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Design, synthesis and biological characterization of thiazolidin-4-one derivatives as promising inhibitors of *Toxoplasma gondii*

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ABSTRACT

We designed and synthesized a large number of novel thiazolidin-4-one derivatives for the evaluation of their anti-*Toxoplasma gondii* activity. This scaffold was functionalized at the *N*1-hydrazine portion with aliphatic, cycloaliphatic and (hetero)aromatic moieties. Then, a benzyl pendant was introduced at the lactamic NH of the core nucleus to evaluate the influence of this chemical modification on biological activity. The compounds were subjected to several *in vitro* assays to assess their anti-parasitic efficacy, cytotoxicity on fibroblasts, inhibition of tachyzoite invasion/attachment and replication after treatment. Results showed that fourteen of these thiazole-based compounds compare favorably to control compound trimethoprim in terms of parasite growth inhibition.

1. Introduction

The World Health Organization considers toxoplasmosis, a condition caused by the intracellular protozoan *Toxoplasma gondii*, one of the major parasitic diseases of humankind [1,2]. This status is warranted by the parasite's worldwide distribution and broad range of intermediate hosts [3] that have resulted in the infection of up to 1 billion people [4]. Humans acquire the parasite by ingestion of improperly cooked meat bearing parasite tissue cysts, by consumption of food or water contaminated with oocysts shed by infected cats, or *via* the placenta in pregnant women [5].

T. gondii has a complex life cycle that is characterized by an interconversion phenomenon, which is the ability of the parasite to differentiate from a rapidly multiplying form (tachyzoites) to a slowly replicating form (bradyzoite) enclosed in a tissue cyst and *vice versa*. The presence of bradyzoite-bearing tissue cysts in the host constitutes the chronic form of toxoplasmosis, typically asymptomatic but potentially fatal in immunocompromised individuals. In fact, in the otherwise healthy individual, the immune system keeps the parasite in the asymptomatic dormant bradyzoite state, establishing a reservoir of tissue cysts in the brain and other tissues. However, following immune system suppression, the conversion of bradyzoites to the rapidly growing tachyzoites causes reemergence of acute toxoplasmosis disease, one manifestation of which is toxoplasmic encephalitis [6]. *T. gondii* is implicated in several maladies in humans, including spontaneous abortions in pregnant women, and ocular disease. Recent studies have shown that it is implicated in the development of schizophrenia [7,8].

Current therapies for treating *T. gondii* infections show limited efficacy and are often associated with severe side effects [9,10]. These therapies include inhibition of folate metabolism, protein synthesis, and electron transport. Antifolate combination therapies employing diaminopyrimidines, such as trimethoprim or pyrimethamine, combined with sulfonamides, such as sulfadiazine or sulfamethoxazole, act synergistically against various bacterial and parasitic microorganisms.

Recently developed *Toxoplasma* inhibitors have shown a broad range of efficacy, measured by therapeutic index [TI = median cytotoxic dose (TD_{50})/median inhibitory concentration (IC_{50})], and

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toxicity [11–17]. The limited efficacy of current therapies due to patient drug tolerance and the relatively large quantities of drug(s) required to treat the disease necessitates the development of well-tolerated alternatives with little toxicity. Ideally these alternatives will inhibit both the tachyzoite and bradyzoite form in all intermediate hosts and will have selectivity toward the parasite with minimal effect on human host cell metabolism. Finally, therapies that target specific stages in the parasitic life cycle provide additional possibilities for synergistic combinations as well as for clarification of the drug's mechanism of action.

In the attempt of finding new anti-*Toxoplasma* agents with better inhibitory activity and minor cell toxicity, a special interest has been oriented towards thiazole derivatives obtaining encouraging results [18–24]. Recently, we published a new series of hydrazothiazoles with an acyl function at C4 of the ring which presented either a promising anti-*Toxoplasma* activity in the micromolar range but higher toxicity or a moderate ability to inhibit the penetration of parasite into the host cell [25].

Starting from these results and on the basis of recent data reported in literature about the antimicrobial activity of thiazolidinone scaffold [26], we decided to synthesize 74 novel thiazolidinone derivatives and to test them for *in vitro* anti-parasitic activity using *T. gondii* tachyzoites. The newly synthesized compounds with respect to the previous series present a slightly modified pharmacophore with a thiazolidinone instead of a thiazole incorporating the carbonylic function into the ring at C4 (Fig. 1).

The broad range of derivatives that we have prepared comprehends several different carbonyl moieties on the hydrazonic nitrogen such as linear or branched aliphatic chains from three to eight carbon atoms, unsubstituted or alkylated cycloaliphatic rings from cyclopentane to cyclooctane and (hetero)aryl or bicyclic rings like furan, thiophene, and so on. In general, the presence or less of a benzyl pendant (R) on the lactamic nitrogen of the thiazolidinone core could modulate the chemical and physical characteristics of the molecules and their specific interactions with the potential target.

Compounds were evaluated for parasite growth inhibition and cytotoxicity, inhibition of parasite invasion of host cells, and inhibition of intracellular replication. Additionally, two promising compounds were evaluated for parasiticidal potential and direct effects on extracellular tachyzoites.

2. Chemistry

As outlined in Scheme 1, for the synthesis of compounds **1A**–**37A** and **1B**–**37B**, different carbonyl compounds (1 eq) were dissolved in ethyl alcohol (100 mL) and reacted directly with thiosemicarbazide (1 eq) in the presence of catalytic amounts of acetic acid as previously reported by us [27]. The suspension was filtered and the crude solid purified by column chromatography on silica gel. The resulting intermediate thiosemicarbazones (1 eq) were reacted with ethyl-bromoacetate (1 eq) in methanol (50 mL) and sodium acetate (1 eq) at room temperature in order to obtain the

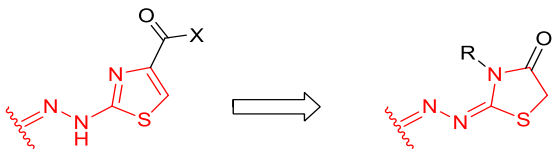
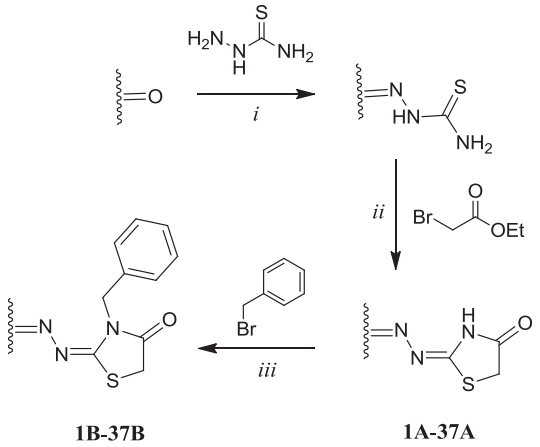


Fig. 1. Chemical modifications (black) and structural requirements (red) in this new scaffold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Scheme 1. General synthesis of the derivatives **1A**–**37A** and **1B**–**37B**. Reagents and conditions: (*i*) EtOH, acetic acid (cat.), rt; (*ii*) MeOH, CH_3COONa, rt; (*iii*) dry acetone, anhydrous K_2CO_3, rt.

1,3-thiazolidin-4-one derivatives (**1A**–**37A**) in high yields. Finally, the reaction between the resulting products (1 eq) and benzyl bromide (1.1 eq) in anhydrous acetone (50 mL) and potassium carbonate (1 eq) gave the *N*-benzyl derivatives (**1B**–**37B**). All the synthesized compounds were washed with petroleum ether and diethyl ether and purified by column chromatography before characterization by spectroscopic methods and elemental analysis.

In general, in the IR spectrum of derivatives **1A**–**37A** showed a strong band at about 3144 cm^{-1} due to the stretching of the lactam NH, at about 1697 cm^{-1} due to the stretching of C=O bond, and at about 1639 cm^{-1} for the stretching of C=N; for derivatives **1B**–**37B** there was a strong band at about 1693 cm^{-1} due to the stretching of C=O bond, at about 1622 cm^{-1} for the stretching of C=N bond and at about 1582 and 1447 cm^{-1} for C=C stretching.

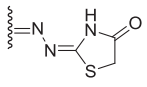
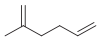
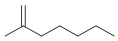
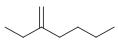
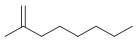
3. Biological characterization

The *in vitro* inhibition of tachyzoite growth by all of the prepared thiazolidinones derivatives (Tables 1 and 2) as well as the inhibition of tachyzoite invasion of host cells (Fig. 2) and intracellular replication (Fig. 3) were determined using published methods [8,20]. Further examination of the most active compounds **34A** and **37B** for parasitic potential, *i.e.*, the ability to kill the tachyzoites, was accomplished by a modification of the replication assay and the evaluation on extracellular tachyzoites as outlined in the Experimental protocols.

4. Experimental protocols

The chemicals, solvents for synthesis, and spectral grade solvents were purchased from Aldrich (Italy) and used without further purification. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Melting points (uncorrected) were determined automatically on an FP62 apparatus (Mettler-Toledo). Neat IR spectra were registered on a Perkin Elmer FT-IR Spectrometer Spectrum 1000. ^1H NMR spectra were recorded at 400 MHz on a Bruker spectrometer using $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts are expressed as δ units (parts per millions) relative to the solvent peak. Coupling constants *J* are valued in Hertz (Hz). Exchangeable protons (O*H*, N*H*) were assigned upon D_2O addition. Elemental analyses for C, H, and N were recorded on a Perkin–Elmer 240 B microanalyzer and the analytical results were within ±0.4% of the theoretical values for all

Table 1
Five-days growth inhibition assay for unsubstituted thiazolidinone **1A**–**37A** derivatives (**A series**).

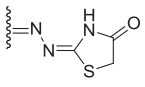
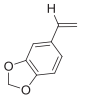
Compound		IC_{50}[a] μM	IC_{90}[b] μM	TD_{50}[c] μM	TI[d]
1A		210	852	≥320	3
2A		133	1163	≥320	4
3A		262	1064	≥320	2
4A		126	580	≥320	4
5A		97	316	≥320	6
6A		195	2219	≥320	3
7A		23	126	290	13
8A		110	686	320	3
9A		89	288	≥320	6
10A		31	188	≥320	18
11A		27	175	≥320	21
12A		0.9	2	35	39
13A		24	154	≥320	23
14A		28	278	≥320	20
15A		247	785	≥320	2
16A		36	310	202	6
17A		226	777	≥320	2
18A		218	693	≥320	3
19A		111	387	≥320	5
20A		79	290	309	4
21A		55	283	≥320	10
22A		126	284	≥320	4
23A		≥320	ND	≥320	ND[e]
24A		135	2683	≥320	4
25A		64	387	≥320	9

compounds. All reactions were monitored by TLC performed on 0.2 mm thick silica gel plates (60 F_{254} Merck).

4.1. 1-(Propan-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (*1A*)

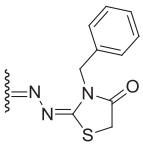
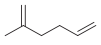
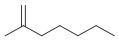
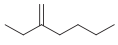
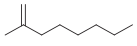
[28].

