175–177 °C. ^{1}H NMR (DMSO-d_6) δ 13.25 (br s, 1H, CH_2N*H*CS); 11.32 (br s, 1H, CSN*H*CO); 8.20 (bm, 1H, CH_2N*H*CO); 7.91 (dd, 2Ho, *J* = 7.2 and 1.4 Hz); 7.68 (dd, 1Hp, *J* = 7.4 and 1.4 Hz); 7.58 (dd, 2Hm, *J* = 7.4 and 7.2 Hz); 7.40–7.20 (m, 5H, CH_2Ph); 4.42 (d, 2H, *J* = 5.8 Hz, C*H*$_2$NH). ^{13}C NMR (DMSO-d_6) δ 178.8 (C2); 153.5 (C4); 166.6 (*C*OPh); 133.3 (Ci); 132.8 (Cp); 128.9 (2Cm); 127.9 (2Co); 138,3 (Ci); 128.5 (2Cm); 127.2 (2Co); 126.9 (Cp); 42.8 (*C*H_2Ph). MS (EI): *m*/*z* 313 (M^+, 8).

5.1.2. General procedure for the synthesis of non-benzoylyc thiobiurets (4–12). One millimole of the appropriate isothiocyanate was added over a solution of 1 mmol of the corresponding urea in 2.5 ml of anhydrous DMF. The mixture was refluxed during 10–24 h depending on the product. The reaction mixture was allowed to room

temperature and then 2.5 ml of distilled water was added. The resulted solid was filtered and purified as indicated in each case.

5.1.2.1. 1-Benzyl-2-thiobiuret (4). Reagents: urea 48 mg (1 mmol), benzylisothiocyanate (0.13 ml, 1 mmol). Isolation: addition of cold water and filtration of the precipitate. Purification: silica gel column chromatography using hexane/AcOEt (1:1) as eluent. Yield: 84 mg (40%). Mp 178–180 °C. ^{1}H NMR (DMSO-d_6) δ 10.83 (bm, 1H, CH_2NHCS); 9.88 (br s, 1H, $CONH_2$); 7.50–7.20 (m, 5H, Ph); 4.79 (d, 2H, J = 5.5 Hz, CH_2–Ph); 6.96 (br s, 1H, NH_2); 6.38 (br s, 1H, NH_2). ^{13}C NMR (DMSO-d_6) δ 180.7 (C2); 155.6 (C2); 137.8 (Ci); 128.5 (2Cm); 127.5 (2Co); 127.2 (Cp); 47.6 (CH_2Ph). MS (EI): m/z 209 (M^+, 40).

5.1.2.2. 1-Benzyl-5-methyl-2-thiobiuret (5). Reagents: methylurea (74 mg, 1 mmol), benzylisothiocyanate (0.13 ml, 1 mmol). Isolation: addition of cold water and filtration of the precipitate. Purification: silica gel column chromatography using hexane/AcOEt (4:1) as eluent. Yield: 160 mg (70%). Mp 177–179 °C. ^{1}H NMR (DMSO-d_6) δ 10.74 (bm, 1H, CH_2NHCS); 9.93 (br s, 1H, $CSNHCO$); 6.69 (bm, 1H, $CONHCH_3$); 7.30–7.20 (m, 5H, Ph); 4.76 (d, 2H, J = 5.5 Hz, CH_2Ph); 2.62 (d, 3H, J = 4.6 Hz, CH_3NH). ^{13}C NMR (DMSO-d_6) δ 179.5 (C2); 160.2 (C4); 140.2 (Ci); 130.1 (2Cm); 129.0 (2Co); 128.2 (Cp); 55.2 (CH_2Ph); 25.1 (CH_3). MS (EI): m/z 223 (M^+, 100).

5.1.2.3. 1,5-Dibenzyl-2-thiobiuret (6). Reagents: benzylurea (150 mg, 1 mmol), benzylisothiocyanate (0.13 ml, 1 mmol). Isolation: addition of cold water and filtration of the precipitate. Purification: silica gel column chromatography using hexane/AcOEt (5:1) as eluent. Yield: 100 mg (35%). Mp 95–97 °C. ^{1}H NMR (DMSO-d_6) δ 10.71 (bt, J = 5.5 Hz, 2× CH_2NHCS); 9.96 (br s, 1H, $CSNHCO$); 7.40–7.30 (m, 5H, Ph); 4.77 (d, 2H, J = 5.5 Hz, CH_2Ph); 7.30–7.20 (m, 5H, Ph); 4.27 (d, 2H, J = 5.8 Hz, CH_2Ph). ^{13}C NMR (DMSO-d_6) δ 180.2 (C2); 154.5 (C4); 137.7 (Ci); 128.5 (2Cm); 127.5 (2Co); 127.2 (Cp); 47.7 (CH_2Ph); 138.7 (Ci); 128.5 (2Cm); 127.1 (2Co); 127.0 (Cp); 42.4 (CH_2Ph). MS (EI): m/z 219 (M^+, 52).

5.1.2.4. 1,5-Dimethyl-2-thiobiuret (7). Reagents: methylurea (74 mg, 1 mmol), methylisothiocyanate (0.068 ml, 1 mmol). Purification: silica gel column chromatography using hexane/AcOEt (1:1) as eluent. Yield: 52 mg (35%). Mp 100–102 °C. ^{1}H NMR (DMSO-d_6) δ 10.22 (bm, 1H, CH_3NHCS); 9.78 (br s, 1H, $CSNHCO$); 6.61 (bm, 1H, CH_3NHCS); 2.97 (d, 3H, J = 4.6 Hz, $NHCH_3$); 2.60 (d, 3H, J = 4.7 Hz, CH_3NH). ^{13}C NMR (DMSO-d_6) δ 180.6 (C2); 155.0 (C4); 31.4 (CH_3); 25.7 (CH_3). MS (EI): m/z 147 (M^+, 100).

5.1.2.5. 1-*p*-Bromophenyl-5-methyl-2-thiobiuret (8). Reagents: methylurea (74 mg, 1 mmol), *p*-bromophenylisothiocyanate (214 mg, 1 mmol). Purification: silica gel column chromatography using hexane/AcOEt (4:1) as eluent. Yield: 147 mg (51%). Mp 185–187 °C. ^{1}H NMR (DMSO-d_6) δ 12.42 (br s, 1H, *p*-BrPhNHCS); 10.22 (br s, 1H, $CSNHCO$); 5.92 (bm, 1H, CH_3NHCS); 7.60–7.50 (d, 2Ho, J = 8.2 Hz); 7.30–7.40 (d, 2Hm, J = 8.2 Hz); 2.67 (d, 3H, J = 4.6 Hz, CH_3NH).^{13}C NMR (DMSO-d_6) δ 179.1 (C2); 156.2 (C4); 138.2 (Ci); 133.0 (2Cm); 128.2 (2Co); 120.2 (Cp); 26.2 (CH_3). MS (EI): m/z 289 (M^+, 92).

5.1.2.6. 5-Methyl-1-phenyl-2-thiobiuret (9). Reagents: methylurea (74 mg, 1 mmol), phenylisothiocyanate (0.12 ml, 1 mmol). Purification: preparative centrifugal circular thin-layer chromatography using hexane/AcOEt (3:1) as eluent. Yield: 79 mg (38%). Mp 153–155 °C. ^{1}H NMR (DMSO-d_6) δ 12.27 (br s, 1H, PhNHCS); 10.20 (br s, 1H, $CSNHCO$); 6.00 (br s, 1H, CH_3NHCS); 7.53 (dd, 2Ho, J = 7.5 and 1.3 Hz); 7.40 (dd, 2Hm, J = 7.5 and 6.6 Hz); 7.28 (dd, 1Hp, J = 7.5 and 1.3 Hz); 2.78 (d, 3H, J = 4.9 Hz, CH_3NH). ^{13}C NMR (DMSO-d_6) δ 179.2 (C2); 155.0 (C4); 137.3 (Ci); 128.8 (2Cm); 127.0 (Cp); 125.0 (2Co); 26.4 (CH_3). MS (EI): m/z 209 (M^+, 100).

5.1.2.7. 5-Methyl-1-phenethyl-2-thiobiuret (10). Reagents: methylurea (74 mg, 1 mmol), phenetylisothiocyanate (0.15 ml, 1 mmol). Purification: silica gel column chromatography using hexane/AcOEt (3:1) as eluent. Yield: 162 mg (68%). Mp 146–148 °C. ^{1}H NMR (DMSO-d_6) δ 10.29 (bm, 1H, CH_2NHCS); 9.71 (br s, 1H, $CSNHCO$); 6.52 (br s, 1H, CH_3NHCS); 7.40–6.80 (m, 5H, Ph); 3.60 (m, 2H, CH_2CH_2–NH); 2.70 (m, 2H, CH_2CH_2–Ph); 3.20 (d, 3H, J = 4.5 Hz, CH_3NH). ^{13}C NMR (DMSO-d_6) δ 179.9 (C2); 155.0 (C4); 138.9 (Ci); 128.6 (2Cm); 128.4 (2Co); 126.3 (Cp); 45.6 (CH_2NH); 33.9 (CH_2Ph); 25.7 (CH_3). MS (EI): m/z 237 (M^+, 21).

