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ARTICLE

Synthesis of bicyclic 1,4-thiazepines as novel anti-*Trypanosoma brucei brucei* agents.

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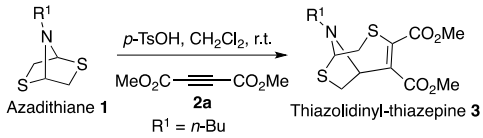
1,4-Thiazepines derivatives are pharmacologically important heterocycles with different applications in medicinal chemistry. In the present work, we describe the preparation of new bicyclic thiazolidinyl-1,4-thiazepines **3** by reaction between azadithiane compounds and Michael acceptors. The reaction scope was explored and the yields were optimized. The activity of new compounds was evaluated against *Nippostrongylus brasiliensis* and *Caenorhabditis elegans* as anthelmintic models and *Trypanosoma brucei brucei.* The most active compound was **3l**, showing an EC_{50} = 2.8 ± 0.7 µM against *T. b. brucei* and a selectivity index >71.

Introduction

Parasitic diseases affect hundreds of millions of people worldwide and result in significant mortality and devastating social and economic consequences.[1] However, most of the drugs available are decades old and have several limitations, such as poor efficacy, toxicity and the emergence and spread of drug resistance. The scientific community is concerned about this problem and fortunately has showed an increasing interest in this field, including efforts to identify new targets leading to the development of potential drug candidates.[2] In this context, our recent research is focused on the preparation of discrete libraries designed against specific key targets of parasites and bacteria. These include the essential redox enzymes thioredoxin glutathione reductase (TGR) of platyhelminths,[3] the cysteine protease expressed by *Trypanosoma cruzi* cruzipain,[4] and the metallo-β-lactamase enzymes of bacteria.[5]

Over the past decade, the design and preparation of new libraries of compounds has been directed to increasing molecular diversity,[6] representing a challenge for organic chemists.[7] In this context, our group has previously prepared small libraries containing N, O- and S-fused heterocycles.[3,8] In an earlier approach, we synthesized a novel bicyclic scaffold: thiazolidinyl-2,3,4,5-tetrahydro-1,4-thiazepine **3a**. The compound was prepared by condensation of azadithiane **1a** and dimethylacetylene-dicarboxylate **2a** through a domino process (see Scheme 1).[9,10]



Scheme 1. Previously prepared thiazolidinyl-thiazepine 3, 3a: R^1 = *n*-Bu.

Thiazepinone and thiazepine derivatives are pharmacologically important compounds with potential applications in the treatment of cancer and inflammatory diseases.[11,12] Benzothiazepines (A) are privileged scaffolds that exhibit interesting biological activities,[13] thiazolothiazepines (B) and 1,3-thiazepine derivatives (C) have shown antiviral activity,[14] see Figure 1.

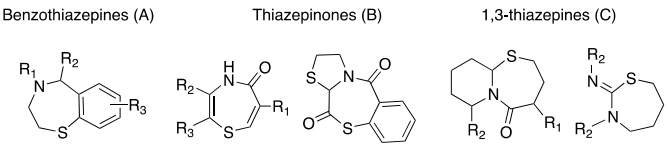


Figure 1. Representative structures of 1,4 and 1,3-thiazepines with biological interest.

Thus, the thiazolinindyl-2,3,4,5-tetrahydro-1,4-thiazepines **3** are new and therefore unexplored bicycles that represent a promising scaffold for medicinal chemistry. Hence we designed and synthesized a library of bicyclic thiazolidinyl-1,4-

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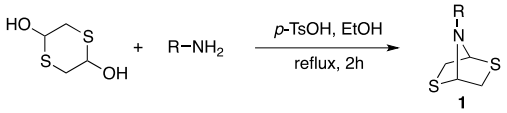
thiazepines, introducing different functional groups at the N-moiety and/or at the ring substituents. A primary screening of compounds as potential antiparasitic agents against helminths and protozoa was undertaken.

