Synthesis and Antiprotozoal Properties of 1,2,6-Thiadiazine 1,1-Dioxide Derivatives

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The synthesis and spectroscopical data of 1,2,6-thiadiazine 1,1-dioxides, designed as antiprotozoal agents, are reported. The *in vitro* trichomonacidal and trypanocidal activities of new compounds and their precursors were evaluated against *Trichomonas vaginalis* and *Trypanosoma cruzi*. The chemoprophylactic activity on mice treated with blood infected with *T. cruzi* and their mortality percentage were tested. Compounds **2** and **8b** show a higher chemoprophylactic activity and lower mortality percentage than Nifurtimox used as reference drug.

Synthese und Wirkung von 1,2,6-Thiadiazin-1,1-dioxiden gegen Protozoen

Über Synthese und spektroskopische Eigenschaften von 1,2,6-Thiadiazin-1,1-dioxiden, entwickelt als Antiprotozoenmittel, wird berichtet. Die trichomonacide und trypanocide Aktivität dieser neuen Verbindungen wurde *in vitro* gegen *Trichomonas vaginalis* und *Trypanosoma cruzi* geprüft. Die chemoprophylaktische Wirkung an Mäusen, behandelt mit *T. cruzi* infiziertem Blut, sowie die Sterblichkeitsraten wurden festgelegt. Die Verbindungen **2** und **8b** zeigen höhere chemoprophylaktische Wirkungen und niedrigere Sterblichkeitsraten als der Vergleich Nifurtimox.

Nifurtimox (**1**) is one of the most effective drugs used in the treatment of *Chagas'* disease. However, this drug presents many inconveniences and none of the compounds in clinical trial are considered to be safe, effective, and inexpensive chemotherapeutic agents for use in man, not even to prevent transmission during blood transfusion[1]. For these reasons *Chagas'* disease, which is frequent in many undeveloped areas, is not a resolved problem.

On the other hand trichomoniasis, a typical infection of rich countries, is needing new effective agents.

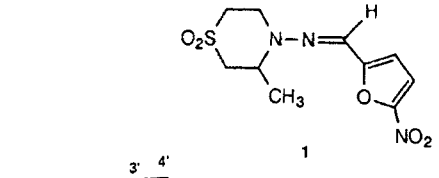
Nifurtimox as well as many of the trichomonacides include a nitropentaheterocycle moiety in their structure. The structural variation of antiprotozoal agents has given good results in finding new active compounds.

In the present work, the synthesis and assays against *T. cruzi* and *T. vaginalis* of compounds **2-5**, in which the thiazine 1,1-dioxide ring of **1** was replaced by 1,2,6-thiadiazine 1,1-dioxide derivatives and the nitroheterocycle counterpart was substituted by thiophene ring in some cases, are reported.

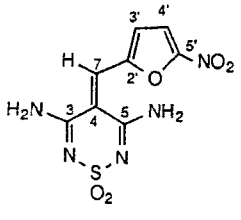
Results and Discussion

Chemistry

In the synthesis of new compounds, the last step involves an electrophilic attack of the corresponding aldehyde to the acidic 4-methylene group of 1,2,6-thiadiazine 1,1-dioxide derivatives **6** and **8**.



1



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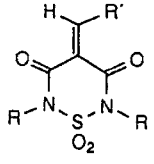
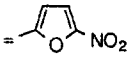


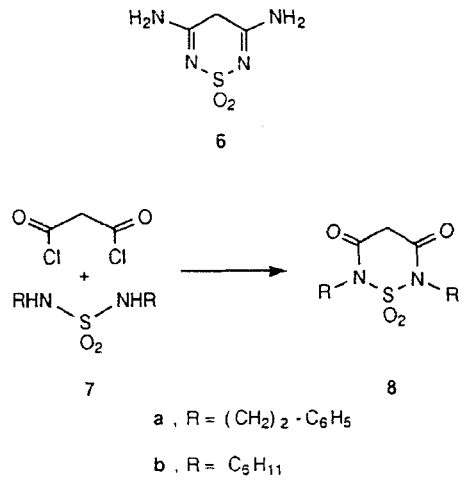
3, $R = (CH_2)_2\text{-}C_6H_5$, $R' =$ 

4, $R = (CH_2)_2\text{-}C_6H_5$, $R' =$ 

5, $R = C_6H_{11}$, $R' =$ 

Scheme 1

  0365-6233/92/0808-0509 $ 3.50 + .25/0



Scheme 2

Compounds **6**[2] and **8b**[3] are known, **8a** was synthesized for the first time following the procedure described for **8b**, in which dicyclohexylsulfamide (**7b**) was substituted by diphenethylsulfamide (**7a**). Reaction of **7a** with malonyl chloride afforded heterocycle **8a** in a good yield.

Condensation of **6** with 5-nitro-2-furaldehyde in aqueous acetic acid gave **2** in 80% yield. No other aldehyde condensed with **6**, in spite of forcing the reaction conditions and changing the catalyst.

The enhanced reactivity of heterocycles **8a** and **8b** allowed condensations with aldehydes such as 5-nitro-2-furaldehyde and thienylcarbaldehyde to the derivatives **3**, **4**, and **5**, using piperidine as catalyst following the procedure for 4-heteroarylidenpyrazole-3,5-diones[4].