Table 1 (*continued*)

Compound		IC_{50}[a] μM	IC_{90}[b] μM	TD_{50}[c] μM	TI[d]
26A		114	262	≥320	5
27A		2.9	7	16	6
28A		52	268	≥320	11
29A		74	406	≥320	8
30A		20	129	≥320	28
31A		53	401	≥320	11
32A		166	474	≥320	3
33A		≥320	ND	≥320	ND
34A		3.9	31	≥320	144
35A		56	175	≥320	10
36A		8	49	≥320	70
37A		105	239	≥320	5
Trimethoprim		13	73	≥320	43

[a] IC_{50} = Median inhibitory concentration, a measure of tachyzoite inhibition.
[b] IC_{90} = Concentration at which 90% of the tachyzoite growth is inhibited.
[c] TD_{50} = Median toxicity dose, a measure of cytotoxicity.
[d] TI = Therapeutic index, a measure of efficacy, calculated by TD_{50}/IC_{50}. When $TD_{50} > 320$ (*i.e.*$>10^{2.5}$) TI = 562 (*i.e.*$10^{2.75}$)/IC_{50}.
[e] ND = Not determined.

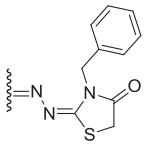
Table 2
Five-days growth inhibition assay for *N*-benzylated thiazolidinone **1B**–**37B** derivatives (**B series**).

Compound		IC_{50}[a] μM	IC_{90} μM	TD_{50} μM	TI
1B		232	636	≥320	2
2B		99	392	≥320	6
3B		47	214	≥320	12
4B		36	243	≥320	16
5B		23	186	67	3
6B		207	885	≥320	3
7B		23	188	220	10
8B		19	120	88	5
9B		15	121	≥320	37
10B		20	88	109	6
11B		105	330	≥320	5
12B		53	329	≥320	11
13B		184	845	≥320	3
14B		255	662	≥320	2
15B		43	171	≥320	13
16B		58	248	95	2
17B		88	242	286	3
18B		102	295	≥320	6
19B		255	1532	≥320	2
20B		43	508	205	5
21B		24	71	60	3
22B		111	396	≥320	5
23B		76	212	≥320	7
24B		270	1661	54	<1

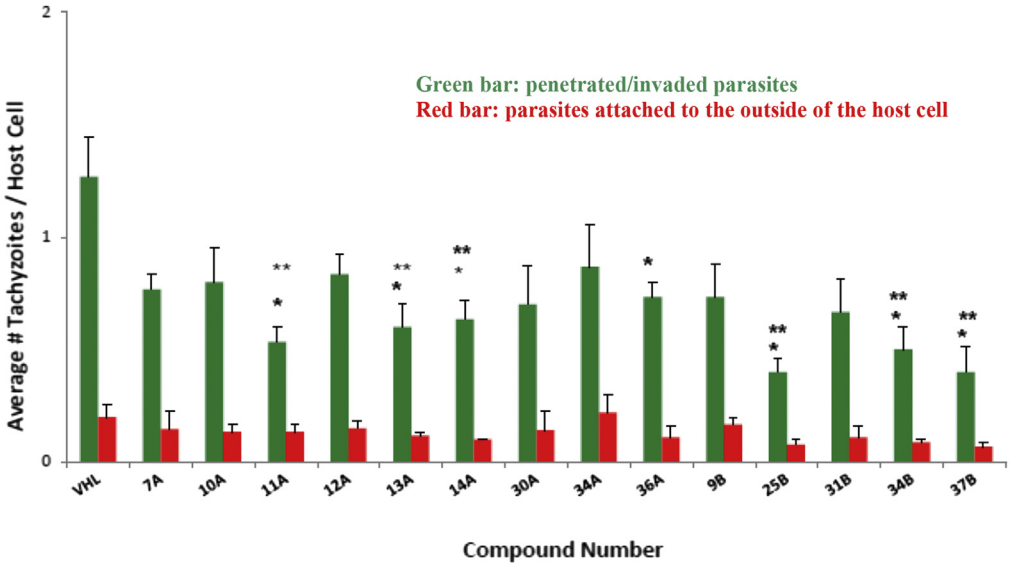
*4.2. 1-(Butan-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene)hydrazine (**2A**)*

Light yellow powder, mp 70–72 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15–1.19 (m, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.38–2.43 (m, 2H, CH_2), 3.83 (s, 2H, CH_2-thiaz.), 11.92 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.40, 17.80, 30.42,

Table 2 (*continued*)

Compound		IC_{50}[a] μM	IC_{90} μM	TD_{50} μM	TI
25B		37	84	≥320	15
26B		61	294	≥320	9
27B		≥320	ND	≥320	ND
28B		111	245	239	2
29B		82	181	147	3
30B		118	304	203	2
31B		36	103	183	5
32B		52	177	102	2
33B		290	2139	≥320	2
34B		38	79	≥320	15
35B		109	499	≥320	5
36B		≥320	ND	≥320	ND
37B		8.5	21	≥320	66
Trimethoprim		13	73	≥320	43

[a] See Table 1 legend.



*Compounds that significantly inhibit tachyzoite invasion/penetration ($P \leq 0.05$, two tailed Students' *t*-test). **Compounds that significantly inhibit tachyzoite attachment ($P \leq 0.05$, two tailed Students' *t*-test). Each value is the mean of triplicate experiments.

Fig. 2. Invasion/attachment assay at 10 μM of the most active and representative compounds.

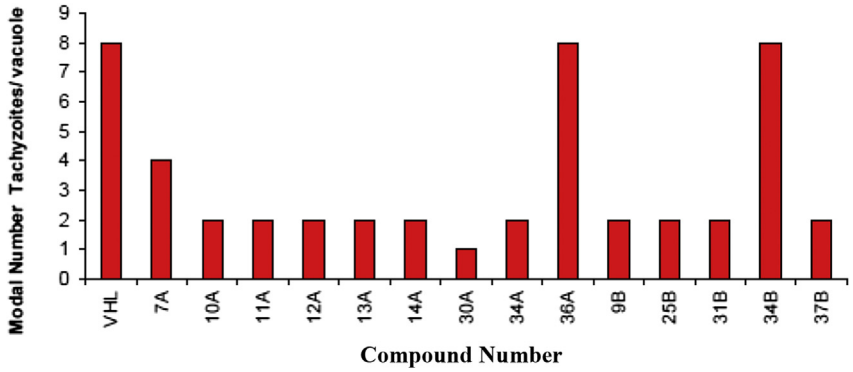


Fig. 3. Replication assay at 10 μM of the most active and representative compounds.

39.02, 163.03, 168.32, 174.63. Anal. Calcd. for $C_7H_{11}N_3OS$: C, 45.39; H, 5.99; N, 22.68. Found: C, 45.72; H, 6.14; N, 22.93.

*4.3. 1-(Pentan-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**3A**)*

Orange powder, mp 90–95 °C, 86% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91–0.99 (m, 3H, CH_3), 1.48–1.67 (m, 2H, CH_2), 2.01 (s, 3H, CH_3), 2.29–2.33 (m, 2H, CH_2), 3.75 (s, 2H, CH_2-thiaz.), 9.57 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.04, 17.45, 19.51, 33.08, 39.33, 161.83, 166.44, 174.54. Anal. Calcd. for $C_8H_{13}N_3OS$: C, 48.22; H, 6.58; N, 21.09. Found: C, 48.44; H, 6.34; N, 20.91.

*4.4. 1-(Pentan-3-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**4A**)*

Light yellow powder, mp 119–121 °C, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04–1.08 (t, 3H, CH_3), 1.13–1.17 (m, 3H, CH_3), 2.31–2.40 (m, 2H, CH_2), 2.43–2.51 (m, 2H, CH_2), 3.76 (s, 2H, CH_2-thiaz.), 8.78 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.41, 14.49, 19.51, 19.68, 39.06, 161.42, 166.56, 174.50. Anal. Calcd. for $C_8H_{13}N_3OS$: C, 48.22; H, 6.58; N, 21.09. Found: C, 48.03; H, 6.82; N, 21.33.

*4.5. 2-(2-(4-Methylpent-3-en-2-ylidene)hydrazono)thiazolidin-4-one (**5A**)*

Yellow powder, mp 100–105 °C, 77% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.72 (s, 6H, 2 × CH_3), 2.02 (s, 3H, CH_3), 3.81 (s, 2H, CH_2-thiaz.), 5.01 (s, 1H, CH=), 11.65 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 19.45, 25.41, 25.63, 33.10, 118.52, 142.34, 163.39, 164.59, 174.02. Anal. Calcd. for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20; N, 19.89. Found: C, 51.35; H, 6.51; N, 20.04.

*4.6. 1-(Hex-5-en-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**6A**)*

White powder, mp 80–82 °C, 79% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.92 (s, 3H, CH_3), 2.27–2.31 (m, 2H, CH_2), 2.34–2.37

(m, 2H, CH_2) 3.79 (s, 2H, CH_2-thiaz.), 5.04–5.09 (m, 2H, CH_2=), 5.81–5.91 (m, 1H, CH=), 11.70 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.66, 30.23, 33.07, 37.53, 115.55, 117.71, 138.39, 165.75, 174.12. Anal. Calcd. for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20; N, 19.89. Found: C, 51.37; H, 6.43; N, 19.67.

4.7. 1-(4-Methylpentan-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**7A**)

Light orange powder, mp 141–145 °C, 82% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 0.89 (d, 6H, J = 8.0 Hz, 2 × CH_3), 1.91 (s, 3H, CH_3), 1.93–2.00 (m, 1H, CH), 2.12 (d, 2H, J = 4.0 Hz, CH_2), 3.78 (s, 2H, CH_2-thiaz.), 11.68 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.73, 22.84, 25.79, 33.07, 47.43, 154.45, 165.71, 174.36. Anal. Calcd. for $C_9H_{15}N_3OS$: C, 50.68; H, 7.09; N, 19.70. Found: C, 50.91; H, 6.87; N, 19.55.

4.8. 1-(Heptan-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**8A**)

Light brown powder, mp 94–96 °C, 84% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 0.85–0.89 (m, 3H, CH_3), 1.27–1.30 (m, 4H, 2 × CH_2), 1.50–1.53 (m, 2H, CH_2), 1.92 (s, 3H, CH_3), 2.22–2.26 (m, 2H, CH_2), 3.78 (s, 2H, CH_2-thiaz.), 11.68 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.32, 17.49, 22.42, 25.79, 31.32, 33.06, 38.19, 161.85, 166.26, 174.34. Anal. Calcd. for $C_{10}H_{17}N_3OS$: C, 52.83; H, 7.54; N, 18.48. Found: C, 52.54; H, 7.22; N, 18.73.

4.9. 1-(Heptan-3-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**9A**)

White powder, mp 77–79 °C, 81% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 0.88–0.92 (m, 3H, CH_3), 0.97–1.01 (m, 3H, CH_3), 1.28–1.36 (m, 2H, CH_2), 1.47–1.53 (m, 2H, CH_2), 2.25–2.29 (m, 2H, CH_2), 2.36–2.42 (m, 2H, CH_2), 3.78 (s, 2H, CH_2-thiaz.), 11.67 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 11.29, 14.28, 22.32, 24.24, 28.27, 33.00, 35.41, 163.09, 170.44, 174.34. Anal. Calcd. for $C_{10}H_{17}N_3OS$: C, 52.83; H, 7.54; N, 18.48. Found: C, 53.05; H, 7.81; N, 18.19.

4.10. 1-(Octan-2-ylidene)-2-(4-oxo-thiazolidin-2-ylidene) hydrazine (**10A**)

Yellow powder, mp 106–110 °C, 87% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 0.87 (s, 3H, CH_3), 1.27 (bs, 6H, 3 × CH_2), 1.49–1.51 (m, 2H, CH_2), 1.92 (s, 3H, CH_3), 2.22–2.26 (m, 2H, CH_2), 3.78 (s, 2H, CH_2-thiaz.), 11.69 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.42, 17.51, 22.52, 26.08, 28.81, 31.60, 32.33, 38.17, 160.11, 167.99, 172.58. Anal. Calcd. for $C_{11}H_{19}N_3OS$: C, 54.74; H, 7.93; N, 17.41. Found: C, 54.35; H, 8.14; N, 17.10.