5.1.2.8. 1-Ethoxycarbonylmethyl-5-methyl-2-thiobiuret (11). Reagents: methylurea (74 mg, 1 mmol), ethoxycarbonylisothiocyanate (0.12 ml, 1 mmol). Purification: silica gel column chromatography using hexane/AcOEt (3:1) as eluent. Yield: 140 mg (65%). Mp 108–110 °C. ^{1}H NMR (DMSO-d_6) δ 10.82 (br s, 1H, CH_2NHCS); 9.82 (br s, 1H, $CSNHCO$); 5.95 (br s, 1H, CH_3NHCS); 4.41 (d, 2H, J = 5.1 Hz, CH_2NH); 4.27 (c, 2H, J = 7.1 Hz, CH_2CH_3); 1.32 (t, 3H, J = 7.1 Hz, CH_2CH_3); 2.83 (d, 3H, J = 4.8 Hz, CH_3NH). ^{13}C NMR (DMSO-d_6) δ 180.8 (C2); 154.9 (C4); 168.5 (CO_2); 61.8 (CH_2CO_2); 46.6 (CH_2CH_3); 14.1 (CH_2CH_3); 26.4 (CH_3). MS (EI): m/z 219 (M^+, 86).

5.1.2.9. 1-Ethoxycarbonyl-5-methyl-2-thiobiuret (12). Reagents: methylurea (74 mg, 1 mmol), ethoxycarbonylisothiocyanate (0.12 ml, 1 mmol). Purification: silica gel column chromatography using hexane/AcOEt (1:1) as eluent. Yield: 113 mg (60%). Mp 198–200 °C. ^{1}H NMR (DMSO-d_6) δ 10.40 (br s, 1H, $CSNHCO_2Et$); 9.89 (br s, 1H, $CSNHCO$); 7.65 (br s, 1H, CH_3NHCS); 4.06 (c, 2H, J = 7.1 Hz, CH_2CH_3); 1.18 (t, 3H, J = 7.1 Hz, CH_2CH_3); 3.02 (d, 3H, J = 4.7 Hz, CH_3NH). ^{13}C NMR (DMSO-d_6) δ 178.0 (C2); 149.3 (C4); 151.4 (CO_2); 60.9 (OCH_2CH_3); 14.2 (CH_2CH_3); 27.1 (CH_3). MS (EI): m/z 205 (M^+, 9).

5.1.3. *N*-Polystyrylmethyl-5-(4-benzylamino-3-methoxyphenoxy)-pentanamide (13). To the *N*-polystyrylmethyl-

5-(4-formyl-3-methoxyphenoxy)-pentanamide (940 mg, 1.0 mmol sealed within a polypropylene mesh packet) was added benzylamine (2.2 ml, 20.0 mmol), $CH(OCH_3)_3$ (2.2 ml, 20.0 mmol) in anhydrous DMF. The mixture was shaken for 48 h at room temperature. The resin was then washed with DMF (three times) and suspended again in DMF. After that $NBH_4(Bu)_4$ (1.3 g, 5 mmol) and acetic acid were added, and vigorous gas loosening was observed. Once the gas bubbling stopped, the resin was washed with DMF (seven times), DCM (two times), and pentane (three times) and dried under reduced pressure. IR (KBr): 3274 (f), 3060 (f, ps), 3026 (f, ps), 2926 (f, ps), 2850 (f, ps), 2386 (f), 2310 (f), 2272 (f), 1945 (m, ps), 1874 (m, ps), 1805 (m, ps), 1611 (f, ps), 1493 (f, ps), 1452 (f, ps). Anal. Calcd for $C_{97}H_{101}N_2O_3$: C, 86.81; H, 7.58; N, 2.06. Found: C, 82.96; H, 8.24; N, 1.19. Loading: 0.470 mmol/g.

5.1.4. *N*-Polystyrylmethyl-5-{4-[1-benzyl-1-(imidazole-1-carbothioyl)-amino]-3-methoxyphenoxy}-pentanamide (14). The resulting resin-bound compound **13** (2.50 g, 1 mmol) was coupled with thiocarbonyldiimidazol (890 mg, 5 mmol) in anhydrous CH_2Cl_2 at room temperature for 24 h. The resin was washed with DMF (seven times), CH_2Cl_2 (three times), and pentane (three times) and dried under reduced pressure. IR (KBr): 3487 (f), 3378 (f), 3059 (f, ps), 3025 (f, ps), 2923 (f, ps), 2850 (f, ps), 1945 (m, ps), 1872 (m, ps), 1804 (m, ps), 1601 (f, ps), 1493 (f, ps), 1356 (f). Anal. Calcd for $C_{183}H_{185}N_4O_3S$: C, 87.22; H, 7.39; N, 2.21; S, 1.27. Found: C, 79.94; H, 7.42; N, 2.42; S, 1.18. Loading: 0.521 mmol/g.

5.1.5. 1-Methyl-3-{[4-(4-polystyrylmethylcarbamoylbutoxy)-2- methoxyphenyl]-benzyl-thiocarbamoyl}-3H-imidazol-1-ium iodide (15). Resin-bound compound **14** (2.32 g, 1 mmol) was reacted with methyl iodide (1.24 ml, 20 mmol) in CH_2Cl_2 for 48 h at room temperature. The resin was washed with DMF (seven times), CH_2Cl_2 (three times), and pentane (three times) and dried under reduced pressure. IR (KBr): 3274 (f), 3058 (f, ps), 3030 (f, ps), 2924 (f, ps), 2850 (f, ps), 2386 (f), 2313 (f), 2272 (f), 1945 (m, ps), 1875 (m, ps), 1805 (m, ps), 1611 (f, ps), 1493 (f, ps), 1452 (f, ps). Anal. Calcd for $C_{168}H_{172}N_4O_3SI$: C, 82.25; H, 7.06; N, 2.27; S, 1.31. Found: C, 74.25; H, 7.35; N, 2.04; S, 0.75. Loading: 0.460 mmol/g.

5.1.6. 1-Benzyl-1-[4-(4-polystyrylmethylcarbamoylbutoxy)-2-methoxyphenyl]-5-methyl-2-thiobiuret (16). Resin-bound compound **15** (2.78 g, 1 mmol) was coupled with methyl urea (1.24 ml, 20 mmol) using NEt_3 (0.28 ml, 2 mmol) in CH_2Cl_2 for 48 h at room temperature. The resin was washed with DMF (seven times), DCM (three times), and pentane (three times) and dried under reduced pressure. IR (KBr): 3274 (f), 3060 (f, ps), 3026 (f, ps), 2926 (f, ps), 2850 (f, ps), 2386 (f), 2310 (f), 2272 (f), 1945 (m, ps), 1874 (m, ps), 1805 (m, ps), 1611 (f, ps), 1493 (f, ps), 1452 (f, ps). Anal. Calcd for $C_{186}H_{191}N_4O_4S$: C, 86.64; H, 7.46; N, 2.17; S, 1.24. Found: C, 82.41; H, 7.75; N, 1.70; S, 0.77. Loading: 0.340 mmol/g.

5.1.7. General procedure of thiobiurets cyclization (17–28). A suspension of 1 mmol of the corresponding thiobiuret and 1.25 mmol of NBS was refluxed in methanol or ethanol for 6–15 h depending on the product. The reaction mixture was allowed to room temperature (with stirring), and part of the product was obtained as a precipitate, being isolated by filtration. The solvent was removed by evaporation under reduced pressure and the crude was purified employing silica gel column chromatography with the mixture of solvents indicated in each case.

5.1.7.1. 5-Benzoylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (17). Reagents: compound **1** (223 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 6 h. Isolation: filtration of the precipitate. Yield: 144 mg (65%). Mp 221–223 °C (lit.[20] 219–221 °C). ^{1}H NMR (DMSO-d_6) δ 12.00 (br s, 1H, NH); 8.10 (dd, 2Ho, J = 7.1 and 1.3 Hz); 7.63 (dd, 1Hp, J = 7.3 and 1.3 Hz); 7.53 (dd, 2Hm, J = 7.3 and 7.1 Hz); 3.42 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 172.0 (C5); 162.0 (C3); 174.0 (*C*OPh); 133.3 (Ci); 131.9 (2Co); 128.8 (Cp); 128.6 (2Cm). MS (EI): *m*/*z* 221 (M^+, 11). Anal. Calcd for $C_9H_7N_3O_2S$: C, 48.86; H, 3.19; N, 18.99; S, 14.49. Found: C, 48.82; H, 3.36; N, 19.07; S, 14.21.

5.1.7.2. 5-Benzoylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (18). Reagents: compound **2** (237 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 4 h. Isolation: filtration of the precipitate. Yield: 221 mg (94%). Mp 273–275 °C (lit.[35] 267–268 °C). ^{1}H NMR (DMSO-d_6) δ 13.38 (br s, 1H, NH); 8.11 (dd, 2Ho, J = 6.9 and 1.6 Hz); 7.60 (dd, 1Hp, J = 7.2 and 1.6 Hz); 7.50 (dd, 2Hm, J = 7.2 and 6.9 Hz); 3.10 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 165.2 (C5); 156.2 (C3); 169.6 (*C*OPh); 137.0 (Ci); 133.4 (2Co); 132.1 (Cp); 128.8 (2Cm); 29.0 (CH_3). MS (EI): *m*/*z* 235 (M^+, 21). Anal. Calcd for $C_{10}H_9N_3O_2S$: C, 51.05; H, 3.86; N, 17.86; S, 13.63. Found: C, 50.86; H, 3.89; N, 18.27; S, 13.88.