Results and discussion

In order to determine the best conditions for the preparation of thiazolidinyl-1,4-thiazepines **3** (Scheme 1), we prepared five azadithianes **1** to be used as starting materials. We selected primary amines bearing benzyl groups with EWG or EDG and aliphatic amines. The condensation of 1,4-dithiane-2,5-diol with primary amines in *p*-TsOH/EtOH at reflux gave the corresponding azadithianes **1** as a single product. Compounds **1b-f** were obtained in high to very high yields (64 to 93 %), see Table 1.

Table 1. Synthesis of 2,5-dithio-7-azabicyclo[2.2.1]heptane **1**.



Entry	R	Product	Yield %
1	Bn	**1b**	76
2	*p*-OMe Bn	**1c**	64
3	*p*-Cl Bn	**1d**	85
4	*p*-F Bn	**1e**	93
5	CH_2CH_2OH	**1f**	86
6	$CH_2CH_2CH_2COOH$	**1g**	13

Azadithianes **1b-f** were used as nucleophiles with different Michael acceptors in order to prepare thiazepines **3**.

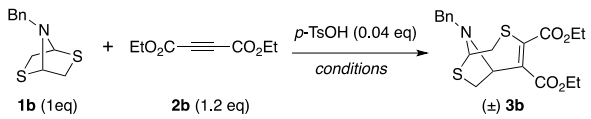
Optimization of thiazepine 3 formation

The optimization was performed studying different reaction conditions. Variables included the catalyst, the solvent, the heating source and the temperature. To determine the optimal reaction conditions, benzyl-azadithiane **1b** and the commercially available diethylacetylene-dicarboxylate **2b** were used as starting materials, see Table 2.

The reaction was firstly performed in CH_2Cl_2 at room temperature and at reflux under conventional thermal heating (Table 2, entries 1 and 2). We then explored the use of microwave as a source of heating and MeCN as solvent, affording better yields (entry 3) compared to conventional heating. Finally a preliminary solvent screening was performed using microwave heating (Table 2, entry 4 to 6).

Solvent optimization included the use of halogenated solvents ($ClCH_2CH_2Cl$ and CH_2Cl_2), polar protic solvents (CF_3CH_2OH, BuOH) and a polar aprotic solvent (MeCN). The best results were obtained using MeCN, a solvent with high dielectric constant and dipolar moment, affording **3b** in 47% yield.

Table 2. Solvent and temperature optimization for **3b** preparation.



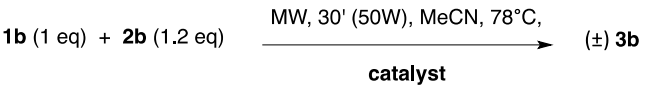
1b (1eq) **2b** (1.2 eq) (±) **3b**

Entry	Solvent	Conditions	**3b** Yield (%)[a]
1	CH_2Cl_2	Overnight, rt	35
2	CH_2Cl_2	4h, reflux	40
3	MeCN	30', 78°C, MW	47
4	$ClCH_2CH_2Cl$	30', 78°C, MW	27
5	CF_3CH_2OH	30', 68°C, MW	5
6	BuOH	30', 110°C, MW	29

[a] Isolated yield.

Once we selected the best solvent, we explored the influence of the catalyst in the reaction; results are shown in Table 3. Based on previous reports, we used a Lewis acid (CF_3SO_3Ag), different bases (DABCO and DMAP),PPh_3 and NH_4OAc as possible catalysts for the thia-Michael addition.[15]

Table 3. Catalyst optimization for **3b** preparation.



Entry	Catalyst	**3b** Yield (%)*
1	No catalyst	36
2	CF_3SO_3Ag (0.04 eq)	29
3	NH_4OAc (0.1 eq)	47
4	PPh_3 (0.2 eq)	42
5	DMAP (0.1 eq)	56
6	DABCO (0.1 eq)	67

* Yields were determined after column purification.