4.11. 2-(2-Cyclopentylidenehydrazono)thiazolidin-4-one (**11A**)

[28].

4.12. 2-(2-(2-Methylcyclopentylidene)hydrazono)thiazolidin-4-one (**12A**)

Light yellow powder, mp 164–166 °C, 89% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.16 (d, 3H, J = 6.4 Hz, CH_3), 1.39–1.41 (m, 2H, cyclopentane), 1.66–1.71 (m, 1H, cyclopentane), 1.87–1.92 (m, 1H, cyclopentane), 2.04–2.07 (m, 1H, cyclopentane), 2.40–2.49 (m, 1H, cyclopentane), 2.57–2.66 (m, 1H, cyclopentane), 3.73 (s, 2H, CH_2-thiaz.), 11.59 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.56, 22.63, 30.24, 33.07, 33.71, 39.26, 160.85, 172.32, 174.62. Anal. Calcd. for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20; N, 19.89. Found: C, 50.89; H, 6.52; N, 20.11.

4.13. 2-(2-(3-Methylcyclopentylidene)hydrazono)thiazolidin-4-one (**13A**)

Yellow powder, mp 189–193 °C, 86% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 1.02 (d, 3H, J = 6.4 Hz, CH_3), 1.26–1.34 (m, 1H, cyclopentane), 1.88–1.93 (m, 1H, cyclopentane), 1.97–2.09 (m, 2H, cyclopentane), 2.26–2.69 (m, 3H, cyclopentane), 3.80 (s, 2H, CH_2-thiaz.), 11.72 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.03, 30.13, 32.89, 33.15, 39.58, 41.34, 161.66, 174.38, 175.80. Anal. Calcd. for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20; N, 19.89. Found: C, 51.44; H, 6.01; N, 19.60.

4.14. 2-(2-Cyclohexylidenehydrazono)thiazolidin-4-one (**14A**)

Light brown powder, mp 171–175 °C, 92% yield; ^1H NMR (400 MHz, $CDCl_3$): δ 1.67–1.89 (m, 6H, 3 × CH_2, cyclohexane), 2.40 (bs, 2H, cyclohexane), 2.62 (bs, 2H, cyclohexane), 3.79 (s, 2H, CH_2-thiaz.), 11.69 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.59, 25.03, 26.84, 27.02, 31.95, 36.22, 164.79, 172.36, 174.44. Anal. Calcd. for $C_9H_{13}N_3OS$: C, 51.16; H, 6.20; N, 19.89. Found: C, 51.38; H, 6.07; N, 19.53.

4.15. 2-(2-(2-Methylcyclohexylidene)hydrazono)thiazolidin-4-one (**15A**)

Yellow powder, mp 159–161 °C, 90% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 1.06 (d, 3H, J = 8.0 Hz, CH_3), 1.21–1.25 (m, 1H, cyclohexane), 1.33–1.36 (m, 1H, cyclohexane), 1.49–1.54 (m, 1H, cyclohexane), 1.69–1.77 (m, 2H, cyclohexane), 1.88–1.98 (m, 2H, cyclohexane), 2.36–2.40 (m, 1H, cyclohexane), 3.10–3.13 (m, 1H, cyclohexane), 3.77 (s, 2H, CH_2-thiaz.), 11.65 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.34, 24.95, 27.02, 27.94, 32.98, 36.36, 39.12, 164.46, 171.07, 174.93. Anal. Calcd. for $C_{10}H_{15}N_3OS$: C, 53.31; H, 6.71; N, 18.65. Found: C, 53.12; H, 6.49; N, 18.91.

4.16. 2-(2-(3-Methylcyclohexylidene)hydrazono)thiazolidin-4-one (**16A**)

Light yellow powder, mp 151–154 °C, 83% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 1.04–1.06 (m, 3H, CH_3), 1.68–1.91 (m, 7H, cyclohexane), 2.28–2.37 (m, 1H, cyclohexane), 3.12–3.19 (m, 1H, cyclohexane), 3.79–3.85 (s, 2H, CH_2-thiaz.), 11.65 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 22.31, 22.52, 25.29, 26.24, 33.15, 34.78, 36.42, 164.68, 169.01, 174.11. Anal. Calcd. for $C_{10}H_{15}N_3OS$: C, 53.31; H, 6.71; N, 18.65. Found: C, 53.05; H, 6.54; N, 18.32.

4.17. 2-(2-(4-Methylcyclohexylidene)hydrazono)thiazolidin-4-one (**17A**)

Yellow powder, mp 183–187 °C, 81% yield; ^1H NMR (400 MHz, DMSO-d_6): δ 0.92 (d, 3H, J = 4.0 Hz, CH_3), 1.00–1.06 (m, 1H, cyclohexane), 1.11–1.15 (m, 1H, cyclohexane), 1.68–1.70 (m, 1H, cyclohexane), 1.77–1.84 (m, 2H, cyclohexane), 1.87–1.96 (m, 1H, cyclohexane), 2.21–2.27 (m, 1H, cyclohexane), 2.32–2.35 (m, 1H, cyclohexane), 3.19–3.23 (m, 1H, cyclohexane), 3.79 (s, 2H, CH_2-thiaz.), 11.65 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 21.78, 22.40, 25.66, 27.01, 33.88, 34.25, 35.19, 163.99, 168.78, 174.45. Anal. Calcd. for $C_{10}H_{15}N_3OS$: C, 53.31; H, 6.71; N, 18.65. Found: C, 53.65; H, 6.50; N, 18.29.

*4.18. 2-(2-(2-Tert-butylcyclohexylidene)hydrazono)thiazolidin-4-one (**18A**)*

White powder, mp 137–143 °C, 75% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 9H, 3 × CH_3), 1.50–1.55 (m, 6H, cyclohexane), 2.04–2.11 (m, 2H, cyclohexane), 3.03–3.08 (m, 1H, cyclohexane), 3.75 (s, 2H, CH_2-thiaz.), 8.87 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 22.89, 26.07, 28.65, 29.84, 30.20, 33.07, 34.90, 54.66, 155.24, 162.13, 170.28. Anal. Calcd. for $C_{13}H_{21}N_3OS$: C, 58.39; H, 7.92; N, 15.71. Found: C, 50.14; H, 8.13; N, 18.02.

*4.19. 2-(2-Cycloheptylidenehydrazono)thiazolidin-4-one (**19A**)*

Light yellow powder, mp 170–175 °C, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58–1.59 (m, 4H, cycloheptane), 1.67–1.68 (m, 4H, cycloheptane), 2.55–2.58 (m, 2H, cycloheptane), 2.65–2.68 (m, 2H, cycloheptane), 3.77 (s, 2H, CH_2-thiaz.), 11.69 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 25.01, 27.17, 29.92, 30.27, 31.77, 33.07, 37.76, 161.39, 171.77, 174.34. Anal. Calcd. for $C_{10}H_{15}N_3OS$: C, 53.31; H, 6.71; N, 18.65. Found: C, 53.60; H, 6.58; N, 18.39.

*4.20. 2-(2-Cyclooctylidenehydrazono)thiazolidin-4-one (**20A**)*

White powder, mp 150–155 °C, 77% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.38–1.44 (m, 2H, cyclooctane), 1.52–1.60 (m, 4H, cyclooctane), 1.77–1.80 (m, 2H, cyclooctane), 1.83–1.85 (m, 2H, cyclooctane), 2.46–2.50 (m, 2H, cyclooctane), 2.56–2.59 (m, 2H, cyclooctane), 3.94 (s, 2H, CH_2-thiaz.), 11.72 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 25.26, 28.41, 28.66, 29.41, 30.68, 30.96, 32.45, 37.12, 161.88, 170.98, 174.10. Anal. Calcd. for $C_{11}H_{17}N_3OS$: C, 55.20; H, 7.16; N, 17.56. Found: C, 55.02; H, 7.41; N, 17.22.

*4.21. 2-(2-(1-Cyclohexylethylidene)hydrazono)thiazolidin-4-one (**21A**)*

White powder, mp 153–158 °C, 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33–1.35 (m, 6H, cyclohexane), 1.71–1.73 (m, 1H, cyclohexane), 1.82–1.85 (m, 4H, cyclohexane), 1.96 (s, 3H, CH_3) 3.75 (s, 2H, CH_2-thiaz.), 11.71 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.93, 26.00, 26.20, 30.24, 33.03, 46.38, 162.31, 169.50, 174.40. Anal. Calcd. for $C_{11}H_{17}N_3OS$: C, 55.20; H, 7.16; N, 17.56. Found: C, 54.98; H, 7.53; N, 17.19.

*4.22. 2-(2-(Furan-2-ylmethylene)hydrazono)thiazolidin-4-one (**22A**)*

Light brown powder, mp 234–235 °C, 85% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (s, 2H, CH_2-thiaz.), 6.57–6.59 (m, 1H, furan), 6.97–6.98 (m, 1H, furan), 7.64–7.65 (m, 1H, furan), 8.45 (s, 1H, CH=), 11.89 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.51, 112.79, 116.11, 142.95, 146.01, 146.31, 149.75, 187.27. Anal. Calcd. for $C_8H_7N_3O_2S$: C, 45.92; H, 3.37; N, 20.08. Found: C, 46.12; H, 3.04; N, 19.83.

*4.23. 2-(2-(1-(Furan-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**23A**)*

Light brown powder, mp 141–146 °C, 80% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 3.70 (s, 2H, CH_2-thiaz.), 6.61–6.63 (m, 1H, furan), 6.98 (s, 1H, furan), 7.83 (s, 1H, furan), 11.91 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.98, 32.40, 111.41, 116.87, 142.01, 145.45, 146.98, 150.23, 183.10. Anal. Calcd. for $C_9H_9N_3O_2S$: C, 48.42; H, 4.06; N, 18.82. Found: C, 48.11; H, 3.87; N, 19.06.

*4.24. 2-(2-(Thiophen-2-ylmethylene)hydrazono)thiazolidin-4-one (**24A**)*

Light yellow powder, mp 230–236 °C, 82% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.05 (s, 2H, CH_2-thiaz.), 7.15–7.17 (m, 1H, thiophene), 7.51 (d, 1H, J = 4.0 Hz, thiophene), 7.70 (d, 1H, J = 4.0 Hz, thiophene), 8.55 (s, 1H, CH=), 11.94 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.45, 128.49, 130.25, 132.40, 139.37, 151.14, 162.15, 174.98. Anal. Calcd. for $C_8H_7N_3OS_2$: C, 42.65; H, 3.13; N, 18.65. Found: C, 42.29; H, 3.38; N, 18.90.

*4.25. 2-(2-(1-(Thiophen-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**25A**)*

Orange powder, mp 163–165 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H, CH_3), 3.81 (s, 2H, CH_2-thiaz.), 7.04–7.08 (m, 1H, thiophene), 7.37–7.39 (m, 2H, thiophene), 11.92 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.33, 33.30, 128.13, 128.79, 129.70, 143.78, 156.67, 163.89, 174.33. Anal. Calcd. for $C_9H_9N_3OS_2$: C, 45.17; H, 3.79; N, 17.56. Found: C, 45.46; H, 3.45; N, 17.89.

*4.26. 2-(2-(1-Phenylethylidene)hydrazono)thiazolidin-4-one (**26A**)*

Light orange powder, mp 154–161 °C, 94% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 3.81 (s, 2H, CH_2-thiaz.), 7.40–7.42 (m, 3H, Ar), 7.86–7.88 (m, 2H, Ar), 11.83 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.09, 32.24, 126.84, 128.86, 130.25, 138.24, 161.10, 165.13, 174.96. Anal. Calcd. for $C_{11}H_{11}N_3OS$: C, 56.63; H, 4.75; N, 18.01. Found: C, 56.89; H, 4.48; N, 18.30.