5.1.7.3. 5-Benzoylamino-2-benzyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (19). Reagents: compound **3** (313 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 8 h. Isolation: filtration of the precipitate. Yield: 199 mg (64%). Mp 255–257 °C. ^{1}H NMR (DMSO-d_6) δ 13.30 (br s, 1H, NH); 8.15 (dd, 2Ho, J = 7.0 and 1.3 Hz); 7.65 (dd, 1Hp, J = 7.4 and 1.3 Hz); 7.45 (dd, 2Hm, J = 7.4 and 7.0 Hz); 7.40–7.20 (m, 5H, Ph); 4.78 (s, 2H, CH_2Ph). ^{13}C NMR (DMSO-d_6) δ 170.1 (C5); 156.4 (C3); 174.9 (*C*OPh); 133.7 (Ci); 132.5 (Cp); 129.1 (2Cm); 128.3 (2Co); 137.3 (Ci); 129.0 (2Cm); 128.2 (2Co); 128.1 (Cp); 46.3 (*C*H_2Ph). MS (EI): *m*/*z* 311 (M^+, 69). Anal. Calcd for $C_{16}H_{13}N_3O_2S$: C, 61.72; H, 4.21; N, 13.50; S, 10.30. Found: C, 61.82; H, 4.36; N, 14.00; S, 10.15.

5.1.7.4. 5-Benzylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (20). Reagents: compound **4** (210 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 24 h. Purification: silica gel column chromatography using dichloromethane/methanol (20:1) as

eluent. Yield: 100 mg (50%). Mp 215–217 °C. ^{1}H NMR (DMSO-d_6) δ 9.43 (br s, 1H, NH); 7.20–7.40 (m, 5H, Ph); 4.53 (s, 2H, CH_2); 5.70 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 174.4 (C5); 165.4 (C3); 137.4 (Ci); 128.6 (2Cm); 127.5 (2Co); 127.1 (Cp); 47.55 (CH_2Ph). MS (EI): *m*/*z* 207 (M^+, 20). Anal. Calcd for $C_9H_9N_3OS$: C, 52.16; H, 4.38; N, 20.27; S, 15.47. Found: C, 51.81; H, 4.13; N, 20.32; S, 15.36.

5.1.7.5. 5-Benzylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (21). Reagents: compound **5** (223 mg, 1 mmol) and NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 12 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 120 mg (55%). Mp 185–187 °C. ^{1}H NMR (DMSO-d_6) δ 8.82 (br s, 1H, NH); 7.20–7.40 (m, 5H, Ph); 4,52 (br s, 2H, CH_2Ph); 3.02 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 169.7 (C5); 166.0 (C3); 137.9 (Ci); 128.6 (2Cm); 127.5 (2Co); 127.4 (Cp), 46.8 (CH_2Ph); 29.9 (CH_3). MS (EI): *m*/*z* 221 (M^+, 46). Anal. Calcd for $C_{10}H_{11}N_3OS$: C, 54.28; H, 5.01; N, 18.99; S, 14.49. Found: C, 54.54; H, 5.29; N, 19.33; S, 14.78.

5.1.7.6. 2-Benzyl-5-benzylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (22). Reagents: compound **6** (300 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 12 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 119 mg (40%). Mp 220–222 °C. ^{1}H NMR (DMSO-d_6) δ 9.27 (br s, 1H, NH); 7.30–7.40 (m, 5H, Ph); 4.69 (s, 2H, CH_2Ph); 7.30–7.20 (m, 5H. Ph); 4.55 (br s, 2H, NHCH_2Ph). ^{13}C NMR (DMSO-d_6) δ 169.6 (C5); 164.0 (C3); 137.2 (Ci); 128.6 (2Cm); 128.5 (2Co); 128.4 (Cp); 46.3 (CH_2Ph); 137.8 (Ci); 127.7 (2Cm); 127.5 (2Co); 127.4 (Cp); 46.9 (CH_2Ph). MS (EI): *m*/*z* 297 (M^+, 4). Anal. Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.18; N, 14.13; S, 17.78. Found: C, 64.16, H, 5.24; N, 13.71; S, 17.35.

5.1.7.7. 2-Methyl-5-methylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (23). Reagents: compound **7** (147 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 24 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 30 mg (20%). Mp 195–197 °C. ^{1}H NMR (DMSO-d_6) δ 8.90 (br s, 1H, NH); 3.20 (bd, 3H, CH_3); 3.13 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 169.6 (C5); 165.2 (C3); 32.0 (CH_3); 30.0 (CH_3). MS (EI): *m*/*z* 145 (M^+, 6). Anal. Calcd for $C_4H_7N_3OS$: C, 33.09; H, 4.86; N, 28.94; S, 22.09. Found: C, 33.26; H, 5.21; N, 29.27; S, 22.38.

5.1.7.8. 5-*p*-Bromophenylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (24). Reagents: compound **8** (288 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 5 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 143 mg (51%). Mp 253–255 °C. ^{1}H NMR (DMSO-d_6) δ 8.80 (br s, 1H, NH); 7.50 (m, 4H, Ph); 3.10 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 167.1 (C5); 165.4 (C3); 139.3 (Ci); 132.4 (2Co); 121.1 (2Cm); 115.7 (Cp); 30.0 (CH_3). MS (ESI, positive): 286 (M^+). Anal. Calcd for $C_9H_8BrN_3OS$: C, 37.78; H, 2.82; N, 14.68; S, 11.21. Found: C, 37.91; H, 2.65; N, 14.88; S, 11.25.

5.1.7.9. 2-Methyl-5-phenylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (25). Reagents: compound **9** (209 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 7 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 93 mg (45%). Mp 229–231 °C. ^{1}H NMR (DMSO-d_6) δ 8.50 (br s, 1H, NH); 7.60 (m, 5H, Ph); 3.00 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 166.2 (C5); 164.4 (C3); 140.3 (Ci); 130.1 (2Cm); 128.0 (Cp); 120.2 (2Co); 29.2 (CH_3). MS (ESI, positive): 207 (M^+). Anal. Calcd for $C_9H_9N_3OS$: C, 52.16; H, 4.38; N, 20.27; S, 15.47. Found: C, 52.53; H, 4.38; N, 19.95; S, 15.85.

5.1.7.10. 2-Methyl-5-phenetylamino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (26). Reagents: compound **10** (237 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in methanol, 6 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 140 mg (54%). Mp 170–172 °C. ^{1}H NMR (DMSO-d_6) δ 9.20 (br s, 1H, NH); 7.50–7.20 (m, 5H, Ph); 5.30 (m, 2H, CH_2Ph); 3.61 (m, 2H, CH_2NH); 2.82 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 168.5 (C5); 163.2 (C3); 138.5 (Ci); 128.7 (2Cm); 128.4 (2Co); 126.4 (Cp); 45.1 (CH_2NH); 34.2 (CH_2Ph); 30.0 (CH_3). MS (EI). *m*/*z* 235 (M^+, 9). Anal. Calcd for $C_{11}H_{13}N_3OS$: C, 56.15; H, 5.57; N, 17.86; S, 13.63. Found: C, 56.47; H, 5.62; N, 18.07; S, 13.94.

5.1.7.11. 5-Ethoxycarbonylmethylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (27). Reagents: compound **11** (220 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in ethanol, 6 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 130 mg (60%). Mp 162–164 °C. ^{1}H NMR (DMSO-d_6) δ 8.50 (br s, 1H, NH); 4.20 (c, 2H, J = 7.0 Hz, CH_2CH$_3$); 3.85 (d, 2H, J = 5.1 Hz, CH_2NH); 1.25 (t, 3H, J = 7.0 Hz, CH$_2$CH_3); 3.10 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 168.9 (C5); 156.1 (C3); 172.9 (CO_2); 61.2 (CH_2CH$_3$); 45.2 (CH_2CO_2Et); 14.0 (CH$_2$CH$_3$); 29.3 (CH_3). MS (EI): *m*/*z* 217 (M^+, 52). Anal. Calcd for $C_7H_{11}N_3O_3S$: C, 38.70; H, 5.10; N, 19.34; S, 14.76. Found: C, 38.41; H, 5.29; N, 19.60; S, 14.73.

5.1.7.12. 5-Ethoxycarbonylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (28). Reagents: compound **12** (205 mg, 1 mmol), NBS (222 mg, 1.25 mmol). Conditions: reflux in ethanol, 24 h. Purification: silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 61 mg (30%). Mp 180–182 °C. ^{1}H NMR (DMSO-d_6) δ 11.36 (br s, 1H, NH); 3.99 (c, 2H, J = 7.1 Hz, OCH_2CH$_3$); 1.13 (t, 3H, J = 7.1 Hz, CH$_2$CH_3); 3.00 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 150.1 (C5); 148.7 (C3); 172.4 (CO_2); 59.7 (OCH_2CH$_3$); 14.1 (CH$_2$CH$_3$); 27.2 (CH_3). MS (EI): *m*/*z* 203 (M^+, 49). Anal. Calcd for $C_6H_9N_3O_3S$: C, 35.46; H, 4.46; N, 20.68; S, 15.78. Found: C, 35.21; H, 4.57; N, 20.60; S, 15.50.

5.1.8. Thiobiuret (29). Compound **1** (223 mg, 1 mmol) was solved in 1 ml of an aq solution of NaOH 1 N and 2 ml of absolute ethanol and the reaction mixture was heated at 50 °C during 10 min. After that, 10 ml of distilled water was added and the reaction mixture was neutralized with HCl 5%. The mixture was extracted with AcOEt (3× 10 ml), the resulting organic phases were dried over anhydrous sodium sulfate, and the solvent was removed by evaporation under reduced pressure. Yield: 101 mg (85%). ^{1}H NMR (DMSO-d_6) δ 9.69 (br s, 1H, CSN*H*CO); 9.47 (br s, 1H, CSNH_2); 8.93 (br s, 1H, CSNH_2); 6.90 (br s, 1H, CONH_2); 6.33 (br s, 1H, CONH_2). ^{13}C NMR (DMSO-d_6) δ 181.7 (C2); 155.1 (C4). MS (EI): *m*/*z* 119 (M^+, 100).