We first assayed the reaction with no catalyst, obtaining **3b** in 36% yield (entry 1). While the use of CF_3SO_3Ag did not improve the yield of the reaction, NH_4OAc slightly increased the yield to 47%, see entry 3. The reaction in presence of catalytic PPh_3 gave 42% yield. The best results were obtained when using bases like DMAP or DABCO (0.1 eq), leading to thiazepine **3b** in 57 and 67% yields respectively; see entries 5 and 6.

Taking these results together, it seems that nucleophilic assistance, in particular by basic-like catalysts (i.e. DABCO or DMAP) improve the addition reaction (see entries 3-6).

The polar nature of the solvent suggests the formation of charged species during the course of the reaction. As depicted in Scheme 2, we propose a mechanism based on a thiolate-iminium **I** formation via azadithiane ring opening. Polar aprotic solvents and temperature could favour the thiolate **I** formation see Scheme 2(a). The base could participate reacting with

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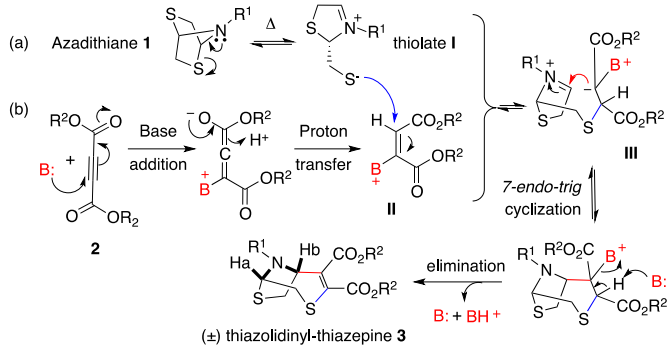
Michael acceptor **2**, affording intermediate **II**, see Scheme 2 (b).[15] Thiolate **I** would attack intermediate **II** affording zwitterion **III**, which could then be trapped through a *7-endo-trig* cyclization to afford the bicyclic product **3** after elimination of the base. This cyclization is favoured according to Baldwin's rules.[16]



Scheme 2. Proposed mechanism for the formation of thiazolidinylthiazepine **3.**

We had previously proposed the structure of thiazolidinyl-thiazepine **3** based on the long-range W coupling between Ha and Hb (^4J = 0.8 to 1.0 Hz). In this paper we report the confirmation of the molecular structure of **3d** by single crystal X-ray diffraction (Figure 2).

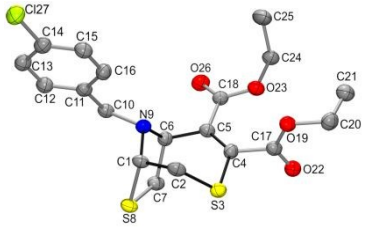
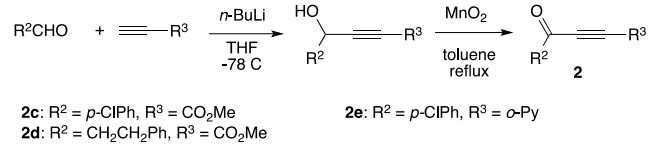


Figure 2. ORTEP diagram of compound **3d**.

Single crystals of compound **3d** were obtained from saturated CH_2Cl_2 solution. Compound **3d** crystallized in the centrosymmetric monoclinic $P2_1/n$ space group with one molecule of **3d** in the asymmetric unit. Table S1 in the Supporting Information contains selected bond distances and angles while Table S2 contains the data collection and refinement details. The molecular structure confirms the insertion of C=C fragment into one of the two S–C(N) bonds and therefore the formation of the seven-membered 1,4-thiazepine ring. Furthermore, as can be expected from the proposed mechanism, both enantiomers of **3d** are observed in the crystal.

Study of the reaction scope

To study the scope and versatility of the reaction, we used different Michael acceptors **2**, prepared by addition of lithium acetylenes to different aldehydes, followed by MnO_2 alcohol oxidation, see Scheme 3.[17]

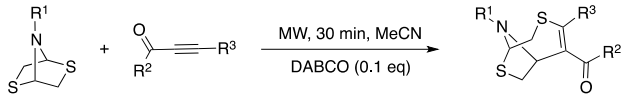


2c: R^2 = *p*-ClPh, R^3 = CO_2Me
2d: R^2 = CH_2CH_2Ph, R^3 = CO_2Me
2e: R^2 = *p*-ClPh, R^3 = *o*-Py

Scheme 3. Synthesis of Michael acceptors **2**.