*4.27. 2-(2-(1-(Pyridin-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**27A**)*

Light brown powder, mp 243–247 °C, 84% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.43 (s, 3H, CH_3), 3.92 (s, 2H, CH_2-thiaz.), 7.57–7.62 (m, 1H, pyridine), 7.92–7.93 (m, 1H, pyridine), 8.07–8.09 (m, 1H, pyridine), 8.14–8.16 (m, 1H, pyridine), 12.18 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.02, 33.36, 120.94, 124.95, 137.18, 149.17, 155.48, 161.77, 166.07, 174.44. Anal. Calcd. for $C_{10}H_{10}N_4OS$: C, 51.27; H, 4.30; N, 23.91. Found: C, 51.01; H, 3.99; N, 23.67.

*4.28. 2-(2-(Pyridin-3-ylmethylene)hydrazono)thiazolidin-4-one (**28A**)*

Light yellow powder, mp 278–279 °C, 88% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.96 (s, 2H, CH_2-thiaz.), 7.88–7.91 (m, 1H, pyridine), 8.57–8.59 (m, 2H, pyridine), 8.83 (s, 1H, pyridine), 9.07 (s, 1H, CH=), 12.18 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.47, 125.02, 133.10, 135.68, 148.77, 151.48, 159.88, 161.62, 174.02. Anal. Calcd. for $C_9H_8N_4OS$: C, 49.08; H, 3.66; N, 25.44. Found: C, 48.87; H, 3.44; N, 25.22.

*4.29. 2-(2-(1-(Pyridin-3-yl)ethylidene)hydrazono)thiazolidin-4-one (**29A**)*

White powder, mp 205–210 °C, 75% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.89 (s, 2H, CH_2-thiaz.), 7.46–7.50 (m, 1H, pyridine), 8.16–8.19 (m, 1H, pyridine), 8.62–8.63 (m, 1H, pyridine), 9.01 (s, 1H, pyridine), 12.00 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.90, 33.31, 124.00, 133.75, 134.12, 148.03,

150.89, 159.05, 160.12, 174.38. Anal. Calcd. for $C_{10}H_{10}N_4OS$: C, 51.27; H, 4.30; N, 23.91. Found: C, 51.08; H, 4.11; N, 23.70.

*4.30. 2-(2-(Pyridin-4-ylmethylene)hydrazono)thiazolidin-4-one (**30A**)*

Yellow powder, mp 258–259 °C, 78% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.95 (s, 2H, CH_2-thiaz.), 7.78 (d, 2H, J = 8.0 Hz, pyridine), 8.47 (s, 1H, CH=), 8.72 (d, 2H, J = 8.0 Hz, pyridine), 12.17 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.71, 122.70, 148.33, 153.95, 169.14, 169.59, 174.46. Anal. Calcd. for $C_9H_8N_4OS$: C, 49.08; H, 3.66; N, 25.44. Found: C, 49.30; H, 3.81; N, 25.62.

*4.31. 2-(2-(1-(Pyridin-4-yl)ethylidene)hydrazono)thiazolidin-4-one (**31A**)*

Light brown powder, mp 259–262 °C, 71% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 3.90 (s, 2H, CH_2-thiaz.), 7.75 (d, 2H, J = 7.6 Hz, pyridine), 8.66 (d, 2H, J = 8.0 Hz, pyridine), 12.09 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.99, 33.11, 123.25, 148.65, 154.80, 168.02, 169.25, 174.49. Anal. Calcd. for $C_{10}H_{10}N_4OS$: C, 51.27; H, 4.30; N, 23.91. Found: C, 51.52; H, 4.65; N, 24.09.

*4.32. 2-(2-((1H-Indol-3-yl)methylene)hydrazono)thiazolidin-4-one (**32A**)*

Yellow powder, mp 299–301 °C, 86% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 2H, CH_2-thiaz.), 7.13–7.22 (m, 2H, indole), 7.44 (d, 1H, J = 8.0 Hz, indole), 7.83 (s, 1H, indole), 8.20 (d, 1H, J = 8.0 Hz, indole), 8.53 (s, 1H, CH=), 11.65 (bs, 1H, NH, D_2O exch.), 11.81 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.43, 112.47, 121.23, 122.56, 123.20, 125.01, 132.18, 137.62, 152.77, 174.77 (two carbons missing due to overlapping signals). Anal. Calcd. for $C_{12}H_{10}N_4OS$: C, 55.80; H, 3.90; N, 21.69. Found: C, 55.56; H, 3.68; N, 21.40.

*4.33. 2-(2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazono) thiazolidin-4-one (**33A**)*

Yellow powder, mp 273–275 °C, 90% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.95 (s, 2H, CH_2-thiaz.), 5.90 (s, 2H, OCH_2O), 6.76–6.77 (m, 1H, benzodioxole), 7.26 (s, 1H, benzodioxole), 7.31–7.32 (m, 1H, benzodioxole), 8.47 (s, 1H, CH=), 11.77 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.42, 102.08, 106.08, 108.99, 114.56, 124.47, 129.08, 148.33, 149.99, 156.19, 174.54. Anal. Calcd. for $C_{11}H_9N_3O_3S$: C, 50.18; H, 3.45; N, 15.96. Found: C, 50.40; H, 3.70; N, 16.15.

*4.34. 2-(2-(Naphthalen-1-ylmethylene)hydrazono)thiazolidin-4-one (**34A**)*

Light yellow powder, mp 260–262 °C, 85% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.96 (s, 2H, CH_2-thiaz.), 7.60–7.67 (m, 3H, Ar), 7.96 (d, 1H, J = 8.0 Hz, Ar), 8.02–8.07 (m, 2H, Ar), 8.97 (s, 1H, CH=), 9.05 (d, 1H, J = 8.0 Hz, Ar), 12.06 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 33.64, 117.78, 125.97, 126.81, 127.99, 129.25, 129.98, 130.63, 130.70, 131.69, 134.06, 162.12, 173.60, 184.95. Anal. Calcd. for $C_{14}H_{11}N_3OS$: C, 62.43; H, 4.12; N, 15.60. Found: C, 62.21; H, 3.92; N, 15.85.

*4.35. 2-(2-(1-(Naphthalen-2-yl)ethylidene)hydrazono)thiazolidin-4-one (**35A**)*

Orange powder, mp 201–206 °C, 87% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.49 (s, 3H, CH_3), 3.88 (s, 2H, CH_2-thiaz.), 7.53–7.57 (m, 2H, Ar), 7.92 (d, 2H, J = 8.0 Hz, Ar), 7.98–8.11 (m, 1H, Ar), 8.12–8.29 (m, 1H, Ar), 8.30 (s, 1H, Ar), 12.00 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.91, 33.31, 123.86, 126.97, 127.29, 127.48, 127.99, 128.17, 129.12, 133.21, 134.03, 135.66, 160.55, 174.41, 187.27. Anal. Calcd. for $C_{15}H_{13}N_3OS$: C, 63.58; H, 4.62; N, 14.83. Found: C, 63.91; H, 4.33; N, 14.55.

*4.36. 2-(2-(1-(2-Oxo-2H-chromen-3-yl)ethylidene)hydrazono) thiazolidin-4-one (**36A**)*

Yellow powder, mp 236–241 °C, 95% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 3.87 (s, 2H, CH_2-thiaz.), 7.36–7.45 (m, 2H, chromene), 7.63–7.67 (m, 1H, chromene), 7.84–7.87 (m, 1H, chromene), 8.17 (s, 1H, chromene), 12.00 (bs, 1H, NH, D_2O exch.); ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.45, 33.34, 116.48, 117.91, 119.17, 125.25, 126.98, 129.79, 133.05, 142.02, 153.97, 154.17, 159.48, 174.84. Anal. Calcd. for $C_{14}H_{11}N_3O_3S$: C, 55.80; H, 3.68; N, 13.95. Found: C, 55.57; H, 3.86; N, 14.14.

*4.37. 2-(2-(1-(Ferrocenyl)ethylidene)hydrazono)thiazolidin-4-one (**37A**)*

Orange powder, mp 235–237 °C, 92% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3), 3.79 (s, 2H, CH_2-thiaz.), 4.12–4.16 (m, 5H, ferrocene), 4.22–4.24 (m, 1H, ferrocene), 4.39–4.42 (m, 2H, ferrocene), 4.65–4.67 (m, 1H, ferrocene), 11.76 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.93, 33.88, 67.44, 69.87, 70.12, 82.66, 106.12, 135.69, 160.45, 164.63, 171.12. Anal. Calcd. for $C_{15}H_{15}FeN_3OS$: C, 52.80; H, 4.43; N, 12.32. Found: C, 53.04; H, 4.27; N, 12.06.

*4.38. 3-Benzyl-2-(2-(propan-2-ylidene)hydrazono)thiazolidin-4-one (**1B**)*

Light yellow powder, mp 59–61 °C, 71% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 3.79 (s, 2H, CH_2-thiaz.), 4.97 (s, 2H, CH_2-benzyl), 7.31–7.34 (m, 3H, Ar), 7.46 (d, 2H, J = 6.4 Hz, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 18.75, 24.97, 32.36, 46.26, 127.95, 128.42, 128.79, 136.67, 159.95, 165.52, 172.55. Anal. Calcd. for $C_{13}H_{15}N_3OS$: C, 59.74; H, 5.79; N, 16.08. Found: C, 59.99; H, 5.56; N, 15.85.

*4.39. 3-Benzyl-2-(2-(butan-2-ylidene)hydrazono)thiazolidin-4-one (**2B**)*

White powder, mp 55–60 °C, 78% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13–1.16 (m, 3H, CH_3), 2.00 (s, 3H, CH_3), 2.32–2.38 (m, 2H, CH_2), 3.75 (s, 2H, CH_2-thiaz.), 4.96 (s, 2H, CH_2-benzyl), 7.27–7.33 (m, 3H, Ar), 7.45–7.47 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 11.00, 17.39, 31.48, 32.31, 46.23, 127.96, 128.40, 128.81, 136.66, 160.32, 168.96, 172.62. Anal. Calcd. for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26. Found: C, 60.81; H, 6.54; N, 15.03.

*4.40. 3-Benzyl-2-(2-(pentan-2-ylidene)hydrazono)thiazolidin-4-one (**3B**)*

White powder, mp 40–42 °C, 74% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90–0.98 (m, 3H, CH_3), 1.56–1.65 (m, 2H, CH_2), 1.99 (s, 3H, CH_3), 2.28–2.38 (m, 2H, CH_2), 3.76 (s, 2H, CH_2-thiaz.), 4.96 (s, 2H, CH_2-benzyl), 7.27–7.33 (m, 3H, Ar), 7.45–7.47 (m, 2H, Ar). ^{13}C

NMR (101 MHz, DMSO-d_6): δ 14.11, 17.50, 19.51, 32.32, 46.24, 127.95, 128.39, 128.81, 136.66, 160.07, 167.91, 172.60 (one carbon missing due to overlapping signals). Anal. Calcd. for $C_{15}H_{19}N_3OS$: C, 62.25; H, 6.62; N, 14.52. Found: C, 62.47; H, 6.34; N, 14.34.