5.1.9. 5-Methyl-2-thiobiuret (30). Compound **2** (237 mg, 1 mmol) was solved in (1 ml, 1 mmol) of an aq solution of NaOH 1 N and 2 ml of absolute ethanol and the reaction mixture was heated at 50 °C during 10 min. After that, 10 ml of distilled water was added, and the reaction mixture was neutralized with HCl 5%. The mixture was extracted with AcOEt (3× 10 ml), the resulting organic phases were dried over anhydrous sodium sulfate and the solvent was removed by evaporation under reduced pressure. Yield: 97 mg (73%). ^{1}H NMR (DMSO-d_6) δ 9.74 (br s, 1H, CSN*H*CO); 9.41 (br s, 1H, CSNH_2); 8.89 (br s, 1H, CSNH_2); 6.65 (bm, 1H, N*H*CH_3); 2.60 (d, 3H, J = 4.6 Hz, C*H*$_3$NH). ^{13}C NMR (DMSO-d_6) δ 181.7 (C2); 155.0 (C4); 26.1 (CH_3). MS (EI): *m*/*z* 134 (M^+, 100).

5.1.10. 5-Amino-3-oxo-2,3-dihydro-1,2,4-thiadiazole (31).[20] Compound **29** (119 mg, 1 mmol) was solved in aq NaOH 2N at 0 °C. After that, H_2O_2 (1 mmol) was added and the reaction mixture was refluxed for 2 h. Then it was acidified with HCl concd to pH 4.5 and a white solid precipitated. The solid was filtered and purified by silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 58 mg (50%). Mp 220–222 °C (lit.[20] 220–222 °C). ^{1}H NMR (DMSO-d_6) δ 9.41 (br s, 2H, NH_2); 8.00 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 176.2 (C5); 168.40 (C3). MS (ESI, positive): 117 (M^+). Anal. Calcd for $C_2H_3N_3OS$: C, 20.51; H, 2.58; N, 35.87; S, 27.38. Found: C, 21.00; H, 2.40; N, 36.07; S, 27.21.

5.1.11. 5-Amino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (32). Compound **30** (134 mg, 1 mmol) was solved in 3 ml of CH_2Cl_2 and 6 ml of AcOEt at 0 °C and a solution Br_2/AcOEt 0.5 M (4 ml, 2 mmol) was added dropwise. The reaction mixture was left stirring at 4 °C for 12 h. The solvent was removed by evaporation under reduced pressure and the crude purified by silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 73 mg (55%). Mp 267–269 °C. ^{1}H NMR (DMSO-d_6) δ 9.43 (br s, 1H, NH); 3.01 (s, 3H, C*H*$_3$). ^{13}C NMR (DMSO-d_6) δ 170.5 (C5); 159.50 (C3); 30.3 (CH_3). MS (ESI, positive): 132 (M^+). Anal. Calcd for $C_3H_5N_3OS$: C, 27.47; H, 3.84; N, 32.04; S, 24.45. Found: C, 27.25; H, 3.46; N, 31.90; S, 24.21.

5.1.12. 1-(2-Hydroxyethyl)-2-methyl-2-thiobiuret (33). Compound **11** (219 mg, 1 mmol) was solved in 12 ml of absolute ethanol and LiCl (127 mg, 3 mmol) and $NaBH_4$ (113 mg, 3 mmol) were added. The reaction mixture was stirred at room temperature during 24 h and then it was acidified to pH 3 with an aq solution of citric acid 20%. The mixture was extracted with AcOEt (3× 10 ml), the resulting organic phases were dried over anhydrous sodium sulfate, and the solvent was removed by evaporation under reduced pressure. Yield: 175 mg (99%). ^{1}H NMR (DMSO-d_6) δ 10.56 (br s, 1H, CH_2N*H*CS); 9.70 (br s, 1H, CSN*H*CO); 6.18 (br s, 1H, CON*H*CH_3); 3.86 (s, 4H, [(CH_2)$_2$]); 3.05 (br s, 1H, OH); 2.79 (d, 3H, J = 4.7 Hz, C*H*$_3$NH). ^{13}C NMR (DMSO-d_6) δ 180.2 (C2); 155.1 (C4); 60.7 (*C*H_2OH); 41.2 (*C*H_2NH); 26.4 (CH_3). MS (EI): *m*/*z* 177 (M^+, 37).

5.1.13. 5-(2-Hydroxyethyl)amino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (34). Compound **33** (177 mg, 1 mmol) was solved in 3 ml of CH_2Cl_2 and 6 ml of AcOEt at 0 °C and a solution Br_2/AcOEt 0.5 M (4 ml, 2 mmol) was added dropwise. The reaction mixture was left stirring at 4 °C for 12 h. The solvent was removed by evaporation under reduced pressure and the crude purified by silica gel column chromatography using dichloromethane/methanol (30:1) as eluent. Yield: 114 mg (65%). Mp 158–160 °C. ^{1}H NMR (DMSO-d_6) δ 8.80 (br s, 1H, NH); 3.38 (br s, 1H, O*H*); 3.51 (m, 4H, CH_2CH_2); 3.01 (s, 3H, C*H*$_3$). ^{13}C NMR (DMSO-d_6) δ 171.1 (C5); 160.8 (C3); 61.0 (*C*H_2OH); 40.8 (NH*C*H_2); 29.7 (CH_3). MS (EI): *m*/*z* 175 (M^+, 24). Anal. Calcd for $C_5H_9N_3O_2S$: C, 34.28; H, 5.18; N, 23.98; S, 18.30. Found: C, 34.43; H, 4.85; N, 24.11; S, 18.22.

5.1.14. 1-Carboxymethyl-5-methyl-2-thiobiuret (35). Compound **11** (219 mg, 1 mmol) was solved in 10 ml of absolute ethanol and (1.2 ml, 1.2 mmol) of an aq solution of NaOH 1 N was added. The reaction mixture was refluxed for 2 h and then after allowing it to room temperature, it was neutralized with HCl 1 N and extracted with AcOEt (3× 10 ml). The resulting organic phases were dried over anhydrous sodium sulfate and the solvent was removed by evaporation under reduced pressure. Yield: 182 mg (95%). ^{1}H NMR (DMSO-d_6) δ 10.67 (br s, 1H, CH_2N*H*CS); 9.56 (br s, 1H, CSN*H*CO); 6.59 (br s, 1H, CON*H*CH_3); 12.70 (s, 1H, CO_2*H*); 4.36 (s, 2H, CH_2); 2.73 (d, 3H, J = 4.7 Hz, C*H*$_3$NH). ^{13}C NMR (DMSO-d_6) δ 182.6 (C2); 156.7 (C4); 172.1 (*C*O_2H); 47.3 (*C*H_2); 26.3 (CH_3). MS (ESI, positive): 192.1 (M^*).

5.1.15. 5-Carboxymethylamino-2-methyl-3-oxo-2,3-dihydro-1,2,4-thiadiazole (36). Compound **35** (191 mg, 1 mmol) was solved in 3 ml of CH_2Cl_2 and 6 ml of AcOEt at 0 °C and a solution Br_2/AcOEt 0.5 M (4 ml, 2 mmol) was added dropwise. The reaction mixture was left stirring at 4 °C for 12 h. The resulting precipitate was isolated by filtration, washed with acetone, and dried in vacuo. Yield: 76 mg (40%). Mp 275–277 °C. ^{1}H NMR (DMSO-d_6) δ 8.86 (br s, 1H, NH); 4.10 (s, 2H, CH_2CO_2H); 3.03 (s, 3H, C*H*$_3$). ^{13}C NMR (DMSO-d_6) δ 172.0 (C5); 161.2 (C3); 169.7 (*C*O_2H); 46.6 (*C*H_2CO_2H); 31.1 (CH_3). MS (ESI, positive): 190.1 (M^+). Anal. Calcd for $C_5H_7N_3O_3S$: C, 31.74; H, 3.73; N, 22.21; S, 16.95. Found: C, 31.48; H, 3.71; N, 22.26; S, 15.97.

5.1.16. General procedure for the synthesis of 5-imino-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole derivatives (37–48). To a solution of 1 mmol of **17**–**19**, **21**, or **24** in anhydrous DMF was added 1 mmol of sodium hydride. The reaction mixture was stirred at room temperature during 1 h. Then, 1 mmol of the appropriate alkylating agent was added and the reaction mixture was refluxed for 4–20 h. depending on the product. The solvent was removed under reduced pressure and the resulting crude was purified as detailed in each case.