We therefore proceed to study the reaction scope between different primary amines **1** (described in Table 1) and the prepared alkynes **2** as Michael acceptors, see Table 4.

Table 4. Study of the reaction scope using different Michael acceptors.



1 **2** (±) 1,4-thiazolidinyl-thiazepines **3**

Entry	R^1	R^2	R^3	Prod	Yield (%)[a]
1	**1b** (R^1 = Bn)	-OEt	$-CO_2Et$	**3b**	67
2	**1c** (R^1 = p-OMeBn)	-OEt	$-CO_2Et$	**3c**	72
3	**1d** (R^1 = p-Cl Bn)	-OEt	$-CO_2Et$	**3d**	75
4	**1e** (R^1 = p-F Bn)	-OEt	$-CO_2Et$	**3e**	61
5	**1f** (R^1=$(CH_2)_2OH$)	-PhpCl	$-CO_2Me$	**3f**	41
6	**1c** (R^1 = p-OMeBn)	-PhpCl	$-CO_2Me$	**3g**	83
7	**1b** (R^1 = Bn)	-PhpCl	$-CO_2Me$	**3h**	34[b]
8	**1b** (R^1 = Bn)	$(CH_2)_2Ph$	$-CO_2Me$	**3i**	65[b]
9	**1b** (R^1 = Bn)	-PhpCl	-2-Py	**3j**	NR[c]

[a] Yields were calculated after purification. [b] Reaction conditions were CH_2Cl_2/*p*-TsOH, reflux. [c] NR = no reaction was observed.

As we can observe in Table 4, the reaction is versatile and occurs in presence of different Michael acceptors **2**. When we used the symmetric diethylacetylene-dicarboxylate **2b** (entries 1 to 4), the reaction occurred as expected, affording the corresponding thiazolidinyl-thiazepines **3** in high to very high yields. When we set up the reaction with alkynyl ketone **2c** (see Scheme 3), containing an ester moiety and a ketone, we observed thiazepines **3g** and **3i** in better yields (entries 6 and 8). This result was expected because the ketone has a more electrophilic centre that favours product formation. The best yield is observed in entry 6, using Michael acceptor **2c**. No reaction was observed with **2e**, probably due to the absence of a di-carbonyl system (entry 9).

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Hybrids design and synthesis

Once we prepared the library of thiazepines **3b-i**, we designed and synthesized hybrid compounds, which combine the thiazepine scaffold with a furoxan ring (Figure 3). We selected furoxans as they are extremely valuable frameworks with interestingly biological properties to target the library against trypanosomatids and helminths. Recently Salinas and co-workers have reported furoxan-containing compounds that inhibit TGRs from *Fasciola hepatica* and *Echinococcus granulosus*, both classes of parasitic flatworms: trematoda and cestoda respectively.[18] With respect to trypanosomatids, the furoxan scaffold is also present in compounds with activity against *T. cruzi*[19] and *T. b. brucei*.[20]

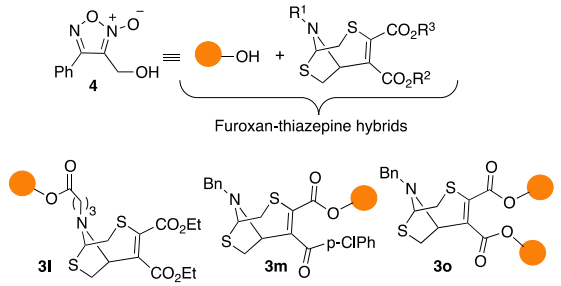
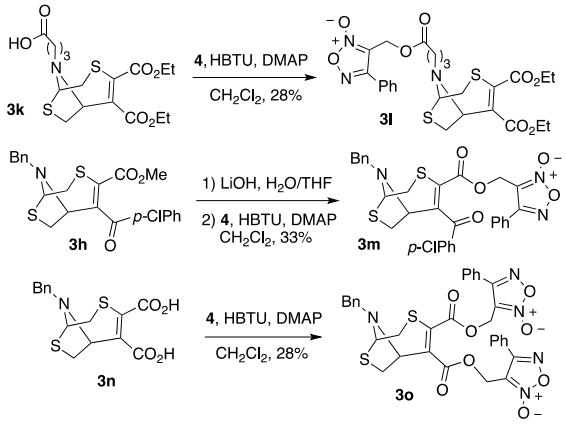