4.41. 3-Benzyl-2-(2-(pentan-3-ylidene)hydrazono)thiazolidin-4-one (**4B**)

Light yellow powder, mp 68–70 °C, 85% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.86–0.90 (m, 3H, CH_3), 1.02–1.05 (m, 3H, CH_3), 2.25–2.36 (m, 4H, 2 × CH_2), 3.98 (s, 2H, CH_2-thiaz.), 4.85 (s, 2H, CH_2-benzyl), 7.26–7.34 (m, 5H, Ar); ^{13}C NMR (101 MHz, DMSO-d_6): δ 10.94, 11.14, 24.50, 29.19, 32.32, 46.26, 127.92, 128.31, 128.78, 136.69, 160.38, 172.59, 173.10. Anal. Calcd. for $C_{15}H_{19}N_3OS$: C, 62.25; H, 6.62; N, 14.52. Found: C, 62.04; H, 6.90; N, 14.78.

4.42. 3-Benzyl-2-(2-(4-methylpent-3-en-2-ylidene)hydrazono) thiazolidin-4-one (**5B**)

Light yellow oil, 70% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (s, 6H, 2 × CH_3), 2.01 (s, 3H, CH_3), 3.79 (s, 2H, CH_2-thiaz.), 4.90 (s, 2H, CH_2-benzyl), 5.04 (s, 1H, CH=), 7.39–7.43 (m, 3H, Ar), 7.50–7.56 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 19.87, 25.02, 25.88, 33.56, 46.27, 119.77, 127.42, 128.44, 128.99, 135.87, 142.34, 162.11, 163.34, 174.45. Anal. Calcd. for $C_{16}H_{19}N_3OS$: C, 63.76 H, 6.35; N, 13.94. Found: C, 63.50; H, 6.14; N, 14.11.

4.43. 3-Benzyl-2-(2-(hex-5-en-2-ylidene)hydrazono)thiazolidin-4-one (**6B**)

Light yellow powder, mp 53–55 °C; 73% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (s, 3H, CH_3), 2.34–2.38 (m, 2H, CH_2), 2.41–2.45 (m, 2H, CH_2), 3.75 (s, 2H, CH_2-thiaz.), 4.96 (s, 2H, CH_2-benzyl), 4.98–5.01 (dd, J_{cis} = 12.0 Hz, J_{gem} = 3.0 Hz, 1H, CH=), 5.05–5.10 (dd, J_{trans} = 16.0 Hz, J_{gem} = 3.0 Hz, 1H, CH=) 5.83–5.93 (m, 1H, CH=), 7.27–7.33 (m, 3H, Ar), 7.43–7.46 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.67, 30.19, 32.34, 37.47, 46.24, 115.58, 127.96, 128.40, 128.81, 136.64, 138.35, 160.45, 167.36, 172.63. Anal. Calcd. for $C_{16}H_{19}N_3OS$: C, 63.76 H, 6.35; N, 13.94. Found: C, 63.98; H, 6.61; N, 13.69.

4.44. 3-Benzyl-2-(2-(4-methylpentan-2-ylidene)hydrazono) thiazolidin-4-one (**7B**)

Light yellow powder, mp 49–50 °C; 87% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (d, 6H, J = 6.8 Hz, 2 × CH_3), 1.27 (d, 2H, J = 7.2 Hz, CH_2), 2.03 (bs, 1H, CH), 2.19 (s, 3H, CH_3), 3.76 (s, 2H, CH_2-thiaz.), 4.94 (s, 2H, CH_2-benzyl), 7.42–7.44 (m, 3H, Ar), 7.55–7.58 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.72, 22.85, 25.76, 32.34, 46.25, 47.36, 127.95, 128.44, 128.77, 136.65, 159.94, 167.30, 172.56. Anal. Calcd. for $C_{16}H_{21}N_3OS$: C, 63.33; H, 6.98; N, 13.85. Found: C, 63.12; H, 7.24; N, 14.01.

4.45. 3-Benzyl-2-(2-(heptan-2-ylidene)hydrazono)thiazolidin-4-one (**8B**)

Yellow powder, mp 39–41 °C, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87–0.92 (m, 3H, CH_3), 1.22–1.35 (m, 4H, 2 × CH_2), 1.55–1.62 (m, 2H, CH_2), 1.99 (s, 3H, CH_3), 2.33–2.36 (m, 2H, CH_2), 3.76 (s, 2H, CH_2-thiaz.), 4.95 (s, 2H, CH_2-benzyl), 7.27–7.33 (m, 3H, Ar), 7.43–7.46 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.34, 17.51, 22.41, 25.77, 31.32, 32.22, 38.12, 46.23, 127.95, 128.42, 128.79, 136.65, 160.12, 168.01, 172.60. Anal. Calcd. for $C_{17}H_{23}N_3OS$: C, 64.32; H, 7.30; N, 13.24. Found: C, 64.60; H, 7.09; N, 13.41.

4.46. 3-Benzyl-2-(2-(heptan-3-ylidene)hydrazono)thiazolidin-4-one (**9B**)

Orange oil, 77% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87–1.03 (m, 6H, 2 × CH_3), 1.27–1.31 (m, 1H, C(H)H), 1.34–1.46 (m, 2H, CH_2), 1.55–1.68 (m, 1H, C(H)H), 2.32–2.40 (m, 2H, CH_2), 2.43–2.48 (m, 2H, CH_2), 3.76 (s, 2H, CH_2-thiaz.), 4.97 (s, 2H, CH_2-benzyl), 7.28–7.34 (m, 3H, Ar), 7.46–7.47 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 10.98, 14.14, 24.56, 28.61, 29.61, 30.98, 32.31, 46.24, 127.88, 128.14, 128.72, 136.67, 160.53, 172.05, 172.61. Anal. Calcd. for $C_{17}H_{23}N_3OS$: C, 64.32; H, 7.30; N, 13.24. Found: C, 64.10; H, 7.55; N, 13.03.

4.47. 3-Benzyl-2-(2-(octan-2-ylidene)hydrazono)thiazolidin-4-one (**10B**)

White powder, mp 59–61 °C, 82% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90–0.93 (m, 3H, CH_3), 1.21–1.50 (m, 8H, 4 × CH_2), 2.00 (s, 3H, CH_3), 2.42–2.48 (m, 2H, CH_2), 3.80 (s, 2H, CH_2-thiaz.), 4.96 (s, 2H, CH_2-benzyl), 7.28–7.33 (m, 3H, Ar), 7.43–7.46 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.43, 17.53, 22.50, 26.06, 28.77, 31.57, 32.33, 38.14, 46.23, 127.96, 128.40, 128.80, 136.65, 160.06, 168.04, 172.61. Anal. Calcd. for $C_{18}H_{25}N_3OS$: C, 65.22; H, 7.60; N, 12.68. Found: C, 64.97; H, 7.89; N, 12.34.

4.48. 3-Benzyl-2-(2-cyclopentylidenehydrazono)thiazolidin-4-one (**11B**)

Yellow powder, mp 112–115 °C, 91% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81–1.83 (m, 4H, cyclopentane), 2.52–2.59 (m, 4H, cyclopentane), 3.81 (s, 2H, CH_2-thiaz.), 4.95 (s, 2H, CH_2-benzyl), 7.27–7.35 (m, 3H, Ar), 7.46–7.48 (d, 2H, J = 7.2 Hz, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 24.58, 24.86, 30.39, 32.37, 33.04, 46.23, 127.99, 128.62, 128.77, 136.61, 159.68, 172.49, 178.18. Anal. Calcd. for $C_{15}H_{17}N_3OS$: C, 62.69; H, 5.96; N, 14.62. Found: C, 62.97; H, 5.69; N, 14.43.

4.49. 3-Benzyl-2-(2-(2-methylcyclopentylidene)hydrazono) thiazolidin-4-one (**12B**)

Dark red oil, 73% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10 (d, 3H, J = 6.8 Hz, CH_3), 1.24–1.29 (m, 1H, cyclopentane), 1.41 (bs, 1H, cyclopentane), 1.56–1.59 (m, 1H, cyclopentane), 1.75–1.79 (m, 1H, cyclopentane), 1.96–1.99 (m, 1H, cyclopentane), 2.26–2.35 (m, 1H, cyclopentane), 2.40–2.48 (m, 1H, cyclopentane), 3.97 (s, 2H, CH_2-thiaz.), 4.77 (s, 2H, CH_2-benzyl), 7.31–7.35 (m, 3H, Ar), 7.43–7.45 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 19.35, 30.66, 32.01, 32.20, 33.54, 41.44, 46.28, 126.13, 127.14, 127.74, 136.25, 160.48, 172.01, 176.66. Anal. Calcd. for $C_{16}H_{19}N_3OS$: C, 63.76; H, 6.35; N, 13.94. Found: C, 63.94; H, 6.68; N, 14.23.

4.50. 3-Benzyl-2-(2-(3-methylcyclopentylidene)hydrazono) thiazolidin-4-one (**13B**)

White powder, mp 109–111 °C, 93% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12–1.14 (m, 3H, CH_3), 1.42–1.47 (m, 1H, cyclopentane), 2.00–2.27 (m, 6H, cyclopentane), 3.89 (s, 2H, CH_2-thiaz.), 4.94 (s, 2H, CH_2-benzyl), 7.32–7.36 (m, 3H, Ar), 7.42–7.51 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 20.03, 30.19, 32.36, 32.49, 32.98, 41.21, 46.22, 127.99, 128.62, 128.79, 136.60, 159.68, 172.51, 177.79. Anal. Calcd. for $C_{16}H_{19}N_3OS$: C, 63.76; H, 6.35; N, 13.94. Found: C, 63.99; H, 6.70; N, 14.18.

*4.51. 3-Benzyl-2-(2-cyclohexylidenehydrazono)thiazolidin-4-one (**14B**)*

Light brown powder, mp 89–91 °C, 77% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65–1.67 (m, 6H, cyclohexane), 2.38–2.42 (m, 2H, cyclohexane), 2.62–2.66 (m, 2H, cyclohexane), 3.77 (s, 2H, CH_2-thiaz.), 4.97 (s, 2H, CH_2-benzyl), 7.28–7.32 (m, 3H, Ar), 7.45–7.49 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 25.80, 26.59, 27.60, 28.52, 32.32, 46.20, 127.94, 128.28, 128.81, 136.67, 170.63, 172.61. Anal. Calcd. for $C_{16}H_{19}N_3OS$: C, 63.76; H, 6.35; N, 13.94. Found: C, 63.53; H, 6.08; N, 13.67.

*4.52. 3-Benzyl-2-(2-(2-methylcyclohexylidene)hydrazono) thiazolidin-4-one (**15B**)*

Light yellow powder, mp 90–92 °C; 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, 3H, J = 8.0 Hz, CH_3), 1.31–1.40 (m, 1H, cyclohexane), 1.45–1.54 (m, 2H, cyclohexane), 1.77–1.85 (m, 2H, cyclohexane), 1.94–2.07 (m, 2H, cyclohexane), 2.38–2.46 (m, 1H, cyclohexane), 3.19–3.25 (m, 1H, cyclohexane), 3.74 (s, 2H, CH_2-thiaz.), 4.97 (s, 2H, CH_2-benzyl), 7.29–7.31 (m, 3H, Ar), 7.45–7.47 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.33, 24.87, 27.03, 27.95, 32.26, 36.32, 39.12, 46.21, 127.92, 128.29, 128.79, 136.66, 161.11, 172.64 (one carbon missing due to overlapping signals). Anal. Calcd. for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32. Found: C, 64.49; H, 6.90; N, 13.12.

*4.53. 3-Benzyl-2-(2-(3-methylcyclohexylidene)hydrazono) thiazolidin-4-one (**16B**)*

Yellow powder, mp 46–47 °C, 91% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00 (d, 3H, J = 8.0 Hz, CH_3), 1.16–1.22 (m, 1H, cyclohexane), 1.40–1.94 (m, 6H, cyclohexane), 2.13–2.21 (m, 1H, cyclohexane), 2.45–2.55 (m, 1H, cyclohexane), 3.75 (s, 2H, CH_2-thiaz.), 4.95 (s, 2H, CH_2-benzyl), 7.28–7.31 (m, 3H, Ar), 7.43–7.47 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 18.55, 25.20, 28.44, 32.12, 34.89, 37.01, 46.29, 127.11, 128.87, 129.55, 137.10, 160.00, 170.89, 172.99 (one carbon missing due to overlapping signals). Anal. Calcd. for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32. Found: C, 64.43; H, 6.99; N, 13.04.