5.1.16.1. 5-Benzoylimino-2,4-dicyanomethyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (37) and 5-benzoylimino-4-cyanomethyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (38). Reagents: compound **17** (221 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), chloroacetonitrile (0.06 ml, 1 mmol). Conditions: reflux, 24 h. Purification: silica gel column chromatography using hexane/AcOEt (3:1). Two compounds were isolated: 1st fraction: compound **37**. Yield: 93 mg (31%). Mp 150–152 °C. ^{1}H NMR (DMSO-d_6) δ 8.24 (dd, 2Ho, J = 7.1 and 1.5 Hz); 7.68 (dd, 1Hp, J = 7.8 and 1.3 Hz); 7.58 (dd, 2Hm, J = 7.8 and 7.1 Hz); 4.85 (s, 2H, CH_2); 5.16 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 169.5 (C5); 150.9 (C3); 178.1 (*C*OPh); 134.1 (Ci); 132.3 (Cp); 129.6 (2Co); 128.8 (2Cm); 115.7 (CH_2*C*N); 31.6 (*C*H_2CN); 114.6 (CH_2*C*N); 32.2 (*C*H_2CN). MS (EI): *m*/*z* 299 (M^+, 12). Anal. Calcd for $C_{13}H_9N_5O_2S$: C, 52.17; H, 3.03; N, 23.40; S, 10.71. Found: C, 52.23; H, 3.17; N, 23.76; S, 10.50. 2nd fraction: compound **38**. Yield: 91 mg (35%). Mp 129–131 °C. ^{1}H NMR (DMSO-d_6) δ 8.20 (dd, 2Ho, J = 7.5 and 1.5 Hz); 7.51 (m, 1Hp); 7.40–7.30 (m, 2Hm); 3.50 (br s, 1H, NH); 5.20 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6) δ 170.0 (C5); 151.9 (C3); 179.0 (*C*OPh); 135.2 (Ci); 133.2 (Cp); 129.4 (2Co); 128.6 (2Cm); 123.1 (CH_2*C*N); 35.0 (*C*H_2CN). MS (EI): *m*/*z* 260 (M^+, 19). Anal. Calcd for $C_{11}H_8N_4O_2S$: C, 50.76; H, 3.10; N, 21.53; S, 12.32. Found: C, 50.39; H, 3.17; N, 21.28; S, 12.47.

5.1.16.2. 5-Benzoylimino-2,4-dibenzyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (39) and 5-benzoylimino-4-benzyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (40). Reagents: compound **17** (221 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), benzylbromide (0.12 ml, 1 mmol). Conditions: reflux, 24 h. Purification: silica gel column chromatography using hexane/AcOEt (3:1). Two compounds were isolated: 1st fraction: compound **39**. Yield: 121 mg (30%). Mp 162–164 °C. ^{1}H NMR (DMSO-d_6) δ 8.26 (dd, 2Ho, J = 7.0 and 1.5 Hz); 7.60 (m, Hp); 7.40–7.50 (m, 2Hm); 7.40–7.20 (m, 5H, CH_2Ph); 5.25 (s, 2H, C*H*$_2$Ph); 7.40–7.20 (m, 5H, Ph); 4.85 (s, 2H, C*H*$_2$Ph). ^{13}C NMR (DMSO-d_6) δ 169.5 (C5); 152.1 (C3); 177.6 (*C*OPh); 133.6 (Ci); 133.3 (Cp); 129.4 (2Co); 129.1 (2Cm); 136.3 (Ci); 128.9 (2Cm); 128.2 (Cp); 128.6 (2Co); 48.11 (*C*H_2Ph); 135.9 (Ci); 128.8 (2Cm); 128.3 (2Co); 128.1 (Cp); 46.9 (*C*H_2Ph). MS (EI): *m*/*z* 402 (M^+, 19). Anal. Calcd for $C_{23}H_{19}N_3O_2S$: C, 68.81; H, 4.77; N, 10.47; S, 7.99. Found: C, 68.40; H, 4.71; N, 10.05; S, 7.61. 2nd fraction: compound **40**. Yield: 90 mg (29%). Mp 145–147 °C. ^{1}H NMR (DMSO-d_6) δ 7.8–8.0 (m, 2Ho); 7.4–7.6 (m, Hp); 7.2–7.4 (m, 2Hm); 7.1–7.0 (m, 5H, Ph); 4.92 (s, 2H, C*H*$_2$Ph). ^{13}C NMR (DMSO-d_6) δ 171.6 (C5); 153.6 (C3); 177.2 (*C*OPh); 134.0 (Ci); 133.3 (Cp); 129.0 (2Co); 128.8 (2Cm); 136.2 (Ci); 128.6 (2Cm); 128.3 (2Co); 128.1 (Cp); 47.7 (*C*H_2Ph). MS (EI): *m*/*z* 312 (M^+, 17). Anal. Calcd for $C_{16}H_{13}N_3O_2S$: C, 61.72; H, 4.21; N, 13.50; S, 10.30. Found: C, 61.98; H, 4.49; N, 13.84; S, 10.59.

5.1.16.3. 5-Benzoylimino-4-benzyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (41). Reagents: compound **18** (235 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), benzylbromide (0.12 ml, 1 mmol). Conditions: room temperature, 36 h. Purification: silica gel column chromatography using hexane/AcOEt (3:1). Yield: 212 mg (65%). Mp 150–152 °C. ^{1}H NMR (DMSO-d_6) δ 8.20–8.10 (m, 2Ho); 7.50–7.40 (m, 1Hp); 7.40–7.30 (m, 2Hm); 3.15 (s, 3H, CH_3); 7.20–7.10 (m, 5H, Ph); 5.10 (s, 2H, C*H*$_2$ Ph). ^{13}C NMR (DMSO-d_6) δ 169.2 (C5); 151.9 (C3); 177.2 (*C*OPh); 133.2 (Ci); 133.1 (Cp); 129.1 (2Co); 128.7 (2Cm); 30.0 (CH_3); 135.6 (Ci); 128.5 (2Cm); 128.1 (2Co); 127.8 (Cp); 48.1 (*C*H_2Ph). MS (EI): *m*/*z* 325 (M^+, 98). Anal. Calcd for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91; S, 9.85. Found: C, 63.06; H, 4.73; N, 12.72; S, 10.04.

5.1.16.4. 5-Benzoylimino-2,4-dimethyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (42). Reagents: compound **18** (235 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), methyliodide (0.062 ml, 1 mmol). Conditions: reflux, 24 h. Purification: silica gel column chromatography using hexane/AcOEt (1:1) as eluent. Yield: 50 mg (45%). Mp 200–202 °C. ^{1}H NMR (DMSO-d_6) δ 8.10–8.00 (m, 2Ho); 7.50–7.40 (m, 1Hp); 7.30–7.20 (m, 2Hm); 3.21 (s, 3H, CH_3); 3.42 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 165.0 (C5); 158.3 (C3); 176.1 (*C*OPh); 136.1 (Ci); 134.8 (Cp); 130.0 (2Co); 129.1 (2Cm); 29.0 (CH_3); 28.4 (NCH_3). MS (EI): *m*/*z* 249 (M^+, 93). Anal. Calcd for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86; S, 12.86. Found: C, 53.17; H, 4.67; N, 17.12; S, 13.17.

5.1.16.5. 5-Benzoylimino-4-ethoxycarbonylmethyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (43). Reagents: compound **18** (235 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), ethyl bromoacetate (0.11 ml, 1 mmol). Conditions: reflux, 40 h. Purification: silica gel column chromatography using hexane/AcOEt (3:2) as eluent. Yield: 212 mg (66%). Mp 166–168 °C. ^{1}H NMR (DMSO-d_6) δ 8.17 (m, 2Ho); 7.75 (m, 1Hp); 7.65 (m, 2Hm); 3.42 (s, 3H, CH_3); 4.95 (s, 2H, C*H*$_2$CO); 4.25 (c, 2H, J = 7.1 Hz, C*H*$_2$CH$_3$); 1.35 (t, 3H, J = 7.1 Hz, CH$_2$C*H*$_3$). ^{13}C NMR (DMSO-d_6) δ 165.3 (C5); 158.3 (C3); 175.2 (*C*OPh); 135.0 (Ci); 133.2 (Cp); 129.0 (2Co); 128.2 (2Cm); 28.4 (CH_3); 171.4 (CO_2); 59.4 (*C*H_2CH$_3$), 46.2 (*C*H_2CO$_2$); 14.1 (CH$_2$*C*H_3). MS (EI): *m*/*z* 321 (M^+, 93). Anal. Calcd for $C_{14}H_{15}N_3O_4S$: C, 52.33; H, 4.70; N, 13.08; S, 9.98. Found: C, 52.56; H, 4.78; N, 13.39; S, 9.89.

5.1.16.6. 5-Benzoylimino-4-cyanomethyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (44). Reagents: compound **18** (235 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), chloroacetonitrile (0.063 ml, 1 mmol). Conditions: reflux, 15 h. Purification: silica gel column chromatography using hexane/AcOEt (3:2). Yield:

110 mg (40%). Mp 219–221 °C. ^{1}H NMR (DMSO-d_6) δ 8.00 (dd, 2Ho, J = 7.0 and 1.4 Hz); 7.74 (dd, 1Hp, J = 7.2 and 1.4 Hz); 7.45 (dd, 2Hm, J = 7.2 and 7.0 Hz); 3.50 (s, 3H, CH_3); 5.22 (s, 2H, CH_2CN). ^{13}C NMR (DMSO-d_6) δ 166.2 (C5); 158.6 (C3); 174.0 (*C*OPh); 135.2 (Ci); 133.1 (Cp); 129.1 (2Co); 128.8 (2Cm); 28.6 (CH_3); 35.2 (CH_2CN); 120.1 (CN). MS (EI): *m*/*z* 274 (M^+, 85). Anal. Calcd for $C_{12}H_{10}N_4O_2S$: C, 52.54; H, 3.67; N, 20.43; S, 11.69. Found: C, 52.31; H, 3.74; N, 20.64; S, 11.88.