Figure 3. Designed furoxan-thiazepine hybrids **3l, 3m** and **3o**.

Furoxan **4** was prepared according to the general procedure described by Maloney group.[21] Hybrid compounds were prepared according to Scheme 4.



Scheme 4. Preparation of hybrids **3l**, **3m** and **3o**.

Anthelmintic activity

1. Thioredoxin glutathione reductase (TGR) inhibition. The selenoenzyme TGR was selected as molecular target because it is ubiquitous and indispensable for flatworm parasites,[22] and because the furoxan moiety of our hybrid compounds had previously shown activity as TGR inhibitor.[18] Prepared compounds were screened as potential inhibitors of TGR using an in vitro spectrophotometric assay. Tested at 50 µM, none of the thiazepines and furoxan-hybrids shows significant inhibitory activity of the enzyme (Table 5).

Complementary, we also screened the prepared compounds against the nematodes *Nippostrongylus brasiliensis* and *Caenorhabditis elegans* as models for anthelmintic discovery. Both species are close relatives to hookworms and to *H. contortus,* one of the most pathogenic nematodes of ruminants[23] since all of them belong to nematode clade V.

2. N. brasiliensis L4. We assayed the library of thiazepines at 20 µM, results are shown in Table 5. Compounds display only modest activity, the best performance was observed for thiazepine **3e**, bearing a *p*-F benzyl group in R^1 (*N. brasiliensis* death of 47%).

3. C. elegans as a model for anthelmintic screening. Traditional screens that rely on parasitic worms are costly and labor intensive. Recently, P. Roy and co-workers established *C. elegans*, a free-living nematode, as an effective and cost-efficient model system for anthelmintic discovery.[24] Compounds were screened at 100 µM, using a sensitive motility assay. The results are shown in Table 5. The library compounds did not show remarkable activity on this model.

Table 5. Anthelmintic evaluation of thiazepines **3b-h** and hybrids **3l**, **3m** and **3o.**

Entry	Cmp	*N. brasiliensis* death % [20µM][a]	% TGR inhibition [50 µM][b]	% *C. elegans* motility inhibition [100 µM][c]
1	**3b**	5.9	0	20
2	**3c**	13.8	36	50
3	**3d**	17.1	0	37
4	**3e**	47.6	41	26
5	**3f**	20.6	0	18
6	**3g**	27.6	29	3
7	**3h**	20.5	0	51
8	**3l**	-	0	22
9	**3m**	-	0	-
10	**3o**	-	-	-
11	Abz	100	N/A	N/A
12	Iver	N/A	N/A	100
13	Aur	N/A	100	N/A

[a] Death percentage of *Nippostrongylus brasiliensis* [L4] at [inh] = 20 µM. Albendazole EC_{50} = 0.34 µM). [b] Percentage of thiorredoxin glutathione reductase (TGR) inhibition (%) was measured at [inh] = 50 µM. Auranofin was used as reference, showing 85% inhibition at 30nM. [c] *C. elegans* motility inhibition assay was performed at [inh] = 100 µM. Reference was performed with 2% DMSO, corresponding to 0% motility inhibition. N/A not assayed.

In summary, we explored the anthelmintic activity of the library through three different model assays, but neither the thiazepine nor the hybrids were active.