*4.54. 3-Benzyl-2-(2-(4-methylcyclohexylidene)hydrazono) thiazolidin-4-one (**17B**)*

Light yellow powder, mp 86–88 °C, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.98 (d, 3H, J = 8.0 Hz, CH_3), 1.12–1.32 (m, 2H, cyclohexane), 1.70–1.76 (m, 1H, cyclohexane), 1.86–2.02 (m, 4H, cyclohexane), 2.19–2.34 (m, 2H, cyclohexane), 3.78 (s, 2H, CH_2-thiaz.), 4.97 (s, 2H, CH_2-benzyl), 7.28–7.31 (m, 3H, Ar), 7.44–7.46 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 21.73, 27.61, 31.77, 32.32, 34.48, 35.50, 46.21, 127.94, 128.28, 128.80, 136.66, 160.41, 170.46, 172.58 (one carbon missing due to overlapping signals). Anal. Calcd. for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32. Found: C, 64.97; H, 6.48; N, 13.64.

*4.55. 3-Benzyl-2-(2-(2-tert-butylcyclohexylidene)hydrazono) thiazolidin-4-one (**18B**)*

Light yellow powder, mp 106–108 °C, 80% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (s, 9H, 3 × CH_3), 1.65–1.68 (m, 3H, cyclohexane), 1.72–1.76 (m, 1H, cyclohexane), 1.94–1.98 (m, 2H, cyclohexane), 2.31–2.40 (m, 1H, cyclohexane), 2.48–2.52 (m, 1H, cyclohexane), 3.33–3.35 (m, 1H, cyclohexane), 3.77 (s, 2H, CH_2-thiaz.), 4.90–5.09 (m, 2H, CH_2-benzyl), 7.24–7.32 (m, 3H, Ar), 7.40–7.42 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 25.42, 26.48, 27.02, 29.36, 30.79, 32.67, 34.03, 35.41, 46.29, 126.90, 128.62, 129.03, 136.11, 161.02, 170.39, 172.22. Anal. Calcd. for $C_{20}H_{27}N_3OS$: C, 67.19; H, 7.61; N, 11.75. Found: C, 66.99; H, 7.32; N, 11.47.

*4.56. 3-Benzyl-2-(2-cycloheptylidenehydrazono)thiazolidin-4-one (**19B**)*

White powder, mp 82–85 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51–1.70 (m, 8H, cycloheptane), 2.55–2.58 (m, 2H, cycloheptane), 2.62–2.65 (m, 2H, cycloheptane), 3.76 (s, 2H, CH_2-thiaz.), 4.95 (s, 2H, CH_2-benzyl), 7.27–7.33 (m, 3H, Ar), 7.44–7.46 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 24.93, 26.98, 29.71, 30.31, 31.79, 32.35, 36.75, 46.27, 127.95, 128.47, 128.77, 136.64, 159.47, 172.53, 173.39. Anal. Calcd. for $C_{17}H_{21}N_3OS$: C, 64.73; H, 6.71; N, 13.32. Found: C, 64.52; H, 6.44; N, 13.03.

*4.57. 3-Benzyl-2-(2-cyclooctylidenehydrazono)thiazolidin-4-one (**20B**)*

White powder, mp 82–84 °C, 77% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37–1.50 (m, 4H, cyclooctane), 1.52–1.59 (m, 2H, cyclooctane), 1.61–1.67 (m, 2H, cyclooctane), 1.82–1.86 (m, 2H, cyclooctane), 2.47–2.50 (t, 2H, cyclooctane), 2.52–2.55 (t, 2H, cyclooctane), 3.79 (s, 2H, CH_2-thiaz.), 4.97 (s, 2H, CH_2-benzyl), 7.28–7.32 (m, 3H, Ar), 7.45–7.46 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 23.01, 25.33, 26.56, 29.11, 30.78, 32.18, 32.88, 36.41, 46.30, 125.55, 128.01, 128.99, 137.50, 160.19, 172.34, 173.39. Anal. Calcd. for $C_{18}H_{23}N_3OS$: C, 65.62; H, 7.04; N, 12.75. Found: C, 65.40; H, 7.29; N, 12.50.

*4.58. 3-Benzyl-2-(2-(1-cyclohexylethylidene)hydrazono) thiazolidin-4-one (**21B**)*

Yellow powder, mp 66–68 °C, 85% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.04–1.23 (m, 6H, cyclohexane), 1.60–1.64 (m, 4H, cyclohexane), 1.78 (s, 3H, CH_3), 3.47 (s, 2H, CH_2-thiaz.), 4.74 (s, 2H, CH_2-benzyl), 7.05–7.12 (m, 3H, Ar), 7.24–7.26 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.92, 25.99, 26.17, 30.22, 32.32, 46.25, 46.34, 127.95, 128.41, 128.80, 136.66, 160.37, 171.18, 172.61. Anal. Calcd. for $C_{18}H_{23}N_3OS$: C, 65.62; H, 7.04; N, 12.75. Found: C, 65.87; H, 6.79; N, 12.91.

*4.59. 3-Benzyl-2-(2-(furan-2-ylmethylene)hydrazono)thiazolidin-4-one (**22B**)*

Brown powder, mp 80–82 °C, 91% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (s, 2H, CH_2-thiaz.), 5.01 (s, 2H, CH_2-benzyl), 6.53 (bs, 1H, furan), 6.85–6.87 (m, 1H, furan), 7.30–7.35 (m, 3H, Ar), 7.45–7.50 (m, 2H, Ar), 7.58–7.60 (m, 1H, furan), 8.30 (s, 1H, CH=). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.60, 46.19, 112.84, 116.76, 128.15, 128.86, 136.37, 146.55, 147.44, 149.59, 163.00, 164.15, 172.70. Anal. Calcd. for $C_{15}H_{13}N_3O_2S$: C, 60.18; H, 4.38; N, 14.04. Found: C, 59.95; H, 4.70; N, 13.71.

*4.60. 3-Benzyl-2-(2-(1-(furan-2-yl)ethylidene)hydrazono) thiazolidin-4-one (**23B**)*

Yellow powder, mp 102–104 °C, 86% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 3.82 (s, 2H, CH_2-thiaz.), 5.01 (s, 2H, CH_2-benzyl), 6.52–6.53 (m, 1H, furan), 6.89–6.90 (m, 1H, furan), 7.28–7.39 (m, 3H, Ar), 7.46–7.49 (m, 2H, Ar), 7.57–7.58 (m, 1H, furan). ^{13}C NMR (101 MHz, DMSO-d_6): δ 16.98, 32.44, 46.26, 113.55, 116.10, 129.47, 130.22, 136.89, 146.03, 148.14, 149.98, 162.14, 165.66, 172.81. Anal. Calcd. for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41. Found: C, 61.59; H, 4.61; N, 13.19.

*4.61. 3-Benzyl-2-(2-(thiophen-2-ylmethylene)hydrazono) thiazolidin-4-one (**24B**)*

White powder, mp 99–101 °C, 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 2H, CH_2-thiaz.), 5.01 (s, 2H, CH_2-benzyl), 7.08–7.11 (m, 1H, thiophene), 7.28–7.37 (m, 4H, Ar), 7.43–7.44 (m, 1H, Ar), 7.50–7.51 (m, 2H, thiophene), 8.57 (s, 1H, CH=). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.56, 46.14, 127.99, 128.08, 128.55, 128.92, 130.60, 132.92, 136.37, 139.07, 152.64, 163.87, 172.73. Anal. Calcd. for $C_{15}H_{13}N_3OS_2$: C, 57.12; H, 4.15; N, 13.32. Found: C, 57.44; H, 4.39; N, 13.60.

*4.62. 3-Benzyl-2-(2-(1-(thiophen-2-yl)ethylidene)hydrazono) thiazolidin-4-one (**25B**)*

Light orange powder, mp 105–107 °C, 77% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 4.03 (s, 2H, CH_2-thiaz.), 4.91 (s, 2H, CH_2-benzyl), 7.10–7.13 (m, 1H, thiophene), 7.14–7.41 (m, 5H, Ar), 7.51–7.53 (m, 1H, thiophene), 7.61–7.62 (m, 1H, thiophene). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.36, 32.57, 46.39, 128.03, 128.20, 128.44, 128.86, 129.20, 129.97, 136.57, 143.43, 158.14, 162.36, 172.70. Anal. Calcd. for $C_{16}H_{15}N_3OS_2$: C, 58.33; H, 4.59; N, 12.76. Found: C, 58.04; H, 4.78; N, 12.48.

*4.63. 3-Benzyl-2-(2-(1-phenylethylidene)hydrazono)thiazolidin-4-one (**26B**)*

Orange powder, mp 93–95 °C, 76%yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 4.04 (s, 2H, CH_2-thiaz.), 4.93 (s, 2H, CH_2-benzyl), 7.28–7.39 (m, 6H, Ar), 7.42–7.44 (m, 2H, Ar), 7.81–7.83 (m, 2H, Ar); ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.18, 32.54, 46.44, 126.93, 128.04, 128.42, 128.88, 130.42, 136.60, 138.06, 139.51, 162.38, 162.91, 172.72. Anal. Calcd. for $C_{18}H_{17}N_3OS$: C, 66.85; H, 5.30; N, 12.99. Found: C, 66.53; H, 5.57; N, 13.31.

*4.64. 3-Benzyl-2-(2-(1-(pyridin-2-yl)ethylidene)hydrazono) thiazolidin-4-one (**27B**)*

White powder, mp 163–165 °C, 89% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, CH_3), 4.06 (s, 2H, CH_2-thiaz.), 4.93 (s, 2H, CH_2-benzyl), 7.27–7.39 (m, 5H, Ar), 7.41–7.44 (m, 1H, pyridine), 7.83–7.87 (m, 1H, pyridine), 8.03–8.05 (m, 1H, pyridine), 8.60–8.61 (m, 1H, pyridine). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.09, 32.63, 46.50, 120.93, 125.09, 128.08, 128.49, 128.89, 136.50, 137.26, 149.32, 152.65, 163.17, 164.46, 172.79. Anal. Calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27. Found: C, 63.21; H, 5.18; N, 17.54.

*4.65. 3-Benzyl-2-(2-(pyridin-3-ylmethylene)hydrazono) thiazolidin-4-one (**28B**)*

Dark brown powder, mp 117–119 °C, 71% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.09 (s, 2H, CH_2-thiaz.), 4.92 (s, 2H, CH_2-benzyl), 7.27–7.36 (m, 5H, Ar), 7.48–7.51 (m, 1H, pyridine), 8.14–8.16 (m, 1H, pyridine), 8.54 (s, 1H, CH=), 8.63–8.64 (m, 1H, pyridine), 8.91 (s, 1H, pyridine). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.54, 46.44, 121.85, 124.52, 128.11, 128.48, 128.82, 130.59, 136.43, 138.19, 139.98, 163.88, 165.47, 172.62. Anal. Calcd. for $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05. Found: C, 62.20; H, 4.84; N, 18.24.