5.1.16.7. 5-Benzoylimino-2-benzyl-4-ethoxycarbonylmethyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (45). Reagents: compound **19** (311 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), ethyl bromoacetate (0.11 ml, 1 mmol). Conditions: reflux, 5 h. Purification: silica gel column chromatography using hexane/AcOEt (1:1) as eluent. Yield: 160 mg (50%). Mp 215–217 °C. ^{1}H NMR (DMSO-d_6) δ 8.18 (dd, 2Ho, J = 7.6 and 1.5 Hz); 7.68 (dd, 1Hp, J = 7.8 and 1.3 Hz); 7.58 (dd, 2Hm, J = 7.9 and 7.5 Hz); 7.4–7.3 (m, 5H, Ph); 4.80 (s, 2H, CH_2Ph); 4.85 (s, 2H, CH_2CO$_2$Et); 4.25 (c, 2H, J = 7.1 Hz, CH_2CH$_3$); 1.28 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). ^{13}C NMR (DMSO-d_6) δ 169.5 (C5); 151.9 (C3); 178.4 (*C*OPh); 135.3 (Ci); 133.2 (Cp); 129.7 (2Co); 128.4 (2Cm); 133.2 (Ci); 128.9 (2Co); 128.3 (2Cm); 133.1 (Cp); 47.7 (*C*H$_2$Ph); 166.5 (*C*O$_2$); 62.1 (CO$_2$*C*H$_2$CH$_3$); 45.3 (*C*H$_2$CO$_2$); 14.1 (CH$_2$*C*H$_3$). MS (EI): *m*/*z* 397 (M^+, 80). Anal. Calcd for $C_{20}H_{19}N_3O_4S$: C, 60.44; H, 4.82; N, 10.57; S, 8.07. Found: C, 60.19; H, 4.93; N, 10.49; S, 7.79.

5.1.16.8. 4-Benzyl-5-benzylimino-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (46). Reagents: compound **21** (221 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), benzylbromide (0.12 ml, 1 mmol). Conditions: reflux, 24 h. Purification: silica gel column chromatography using hexane/AcOEt (3:1). Yield: 124 mg (40%). Mp 220–222 °C. ^{1}H NMR (DMSO-d_6) δ 7.40–7.50 (m, 5H, Ph); 4.87 (s, 2H, CH_2Ph); 3.03 (s, 3H, CH_3); 7.30–7.10 (m, 5H, Ph); 4.19 (s, 2H, CH_2Ph). ^{13}C NMR (DMSO-d_6) δ 166.1 (C5); 158.2 (C3); 137.2 (Ci); 129.4 (2Co); 128.2 (2Cm); 126.1 (Cp); 50.2 (*C*H$_2$Ph); 31.8 (CH_3); 140.1 (Ci); 128.0 (2Cm); 126.8 (2Co); 124.6 (Cp); 47.8 (*C*H$_2$Ph). MS (EI): *m*/*z* 311 (M^+, 75). Anal. Calcd for $C_{17}H_{17}N_3OS$: C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.80; H, 5.57; N, 13.77; S, 10.19.

5.1.16.9. 5-Benzylimino-4-ethoxycarbonylmethyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (47). Reagents: compound **21** (221 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), ethyl bromoacetate (0.11 ml, 1 mmol). Conditions: reflux, 4 h. Purification: silica gel column chromatography using hexane/AcOEt (3:1). Yield: 107 mg (35%) as an oil. ^{1}H NMR (DMSO-d_6) δ 7.20–7.10 (m, 5H, Ph); 4.39 (br s, 2H, CH_2Ph); 3.18 (s, 3H, CH_3); 4.22 (br s, 2H, CH_2CO$_2$Et); 4.14 (c, 2H, J = 7.0 Hz, CH_2CH$_3$); 1.21 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). ^{13}C NMR (DMSO-d_6) δ 167.7 (C5); 165.8 (C3); 133.7 (Ci); 128.74 (2Cm); 127.9 (2Co); 123.5 (Cp); 49.5 (*C*H$_2$Ph); 30.1 (CH_3); 172.1 (CO$_2$); 61.6 (*C*H$_2$CH$_3$); 47.0 (*C*H$_2$CO$_2$); 13.9 (CH$_2$*C*H$_3$). MS (EI): *m*/*z* 307 (M^+, 11). Anal. Calcd for $C_{14}H_{17}N_3O_3S$: C, 54.71; H, 5.57; N, 13.67; S, 10.43. Found: C, 54.87; H, 5.81; N, 13.75; S, 10.68.

5.1.16.10. 5-*p*-Bromophenylimino-2,4-dimethyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (48). Reagents: compound **24** (286 mg, 1 mmol), sodium hydride (24 mg, 1 mmol), methyliodide (0.062 ml, 1 mmol). Conditions: reflux, 7 h. Purification: silica gel column chromatography using hexane/AcOEt (3:1). as eluent. Yield: 126 mg (42%). Mp 120–122 °C. ^{1}H NMR (DMSO-d_6) δ 7.52 (d, 2H, J = 8.5 Hz); 6.92 (d, 2H, J = 8.5 Hz); 3.10 (s, 3H, CH_3); 3.32 (s, 3H, NCH_3). ^{13}C NMR (DMSO-d_6) δ 167.0 (C5); 164.1 (C3); 147.7 (Ci); 132.6 (2Cm); 123.1 (2Co); 120.2 (Cp); 31.8 (CH_3); 30.0 (NCH_3). MS (EI): *m*/*z* 301 (M^+, 100). Anal. Calcd for $C_{10}H_{10}BrN_3OS$: C, 40.01; H, 3.36; N, 14.00; S, 10.68. Found: C, 40.06; H, 3.23; N, 14.14; S, 10.92.

5.1.17. 5-Benzoylimino-4-carboxymethyl-2-methyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (49). A solution of compound **43** (321 mg, 1 mmol) in MeOH (2 ml) was treated with aq. NaOH 1 N (1 ml, 1 mmol). The reaction mixture was heated at 50 °C for 10 min. After that, 10 ml of distilled H_2O was added, neutralized with HCl 5%, and extracted with AcOEt (3× 10 ml). The organic phases were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. Yield: 261 mg (89%). Mp 234–236 °C. ^{1}H NMR (DMSO-d_6) δ 8.20 (dd, 2Ho, J = 6.9 and 1.6 Hz); 7.60 (dd, 1Hp, J = 7.2 and 1.6 Hz); 7.35 (dd, 2Hm, J = 7.2 and 6.9 Hz); 3.20 (s, 3H, CH_3); 4.73 (s, 2H, CH_2CO$_2$H). ^{13}C NMR (DMSO-d_6) δ 166.1 (C5); 158.4 (C3); 175.6 (*C*OPh); 137.0 (Ci); 135.2 (Cp); 129.8 (2Co); 128.4 (2Cm); 30.1 (CH_3); 170.2 (*C*O$_2$H); 53.0 (*C*H$_2$CO$_2$H). MS (EI): *m*/*z* 293 (M^+, 88). Anal. Calcd for $C_{12}H_{11}N_3O_4S$: C, 49.14; H, 3.78; N, 14.33; S, 10.93. Found: C, 49.41; H, 3.96; N, 14.07; S, 11.17.

5.1.18. 5-Benzoylimino-2-benzyl-4-carboxymethyl-3-oxo-2,3,4,5-tetrahydro-1,2,4-thiadiazole (50). A solution of compound **45** (397 mg, 1 mmol) in MeOH (2 ml) was treated with aq NaOH 1 N (1 ml, 1 mmol). The reaction mixture was heated at 50 °C for 10 min. After that, 10 ml of distilled H_2O was added, neutralized with HCl 5% and extracted with AcOEt (3× 10 ml). The organic phases were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. Yield: 350 mg (95%). Mp 236–238 °C. ^{1}H NMR (DMSO-d_6) δ 8.40 (dd, 2H, J = 7.0 and 1.4 Hz); 7.70 (dd, 1H, J = 7.4 and 1.4 Hz); 7.30 (dd, 2H, J = 7.4 and 7.0 Hz); 7.30–7.10 (m, 5H, Ph); 5.30 (s, 2H, CH_2Ph); 4.68 (s, 2H, CH_2CO$_2$H). ^{13}C NMR (DMSO-d_6) δ 168.8 (C5); 154.0 (C3); 177.2 (*C*OPh); 134.0 (Ci); 133.3 (Cp); 129.0 (2Co); 128.8 (2Cm); 133.2 (Ci); 133.1 (Cp); 129.4 (2Co); 128.5 (2Cm); 47.0 (*C*H$_2$Ph); 171.3 (*C*O$_2$H); 52.4 (*C*H$_2$CO$_2$H). MS (EI): *m*/*z* 370 (M^+, 20). Anal. Calcd for $C_{18}H_{15}N_3O_4S$: C, 58.53; H, 4.09; N, 11.38; S, 8.68. Found: C, 58.26; H, 3.89; N, 11.13; S, 8.90.

5.1.19. 3-(4-Methylphenyl)-5-[(4-methylphenyl)-sulfonyl]-1,2,4-thiadiazole (51). A solution of 4-methylbenzenesulfonyl cyanide (0.56 g, 3.1 mmol) in decaline (8 ml)

was heated to 140–160 °C where 3-(4-methylphenyl)-5-oxo-4,1,2-oxathiazole[36] (0.4 g, 2.1 mmol) was added slowly. The reaction mixture was stirred for 2.5 h. The reaction mixture was allowed to cool to room temperature and a cream solid was collected, washed with hexane, and recrystallized from hexane/AcOEt (8:2). Yield: 0.51 g (73%). Mp 125–126 °C. ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.28 (d, 2H, J = 8.1 Hz), 7.44 (d, 2H, J = 8.2 Hz); 8.08 (d, 2H, J = 8.4 Hz), 8.14 (d, 2H, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ 180.3 (C5), 173.8 (C3), 136.7 (Ci), 134.8 (Cp), 131.4 (Cm), 129.9 (Ci), 129.5 (Co), 128.0 (Cm), 130.1 (Co), 126.1 (Cp), 23.8 (CH_3), 24.6 (CH_3). MS (EI): m/z 330 (M^+, 73%), 239 (15%), 176 (39%), 155 (100%). Anal. Calcd for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; N, 8.48; S, 19.41. Found: C, 58.50; H, 4.08; N, 8.78; S, 19.16.