Anti- Trypanosoma activity

The anti-trypanosomal activity of the thiazepines library was evaluated against the bloodstream form of *T. brucei brucei*, causative agent of Nagana disease of cattle and suitable model of *T. b. rhodesiense*, which causes acute Human African trypanosomiasis. The compounds were screened at 30 µM and 5 µM and the % of parasite death determined by flow cytometry (Table 6). Compounds showing *T. brucei* death at 30 µM > 90% were selected for EC_{50} determination against parasites and murine macrophages. The selectivity index was calculated as EC_{50} macrophage/EC_{50} *T. b. brucei*. *Trypanosoma brucei brucei* strain 427, cell line 449 (a kind gift of Dr. R. Luise Krauth-Siegel, Heildeberg University, Germany) and mouse macrophages from the cell line J774 (purchased from the American Type Culture Collection, ATCC® code TIB-67™) were used in the experiments.

Thiazepine scaffolds **3b-h** *evaluation*

With the exception of **3f**, all thiazepines exerted more than 50% parasite death when tested at 30 µM. Thiazepine **3f** was the less active compound; the structural feature is the presence of a hydroxyethyl group instead of the benzyl group in the N bridge. This points out the importance of R^1 substituent for the activity, feature that could guide further exploration at this level. EC_{50} of thiazepines **3b-e** and **3g** were determined, data shown in Table 6. The anti-trypanosomal activity of all the selected compounds is of the same order than that of the reference drug Nfx. Thiazepine **3d** (R^1 = *p*-ClBn) was the best compound displaying an EC_{50} = 8 µM. Thiazepines **3e** and **3g** were toxic for macrophages with SI = 3.6 and 0.6 respectively. Interestingly, at 200 µM our best compound **3d** lacked toxicity against macrophages (SI > 25).

Table 6. Anti-*T. b. brucei* activity of thiazolidinyl-thiazepines **3b-h** and furoxan-thiazepine hybrids **3l, 3m** and **3o**. Values represent the mean ± SD (n = 3).

Comp	death at 30 µM	death at 5 µM	EC_{50} (µM)	MΦ EC_{50} (µM)	SI[a]
3b	98.8 ± 0.3	3.5 ± 3.7	18.9 ± 2.6	200 ± 11	10
3c	97.7 ± 0.3	3.0 ± 0.5	18.0 ± 2.0	>200	> 11
3d	97.3 ± 0.4	2.4 ± 1.2	8.0 ± 0.4	> 200	> 25
3e	99.4 ± 0.1	12.7 ± 1.7	17.8 ± 3.7	64.4 ± 3.5	3.6
3f	3.6± 1.2	1.8± 5.7	-	-	-
3g	95.9± 0.6	0 ± 5.0	16.4 ± 1.5	9.7 ± 0.6	0.6
3h	57.5 ± 2.2	3.4 ± 13.4	-	-	-
3l	97.8 ± 0.5	60.4 ± 1.2	2.8 ± 0.7	> 200	> 71
3m	97.7 ± 0.1	89.4 ± 2.1	2.5 ± 0.2	3.1 ± 0.5	1.2
3o	23.3 ± 1.1	12.2 ± 2.2	-	-	-
Nfx	-	-	15 ± 3	150 ± 5	10

[a] Selectivity index = EC_{50} macrophage/EC_{50} *T. b. brucei*.

Furoxan-hybrids **3l-o** *evaluation*

Hybrids containing one furoxan ring **3l** and **3m** are both more active than Nfx and also more active than thiazepine **3d**. If we compare hybrid **3m** with its precursor thiazepine **3h**, the first is 9-fold more active than the second when assayed at 5 µM. The introduction of furoxan rings in R^1 or R^2 is beneficial for the anti-trypanosomal activity. In contrast, compound **3o** bearing two furoxan rings at R^2 and R^3 is inactive, which suggests that introducing a furoxan at position R^3 is detrimental for activity. The hybrids **3l** and **3m** were selected to determine EC_{50} in *T. b. brucei* (EC_{50} = 2.8 ± 0.7 µM and EC_{50} = 2.5 ± 0.2 µM respectively), see Table 6.