*4.66. 3-Benzyl-2-(2-(1-(pyridin-3-yl)ethylidene)hydrazono) thiazolidin-4-one (**29B**)*

Brown powder, mp 148–150 °C, 99% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, CH_3), 4.07 (s, 2H, CH_2-thiaz.), 4.95 (s, 2H, CH_2-benzyl), 7.30–7.41 (m, 5H, Ar), 7.46–7.49 (m, 1H, pyridine), 8.16–8.19 (m, 1H, pyridine), 8.62–8.63 (m, 1H, pyridine), 8.99–9.00 (m, 1H, pyridine). ^{13}C NMR (101 MHz, DMSO-d_6): δ 15.01, 32.62, 46.45, 124.02, 128.06, 128.42, 128.90, 134.00, 134.23, 136.54, 150.72, 160.63, 163.98, 172.77 (one carbon missing due to overlapping signals). Anal. Calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27. Found: C, 63.26; H, 5.21; N, 17.60.

*4.67. 3-Benzyl-2-(2-(pyridin-4-ylmethylene)hydrazono) thiazolidin-4-one (**30B**)*

Green powder, mp 190–192 °C, 75% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.09 (s, 2H, CH_2-thiaz.), 4.92 (s, 2H, CH_2-benzyl), 7.27–7.37 (m, 5H, Ar), 7.67–7.69 (m, 2H, pyridine), 8.49 (s, 1H, CH=), 8.66–8.67 (m, 2H, pyridine). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.69, 46.28, 121.86, 128.04, 128.95, 136.22, 141.62, 145.21, 150.79, 156.43, 167.02, 172.89. Anal. Calcd. for $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05. Found: C, 61.67; H, 4.22; N, 17.79.

*4.68. 3-Benzyl-2-(2-(1-(pyridin-4-yl)ethylidene)hydrazono) thiazolidin-4-one (**31B**)*

Green powder, mp 137–139 °C, 96% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.34 (s, 3H, CH_3), 4.07 (s, 2H, CH_2-thiaz.), 4.93 (s, 2H, CH_2-benzyl), 7.28–7.39 (m, 5H, Ar), 7.72–7.73 (m, 2H, pyridine), 8.63–8.64 (m, 2H, pyridine). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.77, 32.67, 46.51, 120.96, 128.07, 128.42, 128.90, 136.46, 144.93, 150.53, 160.81, 165.01, 172.77. Anal. Calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27. Found: C, 62.77; H, 4.75; N, 17.03.

*4.69. 2-(2-((1H-Indol-3-yl)methylene)hydrazono)-3-benzylthiazolidin-4-one (**32B**)*

Yellow powder, mp 238–240 °C, 90% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.01 (s, 2H, CH_2-thiaz.), 4.91 (s, 2H, CH_2-benzyl), 7.13–7.83 (m, 10H, indole + Ar), 8.58 (s, 1H, CH=), 11.68 (bs, 1H, NH, D_2O exch.). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.45, 46.12, 112.31, 112.47, 121.28, 122.48, 123.21, 124.98, 127.94, 128.12, 128.94, 132.62, 133.64, 140.96, 154.35, 160.70, 172.72. Anal. Calcd. for $C_{19}H_{16}N_4OS$: C, 65.50; H, 4.63; N, 16.08. Found: C, 65.34; H, 4.46; N, 15.80.

*4.70. 2-(2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazono)-3-benzylthiazolidin-4-one (**33B**)*

Light yellow powder, mp 153–155 °C, 78% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (s, 2H, CH_2-thiaz.), 5.05 (s, 2H, CH_2-benzyl), 6.04 (s, 2H, OCH_2O), 6.84 (d, 1H, J = 7.8 Hz, benzodioxole), 7.14 (d, 1H, J = 7.8 Hz, benzodioxole), 7.31–7.34 (m, 3H, Ar), 7.41 (s, 1H, benzodioxole), 7.49–7.51 (m, 2H, Ar), 8.40 (s, 1H, CH=). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.65, 45.77, 106.17, 108.57, 124.72, 128.01, 128.87, 128.92, 135.92, 148.35, 150.19, 157.41, 161.87, 163.83, 171.09, 172.76. Anal. Calcd. for $C_{18}H_{15}N_3O_3S$: C, 61.18; H, 4.28; N, 11.89. Found: C, 61.05; H, 4.07; N, 12.08.

*4.71. 2-(2-((Naphthalen-1-yl)methylene)hydrazono)-3-benzylthiazolidin-4-one (**34B**)*

Yellow powder, mp 137–139 °C, 83% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (s, 2H, CH_2-thiaz.), 5.09 (s, 2H, CH_2-benzyl), 7.31–7.38 (m, 3H, Ar), 7.52–7.58 (m, 4H, Ar), 7.61–7.66 (m, 1H, Ar), 7.90–7.97 (m, 3H, Ar), 8.93–8.95 (m, 1H, Ar), 9.10 (s, 1H, CH=). ^{13}C NMR (101 MHz, DMSO-d_6): δ 32.71, 46.28, 125.98, 126.84, 128.08, 128.97, 129.28, 129.79, 131.07, 131.94, 134.06, 136.37, 159.32, 163.91, 165.19, 173.09 (three carbons missing due to overlapping signals). Anal. Calcd. for $C_{21}H_{17}N_3OS$: C, 70.17; H, 4.77; N, 11.69. Found: C, 69.98; H, 4.34; N, 12.90.

*4.72. 2-(2-(1-(Naphthalen-2-yl)ethylidene)hydrazono)-3-benzylthiazolidin-4-one (**35B**)*

Light pink powder, mp 142–144 °C, 84% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.58 (s, 3H, CH_3), 3.83 (s, 2H, CH_2-thiaz.), 5.07 (s, 2H, CH_2-benzyl), 7.30–7.37 (m, 3H, Ar), 7.50–7.54 (m, 4H, Ar), 7.83–7.91 (m, 3H, Ar), 8.16–8.19 (m, 2H, Ar). ^{13}C NMR (101 MHz, DMSO-d_6): δ 14.88, 32.33, 46.18, 123.92, 127.52, 128.05, 128.42, 128.91, 133.17, 134.18, 136.61, 161.89, 163.17, 172.77 (five carbons missing due to overlapping signals). Anal. Calcd. for $C_{22}H_{19}N_3OS$: C, 70.75; H, 5.13; N, 11.25. Found: C, 70.50; H, 4.94; N, 11.00.

*4.73. 2-(2-(1-(2-Oxo-2H-chromen-3-yl)ethylidene)hydrazono)-3-benzylthiazolidin-4-one (**36B**)*

Yellow powder, mp 200–202 °C, 73% yield; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 4.05 (s, 2H, CH_2-thiaz.), 4.92 (s, 2H, CH_2-benzyl), 7.28–7.29 (m, 1H, chromene), 7.30–7.39 (m, 5H, Ar), 7.42–7.44 (m, 1H, chromene), 7.63–7.67 (m, 1H, chromene), 7.83–7.85 (m, 1H, chromene), 8.18 (s, 1H, chromene). ^{13}C NMR (101 MHz, DMSO-d_6): δ 17.70, 32.07, 46.44, 116.22, 119.05, 124.95, 126.49, 128.46, 128.89, 132.90, 136.49, 138.29, 139.57, 142.39, 153.94, 159.58, 161.63, 163.94, 172.67. Anal. Calcd. for $C_{21}H_{17}N_3O_3S$: C, 64.43; H, 4.38; N, 10.73. Found: C, 64.20; H, 4.54; N, 10.97.

*4.74. 2-(2-(1-(Ferrocenyl)ethylidene)hydrazono)-3-benzylthiazolidin-4-one (**37B**)*

Red powder; mp 118–120 °C, 74% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (s, 3H, CH_3), 3.77 (s, 2H, CH_2-thiaz.), 4.17 (s, 5H, ferrocene), 4.37–4.38 (m, 2H, ferrocene), 4.71–4.72 (m, 2H, ferrocene), 5.01 (s, 2H, CH_2-benzyl), 7.27–7.36 (m, 3H, Ar), 7.50–7.51 (m, 2H, Ar); ^{13}C NMR (101 MHz, DMSO-d_6): δ 16.21, 32.42, 46.33, 67.79, 69.67, 70.53, 82.98, 107.08, 128.01, 128.50, 128.84, 136.69, 146.41, 159.81, 164.30, 172.65. Anal. Calcd. for $C_{22}H_{21}FeN_3OS$: C, 61.26; H, 4.91; N, 9.74. Found: C, 61.01; H, 4.62; N, 9.50.

4.75. Five-day growth inhibition assay

Compounds were tested for the ability to inhibit tachyzoite growth over a period of 5 days using a published colorimetric assay method [8,25]. Briefly, compounds (20 mM DMSO stock solutions) were added to host human foreskin fibroblast (HFF) cells growing in 96-well plates followed by addition of *T. gondii* RH-2F (50839; ATCC, VA, USA). Serial 0.5 $\log_{10}$ dilutions (320–0.032 μM) of each compound were tested for anti-*T. gondii* as well as anti-HFF activity on each plate. Data were analyzed and the IC_{50}, IC_{90}, and TD_{50} calculated using Calcusyn software (Biosoft, Cambridge, U.K.). The therapeutic index (TI), a measure of specific anti-*T. gondii* activity, was calculated with the formula shown under Table 1.

4.76. Invasion assay

Selected compounds were examined for activity on purified, extracellular tachyzoites using an established red/green invasion assay [20,25]. Tachyzoites were exposed to compounds (10 μM) for 20 min at room temperature and then were allowed to infect host HFF cells growing on chamber slides. After 1 h the cells were rinsed, fixed and then immunostained using anti-*T. gondii* surface antigen (SAG-1 monoclonal (Abcam, USA) and polyclonal (ABD Serotec)), antibodies followed by fluorescent-labeled secondary antibodies. Invaded/penetrated tachyzoites are labeled green while tachyzoites attached to the surface, but unable to invade, are labeled red. A decrease in the number of penetrated tachyzoites relative to vehicle [VHL (DMSO)] is indicative of invasion inhibition. A decrease in the number of penetrated plus attached tachyzoites relative to VHL is indicative of inhibition of attachment. Data shown are mean values ± S.E.M. of three independent experiments.

4.77. Replication assay

The same selected compounds that were tested for invasion inhibition were further tested for inhibitory activity against intracellular parasites that had been allowed to invade host cells and establish an infection before the addition of compound using an established fluorescence-based protocol [20,25]. Compounds (10 μM) were added to established infections. After 24 h, the cells were rinsed, fixed and immunolabelled. The numbers of parasitophorous vacuoles (PV) containing 1, 2, 4, or 8+ tachyzoites/vacuole were enumerated. The modal number of tachyzoites/vacuole is compared to that of the VHL treated infected cells. A decrease in the number of tachyzoites in a vacuole indicates inhibition of replication. Data shown are mean values from three independent experiments.

4.78. Recovery assay

The Recovery assay is an extension of the Replication assay that can illuminate the ability of a compound to permanently rid the host cells of the parasite infection [20]. After the 24 h exposure to compounds described above, the cells are rinsed to remove the compound and then the infection is allowed to continue for 96 h. At this time the cells are rinsed, immunolabelled and examined for PV as described above and compared to the VHL. Compounds are considered potentially parasiticidal if the infection has not progressed, *i.e.*, there are no parasites or only singlet intracellular tachyzoites indicating no recovery from treatment.

4.79. Effect on extracellular tachyzoites

An extension of the Invasion assay in combination with the Replication assay was used to further explore the direct effect of the most active compounds on extracellular tachyzoites. Exposure of purified tachyzoites to compound was extended to 24 h at 4 °C before adding them to HFF cells. The infection was allowed to proceed for 24 h and then the cells were rinsed, fixed and processed as in the Replication assay. Cells were examined for a decrease in the number of tachyzoites/vacuole compared to VHL control.