5.1.20. 3-(4-Methylphenyl)-5-oxo-4,5-dihydro-1,2,4-thiadiazole (52). To a mixture of potassium *tert*-butoxide in 25 ml of *tert*-butyl alcohol was added thiadiazole **51** (268 mg, 0.6 mmol) and refluxed for 15 min. The reaction mixture was diluted with 30 ml of AcOEt, washed with brine (2× 50 ml), dried over anhydrous sodium sulfate and the solvent was removed by evaporation under reduced pressure. The solid was triturated with Et_2O/hexane to yield 132 mg (71%). Mp 216–217 °C; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 7.31 (d, 2H, ArH, J = 8.3 Hz), 7.92 (d, 2H, ArH, J = 8.4 Hz), 11.10 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ 178.8 (C3), 153.9 (C5), 132.4 (Cp), 129.4 (2Cm), 128.4 (2Co), 126.1 (Ci), 24.2 (CH_3). MS (ESI, positive): 193.0 (MH^+). Anal. Calcd for $C_9H_8N_2OS$: C, 56.23; H, 4.19; N, 14.57; S, 16.68. Found: C, 56.09; H, 3.97; N, 14.33; S, 16.29.

5.1.21. 4-Ethoxycarbonylmethyl-3-(4-methylphenyl)-1,2,4-thiadiazole (53). A solution of **52** (0.1 g, 0.52 mmol) in 5 ml of DMF was treated with NaH (21 mg, 0.52 mmol) at room temperature for 30 min. Then, ethyl 2-bromoacetate (0.6 ml, 0.52 mmol) was added and the resulting solution was heated for 1 h at 80 °C. The reaction was cooled to room temperature, mixed with brine solution, and extracted with AcOEt (3× 20 ml), the resulting organic phases were dried over anhydrous sodium sulfate and the solvent was removed by evaporation under reduced pressure. The crude was purified by silica gel column chromatography using hexane/AcOEt (9:1) as eluent. Yield (65 mg, 45%). Mp 68–69 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 4.09 (q, 2H, CH_2), 7.24 (d, 2H, J = 8.3 Hz), 7.76 (d, 2H, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6) δ 176.7 (C5), 168.2 (C3), 131.8 (Cp), 129.1 (2Cm), 128.6 (2Co), 126.3 (Ci), 62.4 (CH_2CH_3), 46.8 (CH_2CO_2Et), 14.2 (CH_2CH_3). MS (ESI, positive) 279 (M^+). Anal. Calcd for $C_{13}H_{14}N_2O_3S$: C, 56.10; H, 5.07; N, 10.06; S, 11.52. Found: C, 55.82; H, 4.73; N, 9.85; S, 11.21.

5.1.22. General procedure for the synthesis of 3,5-diamino-1,2,4-thiadiazoles (56–59). [27] Over a suspension of 1 mmol of monosubstituted thiourea in chloroform 1 mmol of DIB was added. The reaction mixture was stirred at room temperature for 4 h. The resulting precipitate was filtered off and purified as indicated in each case.

5.1.22.1. 3,5-Diethylamino-1,2,4-thiadiazole (56). Reagents: ethylthiourea (104 mg, 1 mmol), DIB (322 mg, 1 mmol). Conditions: room temperature, 3 h. Purification: silica gel column chromatography using hexane/AcOEt (10:1). Yield: 95 mg (55%). Mp 198–200 °C. ^{1}H NMR (DMSO-d_6) δ 12.23 (br s, 1H, NH); 8.61 (br s, 1H, NH); 3.15 (c, 2H, CH_2); 1.07 (t, 3H, CH_3); 3.22 (c, 2H, CH_2); 1.13 (t, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 182.7 (C5); 168.4 (C3); 38.4 (NCH_2); 14.6 (CH_3); 41.1 (NCH_2); 15.2 (CH_3). MS (ESI, positive): 173.0 (M^+). Anal. Calcd for $C_6H_{12}N_4S$: C, 41.84; H, 7.02; N, 32.53; S, 18.62. Found: C, 42.17; H, 7.09; N, 32.60; S, 18.64.

5.1.22.2. 3,5-Diacetylamino-1,2,4-thiadiazole (57). [27] Reagents: acetylthiourea (118 mg, 1 mmol), DIB (322 mg, 1 mmol). Conditions: room temperature, 3 h. Purification: Recrystallization from ethanol. Yield: 90 mg (45%). Mp 340–342 °C. (lit.[27] 336–338 °C). ^{1}H NMR (DMSO-d_6) δ 12.41 (br s, 1H, NH); 10.75 (br s, 1H, NH); 2.08 (s, 3H, C*H*3); 2.21 (s, 3H, C*H*3). ^{13}C NMR (DMSO-d_6) δ 174.4 (C5); 168.0 (C3); 170.8 (CO); 23.8 (CH_3); 170.8 (CO); 22.10 (CH_3). MS (ESI, positive): 201.0 (M^+). Anal. Calcd for $C_6H_8N_4O_2S$: C, 35.99; H, 4.03; N, 27.98; S, 16.02. Found: C, 36.04; H, 4.27; N, 28.25; S, 16.13.

5.1.22.3. 3,5-Dianilin-1,2,4-thiadiazole (58). [27] Reagents: phenylthiourea (152 mg, 1 mmol), DIB (322 mg, 1 mmol). Conditions: room temperature, 4 h. Purification: silica gel column chromatography using hexane/AcOEt (2:1) as eluent. Yield: 121 mg (45%). Mp 203–205 °C. (lit.[27] 200–202 °C).^{1}H NMR (DMSO-d_6) δ 9.34 (br s, 1H, NHR_1); 5.62 (br s, 1H, NHR_2); 7.16 (t, 2H, J = 7.5 Hz, arom); 6.80 (t, 1H, J = 7.5 Hz, arom); 7.70 (d, 2H, J = 7.5 Hz, arom); 7.55 (dd, 2H, J = 7.5 Hz, arom); 7.35 (t, 1H, J = 7.5 Hz, arom); 7.00–7.10 (m, 2H, J = 7.5 Hz, arom). ^{13}C NMR (DMSO-d_6) δ 175.1 (C5); 159.6 (C3); 141.5 (Ci); 128.3 (Cm); 119.8 (Cp); 116.5 (Co); 146.4 (Ci); 129.4 (Cm); 129.3 (Cp); 122.8 (Co). MS (ESI, positive): 269.0 (M^+). Anal. Calcd for $C_{14}H_{12}N_4S$: C, 62.66; H, 4.51; N, 20.88; S, 11. 95. Found: C, 62.97; H, 4.46; N, 21.09; S, 12.18.

5.1.22.4. 3,5-Bis(4-pyridylamino)-1,2,4-thiadiazole (59). Reagents: pyridinylthiourea (153 mg, 1 mmol), DIB (322 mg, 1 mmol). Conditions: room temperature, 2 h. Purification: silica gel column chromatography using hexane/AcOEt (2:1). Yield: 68 mg (25%). Mp 188–190 °C. ^{1}H NMR (DMSO-d_6) δ 12.20 (br s, 1H, NH); 10.60 (br s, 1H, NH); 8.20–7.00 (m, 4H, arom); 8.10–6.50 (m, 4H, arom). ^{13}C NMR (DMSO-d_6) δ 176.2 (C5); 167.8 (C3); 160.0 (Ci); 149.8 (Cm); 138.5 (Cm); 113.5 (Cp); 109.0 (Co); 164.0 (Ci); 150.6 (Cm); 140.3 (Cm); 115.7 (Cp); 112.0 (Co). MS (ESI, positive): 271.0 (M^+). Anal. $C_{12}H_{10}N_6S$: C, 53.32; H, 3.73; N, 31.09; S, 11.88. Found: C, 53.26; H, 3.50; N, 31.37; S, 11.48.

5.1.23. General procedure for the synthesis of 3,5-disubstituted-1,2,4-thiadiazoles (73, 77, and 80). A solution

of the corresponding nitrile in appropriate solvent was heated to 100–160 °C where the oxathiazolone was added slowly. The reaction mixture was stirred for 2–36 h. The solvent was removed in vacuo and the residue purified as indicated in each case.

5.1.23.1. 5-Chloromethyl-3-phenyl-1,2,4-thiadiazole (73). Reagents: chloroacetonitrile (2.5 ml, 40 mmol) oxathiazolone **66**[36] (0.2 g, 1.1 mmol). Conditions: reflux, 36 h. Purification: silica gel column chromatography using hexane/AcOEt (9:1) as eluent. Yield: 27 mg (12%). Mp 58–59 °C. ^{1}H NMR ($CDCl_3$) δ 5.00 (s, 2H, CH_2), 7.42–7.48 (m, 3H, ArH), 8.22–8.28 (m, 2H, ArH). ^{13}C NMR (DMSO-d_6) δ 179.8 (C5), 174.2 (C3), 131.8 (Ci), 130.4 (Co), 128.1 (Cm, Cp), 50.2 (CH_2Cl). MS (EI)**:** *m*/*z* 210 (M^+, 30%), 135 (100%), 103 (64%). Anal. Calcd for $C_9H_7ClN_2S$: C, 51.31; H, 3.35; N, 13.30; S, 15.22. Found: C, 51.64; H, 3.15; N, 13.63; S, 15.04.