Strikingly, while **3l** lacked cytotoxicity at 200 µM, **3m** resulted highly toxic to macrophages (EC_{50} = 3.1 µM). Compound **3l** displayed the highest selectivity against *T. brucei* (SI>71).

Drug-like properties

Finally, we calculated Lipinski parameters[25] to analyse the chemical and physical properties of the thiazepine library and evaluate its druglikeness (Table 7).

Thiazepines **3b-h** follow the rule of 5: log P under 5, MW under 500 dalton, HBA<10 and HBD<5. This suggests that bicyclic scaffolds would have good bioavailability when administered orally, which could encourage the exploration of other relevant biological activities for these compounds.

Table 7. Lipinski's parameters[a] for thiazepines **3** and % *T. b. brucei* death at 30 µM.

Entry	Comp	log P	MW	HBD	HBA	% Death [30 µM]
1	**3b**	2.8	393	0	5	98.8 ± 0.3
2	**3c**	2.68	423	0	6	97.7 ± 0.3
3	**3d**	3.36	427	0	5	97.3 ± 0.4
4	**3e**	2.96	411	0	5	99.4 ± 0.1
5	**3f**	1.82	399	1	6	3.6± 1.2
6	**3g**	3.95	475	0	6	95.9± 0.6
7	**3h**	4.07	445	0	5	57.5 ± 2.2
8	**3l**	3.57	563	0	10	97.8 ± 0.5
9	**3m**	5.48	605	0	7	97.7 ± 0.1
10	**3o**	4.93	685	0	11	76.7 ± 1.1

[a]Calculated according to the method of Ghose, Pritchett and Crippen in Spartan 10.

The compound with the best inhibition profile **3l** breaks one of the rules (MW). Nonetheless, there is a significant number of drugs in the 500 < MW < 600 range; molecules are still considered drug-like despite breaking one of the rules. According to Roughley's analyses, 36% of drugs break at least one rule.[26]

Conclusions

In conclusion, we optimized the synthesis of thiazepines **3**, using DABCO as catalyst and microwave heating. X-Ray single-crystal diffraction confirmed the bicyclic structure of compound **3b** and a mechanism for the base catalysis was proposed.

We were able to prepare a small library of thiazolidinyl-thiazepines **3b-h** and furoxan-hybrids **3l, 3m** and **3o**, whose antiparasitic activity was evaluated against four different

targets. Thiazepine scaffolds **3b-d** show good activity against the infective form of *T. b. brucei* and low cytotoxicity against murine macrophages, the best compound was **3d** (EC_{50} = 8.0 ± 0.4 and SI > 25). Hybrids **3l** and **3m** bearing one furoxan ring in different position, present the highest potency (EC_{50} = 2.8 ± 0.7 and 2.5 ± 0.2 μM respectively). Taking into account the cytotoxicity against a mammalian cell model, the most selective compound is **3l** with an excellent SI (>71).

These results are encouraging due to the good anti-*T. b. brucei* activity and the novelty of the thiazepine scaffold. Compound **3d** and derivatives could be used as leaders for further studies against trypanosomatids.

Supplementary data

Supplementary data to this article can be found online at…
CCDC-1885443 contains the supplementary crystallographic data for this paper. Copies of the data can be obtained free of charge via http://www.ccdc.cam.ac.uk/const/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Conflicts of interest

There are no conflicts to declare.

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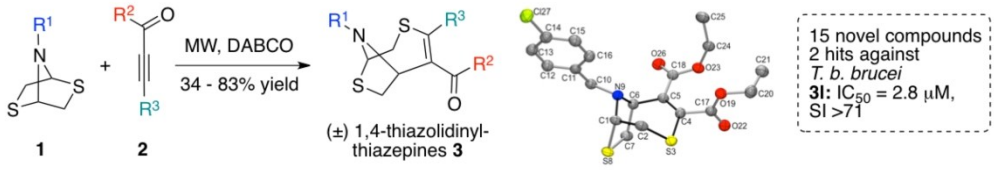

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