5. Results and discussion

Collectively, we synthesized 74 novel thiazolidin-4-one derivatives in high yield exploring several and different substituents at the *N*1-hydrazine portion of the scaffold (ranging from small aliphatic chains to aromatic and bicyclic rings) and the influence of a benzyl group at the lactamic NH of the core nucleus on the biological activity. We have demonstrated that this new thiazolidin-4-one scaffold can be as effective at micromolar concentration as the reference drug (trimethoprim) in the inhibition of infection by *T. gondii*. More in detail:

a. In the five-day growth inhibition assay, HFF (human foreskin fibroblast) cells are grown in the presence of test compounds and tachyzoites for five days. Among the unsubstituted thiazolidinones (A series reported in Table 1), some compounds, especially in the (cyclo)aliphatic and (hetero)aryl derivatives, displayed at least moderate inhibition, while compounds **7A**, **10A**–**14A**, **27A**, **30A**, **34A** and **36A** were endowed with an encouraging inhibitory activity similar to or better than that of trimethoprim. In particular, derivatives **12A** and **27A** presented

IC_{50} values (0.9 and 2.9 μM, respectively) lower than that of trimethoprim (13 μM), although they were characterized by greater cytotoxicity. Moreover, the bicyclic aromatic compounds **34A** and **36A** demonstrated to possess not only an inhibitory activity (3.9 μM and 8.0 μM, respectively) better than that of the reference drug, but also a great selective effect against the parasite with high therapeutic index levels. The TI is a measure of specificity of a drug expressed as a ratio of the median cytotoxic dose (TD_{50}) divided by the median inhibitory concentration (IC_{50}). As far as the results of B series (N-benzylthiazolidinones, shown in Table 2), the aliphatic derivatives **3B**, **4B** and **9B**, the cycloaliphatic derivative **15B**, the (hetero)aryl derivatives **25B** and **31B**, and the bicyclic derivatives **34B** and **37B** have proven to be effective in this assay as anti-*Toxoplasma* agents, especially the metallocene **37B** which was endowed with an outstanding inhibitory effect (8.5 μM) against tachyzoites and low cytotoxicity with respect to the reference drug.

b. *T. gondii* establishes its lytic growth cycle in host cells through the active process of invasion. This process is initiated by attachment of the tachyzoite to the cell and ordinarily quickly progresses to penetration of that cell. The red/green invasion assay quantifies the ability of a compound to affect the attachment and/or the penetration steps of host cell invasion. In this experiment (Fig. 2), cells are incubated with fourteen test compounds for 20 min before being added to HFF cells. Fluorescent staining results in extracellular/attached parasites labeled red and intracellular/penetrated parasites labeled green. Thus, this assay demarcates compounds that can act extracellularly, directly on the parasite, from those that require the intracellular environment inside the host cell, in order to manifest anti-parasitic activity.

Generally, the most active and representative thiazolidin-4-ones selected for this analysis appeared to have the ability to inhibit the tachyzoite attachment and invasion (Fig. 2). Specifically, compounds with one asterisk caused a significant reduction in the number of penetrated (green) parasites. In this assay, an effect on attachment of parasites to host cells is defined as a decrease of numbers of both penetrated (green) and attached (red) parasites relative to that of the vehicle. It is important to note that the inhibition of attachment and the inhibition of penetration are not necessarily interrelated.

Among A series of thiazolidinones, the chromene derivative **36A** significantly inhibited tachyzoite invasion/penetration, whereas the cycloaliphatic products **11A**, **13A** and **14A** significantly inhibited both tachyzoite attachment and invasion/penetration. As regards the thiazolidinones belonging to B series, the heterocyclic derivative **25B** and the bicyclic derivatives **34B** and **37B** affected both parasite attachment and invasion/penetration.

c. The Replication assay is a relatively short duration assay that provides an estimation of the time required for activity onset as well as the compound's ability to inhibit an established intracellular infection. All of the newly synthesized derivatives with the notable exceptions of **36A** and **34B** strongly blocked replication at 10 μM (Fig. 3).

d. Compounds **34A** and **37B** were further tested in the Recovery assay. Neither compound was able to permanently kill the tachyzoites as the parasite, although initially slowed in replication, was able to recover from compound treatment (data not shown).

e. Likewise **37B** was tested for the ability to inhibit tachyzoite replication by direct action on extracellular tachyzoites. Tachyzoites exposed to **37B** for 24 h pre-infection were able to replicate normally indicating that the anti-*T. gondii* activity of **37B** does not appear to be manifested *via* direct action on the tachyzoite (data not shown).

6. Conclusions

We have synthesized a large number of new 1,3-thiazolidin-4-one derivatives to assess their inhibitory activity against *Toxoplasma* parasite. Fourteen of them stood out as promising anti-parasitic agents possessing an activity similar or better than that of trimethoprim against *Toxoplasma* and a comparable or lower cytotoxicity with respect to the reference drug. Furthermore, we have also documented that among the most active compounds, some derivatives strongly blocked the parasite attachment and invasion of the host cell. Finally, it is of note that most of these fourteen derivatives inhibited the parasite replication at 10 μM. These results demonstrated the importance of this thiazolidinone scaffold for the development of new anti-parasitic drugs.

Conflict of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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Appendix A. Supplementary data

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References

[1] (a) P. Kodym, M. Malý, O. Beran, D. Jilich, H. Rozsypal, L. Machala, M. Holub, Epidemiol. Infect. 22 (2014) 1–8;
(b) X.L. Li, H.X. Wei, H. Zhang, H.J. Peng, D.S. Lindsay, PLoS One 9 (2014) e97775.
[2] J.D. Schwartzman, J.H. Maguire, Toxoplasmosis in Tropical Infectious Diseases: Principles, Pathogens and Practice, third ed., W. B. Saunders, Edinburgh, U.K, 2011, pp. 722–728 (Chapter 103).
[3] J.L. Jones, M.E. Parise, A.E. Fiore, Am. J. Trop. Med. Hyg. 90 (2014) 794–799.
[4] A.M. Tenter, A.R. Heckeroth, L.M. Weiss, Int. J. Parasitol. 30 (2000) 1217–1258.
[5] D.E. Hill, S. Chirukandoth, J.P. Dubey, Anim. Health Res. Rev. 6 (2005) 41–61.
[6] S. Kongsaengdao, K. Samintarapanya, K. Oranratnachai, W. Prapakarn, C. Apichartpiyakul, J. Int. Assoc. Physicians AIDS Care 7 (2008) 11.
[7] R.H. Yolken, S. Bachmann, I. Rouslanova, E. Lillehoj, G. Ford, E.F. Torrey, J. Schroeder, Clin. Infect. Dis. 32 (2001) 842–844.
[8] L. Jones-Brando, E.F. Torrey, R. Yolken, Schizophr. Res. 62 (2003) 237–244.
[9] J.B. Rodriguez, S.H. Szajnman, Expert Opin. Ther. Pat. 22 (2012) 311–334.
[10] G. García Liñares, E.L. Ravaschino, J.B. Rodriguez, Curr. Med. Chem. 13 (2006) 335–360.
[11] P. Meneceur, M. Bouldouyre, D. Aubert, I. Villena, J. Menotti, V. Sauvage, J. Garin, F. Derouin, Antimicrob. Agents Chemother. 52 (2008) 1269.
[12] J.S. Doggett, A. Nilsen, I. Forquer, K.W. Wegmann, L. Jones-Brando, R.H. Yolken, C. Bordón, S.A. Charman, K. Katneni, T. Schultz, J.N. Burrows, D.J. Hinrichs, B. Meunier, V.B. Carruthers, M.K. Riscoe, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 15936–15941.
[13] E.J. Mui, G.A. Schiehser, W.K. Milhous, H. Hsu, C.W. Roberts, M. Kirisits, S. Muench, D. Rice, J.P. Dubey, J.W. Fowble, P.K. Rathod, S.F. Queener, S.R. Liu, D.P. Jacobus, R. McLeod, PLoS Negl. Trop. Dis. 2 (2008) 1–13.
[14] A. Gangjee, O.O. Adair, M. Pagley, S.F. Queener, J. Med. Chem. 51 (2008) 6195–6200.
[15] B. Krivogorsky, P. Grundt, R. Yolken, L. Jones-Brando, Antimicrob. Agents Chemother. 52 (2008) 4466–4469.
[16] J.S. Strobl, C.W. Seibert, Y. Li, R. Nagarkatti, S.M. Mitchell, A.C. Rosypal, D. Rathore, D.S. Lindsay, J. Parasitol. 95 (2009) 215–223.
[17] J.G.A. Walton, S. Patterson, G. Liu, J.D. Haraldsen, J.J. Hollick, A.M.Z. Slawin, G.E. Ward, N.J. Westwood, Org. Biomol. Chem. 7 (2009) 3049–3060.
[18] J. Müller, A. Hemphill, Exp. Parasitol. 128 (2011) 145–150.

[19] A.P. Liesen, T.M. de Aquino, C.S. Carvalho, V.T. Lima, J.M. de Araújo, J.G. de Lima, A.R. de Faria, E.J. de Melo, A.J. Alves, E.W. Alves, A.Q. Alves, A.J. Góes, Eur. J. Med. Chem. 45 (2010) 3685–3691.
[20] C.P. Hencken, L. Jones-Brando, C. Bordón, R. Stohler, B.T. Mott, R. Yolken, G.H. Posner, L.E. Woodard, J. Med. Chem. 53 (2010) 3594–3601.
[21] C.S. Carvalho, E.J. de Melo, R.P. Tenório, A.J. Góes, Braz. J. Med. Biol. Res. 43 (2010) 139–149.
[22] T.M. de Aquino, A.P. Liesen, R.E. da Silva, V.T. Lima, C.S. Carvalho, A.R. de Faria, J.M. de Araújo, J.G. de Lima, A.J. Alves, E.J. de Melo, A.J. Góes, Bioorg. Med. Chem. 16 (2008) 446–456.
[23] R.P. Tenório, C.S. Carvalho, C.S. Pessanha, J.G. de Lima, A.R. de Faria, A.J. Alves, E.J. de Melo, A.J. Góes, Bioorg. Med. Chem. Lett. 15 (2005) 2575–2578.
[24] R.A. Tapia, L. Alegria, C.D. Pessoa, C. Salas, M.J. Cortés, J.A. Valderrama, M.E. Sarciron, F. Pautet, N. Walchshofer, H. Fillion, Bioorg. Med. Chem. 11 (2003) 2175–2182.
[25] F. Chimenti, B. Bizzarri, A. Bolasco, D. Secci, P. Chimenti, S. Carradori, A. Granese, D. Rivanera, N. Frishberg, C. Bordón, L. Jones-Brando, J. Med. Chem. 52 (2009) 4574–4577.
[26] A.P. Liesen, T.M. de Aquino, C.S. Carvalho, V.T. Lima, J.M. de Araújo, J.G. de Lima, A.R. de Faria, E.J.T. de Melo, A.J. Alves, E.W. Alves, A.Q. Alves, A.J.S. Góes, Eur. J. Med. Chem. 45 (2010) 3685–3691.
[27] (a) S. Carradori, D. Secci, M. D'Ascenzio, P. Chimenti, A. Bolasco, J. Heterocycl. Chem. (2014), http://dx.doi.org/10.1002/jhet.1856;
(b) D. Secci, A. Bolasco, S. Carradori, M. D'Ascenzio, R. Nescatelli, M. Yáñez, Eur. J. Med. Chem. 58 (2012) 405–417.
[28] S. Carradori, D. Rotili, C. De Monte, A. Lenoci, M. D'Ascenzio, V. Rodriguez, M. Miceli, A.P. Filetici, Nebbioso, L. Altucci, D. Secci, A. Mai, Eur. J. Med. Chem. 80 (2014) 569–578.