5.1.23.2. 5-Ethoxycarbonyl-3-(4-nitrophenyl)-1,2,4-thiadiazole (77). Reagents: ethyl cyanoformate (2.3 ml, 23.2 mmol) in 20 ml of decaline and oxathiazolone **69**[36] (1.0 g, 4.5 mmol). Conditions: 140–160 °C, 36 h. Purification: recrystallization from ethanol. Yield: 0.47g (38%). Mp 120–121 °C. ^{1}H NMR ($CDCl_3$) δ 1.54 (t, 3H, CH_3), 4.42 (q, 2H, CH_2), 8.39 (d, 2H, ArH, J = 8.9 Hz), 8.59 (d, 2H, ArH, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6) δ 178.5 (C5), 174.3 (C3), 158.6 (CO_2Et), 149.9 (Cp), 135.6 (Ci), 128.2 (2Co), 124.1 (2Cm), 63.2 (CH_2CH_3). 14.1 (CH_2CH_3). MS (EI)**:** *m*/*z* 279 (M^+, 65%), 234 (6%), 180 (100%), 149 (32%). Anal. Calcd for $C_{11}H_9N_3O_4S$: C, 47.31; H, 3.25; N, 15.05; S, 11.48. Found: C, 47.08; H, 3.15; N, 14.92; S, 11.23.

5.1.23.3. 3-(4-Methylphenyl)-5-[(4-methylphenyl)-sulfonyl]-1,2,4-thiadiazole (80). Reagents: 4-methylbenzene-sulfonyl cyanide (0.56 g, 3.1 mmol) in decaline (8 ml) and oxathiazolone **72**[36] (0.4 g, 2.1 mmol). Conditions: 140–160 °C, 2.5 h. Purification: the reaction mixture was allowed to cool to room temperature and the cream solid was collected, washed with hexane, and recrystallized from hexane/AcOEt (8:2) Yield: 0.51 g (73%). Mp 125–126 °C. ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.28 (d, 2H, ArH, J = 8.1 Hz), 7.44 (d, 2H, ArH, J = 8.2 Hz); 8.08 (d, 2H, ArH, J = 8.4 Hz), 8.14 (d, 2H, ArH, J = 8.2 Hz). ^{13}C NMR (DMSO-d_6) δ 180.3 (C5), 173.8 (C3), 136.7 (Ci), 134.8 (Cp), 131.4 (Cm), 129.9 (Ci), 129.5 (Co), 128.0 (Cm), 130.1 (Co), 126.1 (Cp), 23.8 (CH_3), 24.6 (CH_3). (MS (EI)): *m*/*z* 330 (M^+, 73%), 239 (15%), 176 (39%), 155 (100%). Anal. Calcd for $C_{16}H_{14}N_2O_2S_2$: C, 58.16; H, 4.27; N, 8.48; S, 19.41. Found: C, 58.50; H, 4.08; N, 8.78; S, 19.16.

5.2. Biological evaluation materials

The recombinant rabbit glycogen synthase kinase 3β (catalog number G 1663) was obtained from Sigma (St. Louis, MO). Ro 31-8220 was supplied by Calbiochem. DEAE–cellulose and phosphocellulose were from Whatman Ltd (London). ATP was purchased from Boehringer-Mannheim. [γ-^{32}P]ATP was purchased from Amersham.

5.3. GSK-3β Inhibition

GSK-3β enzyme (Sigma) was incubated with 15 μM of ATP, 0.2 μCi of [γ-^{32}P]ATP, GS-1 as substrate,[33] and different concentrations of the test compound.

GSK-3 activity was assayed in 50 mm Tris–HCl, pH 7.5, 10 mm $MgCl_2$, 1 mm EGTA, and 1 mm EDTA buffer, at 37 °C, in the presence of 15 μM GS-1 (substrate), 15 μM [γ-^{32}P]ATP in a final volume of 12 μL. After 20 min incubation at 37 °C, 4 μL aliquots of the supernatant were spotted onto 2 × 2 cm pieces of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed four times (for at least 10 min each time) in 1% phosphoric acid. The dried filters were transferred into scintillation vials, and the radioactivity was measured in a liquid scintillation counter. Blank values were subtracted, and the GSK-3β activity was expressed in picomoles of phosphate incorporated in GS-1 per 20 min or in percentage of maximal activity. The IC_{50} (concentration at which a 50% of enzyme inhibition is shown) values are gathered in Table 1.

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References and notes

1. Mount, C.; Downton, C. *Nat. Med.* **2006**, *12*, 780.
2. Grossman, H.; Bergmann, C.; Parker, S. *Mt. Sinai J. Med.* **2006**, *73*, 985.
3. Minino, A. M.; Arias, E.; Kochanek, K. D.; Murphy, S. L.; Smith, B. L. *Natl. Vital. Stat. Rep.* **2002**, *50*, 1.
4. Sivaprakasam, K. *Curr. Med. Chem.* **2006**, *13*, 2179.
5. Lewis, J.; Dickson, D. W.; Lin, W. L.; Chisholm, L.; Corral, A.; Jones, G.; Yen, S. H.; Sahara, N.; Skipper, L.; Yager, D.; Eckman, C.; Hardy, J.; Hutton, M.; McGrowan, E. *Science* **2001**, *293*, 1487.
6. Mi, K.; Johnson, G. V. *Curr. Alzheimer Res.* **2006**, *3*, 449.
7. Larner, A. J. *Exp. Opin. Ther. Patents* **1999**, *9*, 1359.
8. Ferrer, I.; Gomez-Isla, T.; Puig, B.; Freixes, M.; Ribé, E.; Dalfó, E.; Ávila, J. *Curr. Alz. Res.* **2005**, *2*, 3.
9. Doble, B. W.; Woodgett, J. R. *J. Cell Sci.* **2003**, *116*, 1175.
10. Van Waue, J.; Haefner, B. *Drug News Perspect.* **2003**, *16*, 557.
11. Grimes, C. A.; Jope, R. S. *Prog. Neurobiol.* **2001**, *65*, 391.
12. Sun, W.; Qureshi, H. Y.; Cafferty, P. W.; Sobue, K.; Agarwal-Mawal, A.; Neufield, K. D.; Paudel, H. K. *J. Biol. Chem.* **2002**, *277*, 11933.
13. Cho, G. H.; Johnson, G. V. *J. Biol. Chem.* **2003**, *278*, 187.
14. Eldar-Finkelman, H. *Trends Mol. Med.* **2002**, *8*, 126.
15. Martínez, A.; Castro, A.; Dorronsoro, I.; Alonso, M. *Curr. Med. Chem.* **2002**, *22*, 373.
16. Balaramana, Y.; Limayea, A. R.; Leveyb, A. I.; Srinivasana, S. *Cell. Mol. Life Sci.* **2006**, *63*, 1226.
17. Huang, H. C.; Klein, P. S. *Curr. Drug Targets* **2006**, *7*, 1389.

18. Martínez, A.; Alonso, M.; Castro, A.; Pérez, C.; Moreno, F. J. *J. Med. Chem.* **2002**, *45*, 1292.
19. Martínez, A.; Alonso, M.; Castro, A.; Dorronsoro, I.; Gelpí, J. L.; Luque, F. J.; Pérez, C.; Moreno, F. J. *J. Med. Chem.* **2005**, *48*, 7103.
20. Parkanyi, C.; Yuan, H. L.; Cho, N. S.; Jaw, J.-H. J.; Woodhouse, T. E.; Aung, T. *J. Heterocycl. Chem.* **1989**, *28*, 1331.
21. Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441.
22. Grzyb, J. A.; Shen, M.; Yoshina-Ishii, C.; Chi, W.; Brown, R. S. *Tetrahedron* **2005**, *61*, 7153.
23. Singh, R.; Choubey, A. K.; Bhattacharya, A. *J. Indian Chem. Soc.* **1998**, *75*, 430.
24. Hamada, Y.; Shibata, M.; Sugiura, T.; Kato, S.; Shioiri, T. *J. Org. Chem.* **1987**, *52*, 1252.
25. Song, Y.; Connor, D. T.; Sercel, A. D.; Sorenson, R. J.; Doubleday, R.; Unangst, P. C.; Roth, B. D.; Beylin, V. G.; Gilbertsen, R. B.; Chan, K.; Schrier, D. J.; Guglietta, A.; Bornemeier, D. A.; Dyer, R. D. *J. Med. Chem.* **1999**, *42*, 1161.
26. Kohara, Y.; Kubo, K.; Imamiya, E.; Naka, T. A. *J. Heterocycl. Chem.* **2000**, *37*, 1419.
27. Mamaeva, E. A.; Bakibaev, A. A. *Tetrahedron* **2003**, *59*, 7521.
28. Chen, D.; Chen, Z. *Syn. Commun.* **2002**, *32*, 2155.
29. Forlani, L.; Boga, C. *J. Chem. Soc. Perkin Trans. 2* **2002**, 768.
30. Lin, Y.; Lang, S. A.; Petty, S. R. *J. Org. Chem.* **1980**, *45*, 3750.
31. Paton, R. M. *Chem. Soc. Rev.* **1989**, *18*, 33.
32. McKie, M. C.; Paton, R. M. *Arkivoc.* **2002**, *6*, 15.
33. Woodgett, J. R. *Anal. Biochem.* **1989**, *180*, 237.
34. Hers, I.; Tavere, J. M.; Denton, R. M. *FEBS Lett.* **1999**, *460*, 433.
35. Cho, N. S.; Shon, H. I.; Parkanyi, C. *J. Heterocycl. Chem.* **1991**, *28*, 1645.
36. Howe, R. K.; Gruner, T. A.; Carter, L. G.; Black, L. L.; Franz, J. E. *J. Org. Chem.* **1978**, *43*, 3736.