Compound **2** shows interesting complexation properties. The [1:1] complexes with dimethylformamide **2a**, pyridine **2b**, and hexamethylphosphotriamide **2c** were isolated. The stoichiometry of the complexes was determined by analytical data. Compound **2** does not admit recrystallization because it crystallizes under the complexed form with the solvent, and for this reason the elementary analysis was not performed for **2** but for **2a**, **2b**, and **2c**.

Structures of the new compounds were confirmed by ^{1}H- and ^{13}C-NMR spectroscopy (Table 1). Assignments of all the signals were achieved on the basis of data in the lit.[5,8], signal multiplicity and coupling constant values. So, chemical shift assignments of C-3 and C-5 were made taking into account the $^{3}J_{C\text{-}H}$ values corresponding to *cis* and *trans* coupling, respectively. Moreover, C-5 of final products **2-5** suffers a shielding with respect to C-3 and with the equivalent C-3 and C-5 of starting bases **6**[8], **8a**, and **8b**[5], owing to the effect of the pentaheterocycle.

Biological Results

In order to know whether the pentaheterocycle in compounds **2-5** is necessary for the potential antiprotozoal activity, heterocycles **6**, **8a**, and **8b** were tested too. In previous assays[9] compound **6** did not show any trichomonacidal activity.

Trichomonacidal Activity

Compounds **2-5**, **8a**, and **8b** were tested *in vitro* against a recently isolated strain of *Trichomonas vaginalis* (namely

Table 1. Efficacy against *Trichomonas vaginalis* at 24 h and 48 h (in brackets) of contact[a]

Comp. (µg/ml)	Growth rate	Growth %	Reduct %
Control	2.80 (1.00)	100.0 (100.0)	0.0 (0.0)
2 (400)	0.00 (0.00)	0.0 (0.0)	100.0 (100.0)
2 (100)	0.00 (0.00)	0.0 (0.0)	100.0 (100.0)
2 (50)	0.00 (0.00)	0.0 (0.0)	100.0 (100.0)
2 (25)	0.00 (0.00)	0.0 (0.0)	100.0 (100.0)
2 (12.5)	0.29 (0.00)	0.0 (0.0)	88.9 (100.0)
2 (5)	0.60 (0.19)	0.0 (0.0)	39.7 (74.5)
3 (400)	0.54 (0.20)	0.0 (0.0)	80.8 (80.3)
3 (100)	1.77 (0.79)	63.3 (78.9)	0.0 (21.1)
5 (400)	2.50 (0.41)	89.2 (40.6)	0.0 (59.4)
5 (100)	2.02 (2.94)	71.9 (293.8)	0.0 (0.0)
Control	1.28 (1.88)	100.0 (100.0)	0.0 (0.0)
4 (100)	0.07 (0.00)	0.0 (0.0)	94.2 (100.0)
4 (10)	0.88 (1.99)	0.0 (0.0)	11.8 (0.0)
6 (100)	1.42 (1.26)	100.0 (67.0)	0.0 (0.0)
8a (100)	0.20 (0.00)	0.0 (0.0)	84.4 (100.0)
8a (10)	0.73 (0.22)	0.0 (0.0)	43.0 (88.3)
8b (100)	0.06 (0.00)	0.0 (0.0)	95.3 (100.0)
8b (10)	0.53 (0.26)	0.0 (0.0)	58.8 (86.2)
Met.[b] (5)	0.09 (0.01)	0.0 (0.0)	96.8 (99.4)
Met.[b] (2.5)	0.45 (0.07)	0.0 (0.0)	84.1 (93.2)

[a] The tests were carried in triplicate. [b] Metronidazol as standard.

Table 2. Efficacy against epimastigote forms of *Trypanosoma cruzi*[a]

Comp. (μg/ml)	Growth rate	Growth %	Reduct %
Control	4.62	100.0	0.0
2 (100)	0.01	0.0	99.8
2 (10)	1.71	36.3	0.0
3 (100)	0.00	0.0	100.0
3 (10)	5.35	115.7	0.0
5 (100)	1.09	23.6	0.0
5 (10)	4.73	102.2	0.0
Control	2.11	100.0	0.0
4 (100)	0.09	0.0	98.1
4 (10)	1.80	85.2	0.0
6 (100)	3.01	142.5	0.0
6 (10)	3.08	146.0	0.0
8a (100)	0.42	0.0	90.8
8a (10)	1.36	64.4	0.0
8b (100)	0.92	0.0	80.1
8b (10)	1.19	56.5	0.0
Control	2.49	100.0	0.0
1 (100)	0.00	0.0	100.0
1 (10)	0.00	0.0	100.0
1 (1)	1.04	41.7	0.0

[a] Three wells were used for each compound and dose assayed. Nifurtimox (1) as standard.

S). Growth rate was expressed as the relation between the number of viable protozoa in the wells at 24 or 48 h, and the number of trichomonads counted at h zero. A growth rate greater than 1 shows that the protozoa population have had an active multiplication, while less than 1 indicates a reduction in the initial population.

Finally, minimal inhibitory concentration (MIC) and minimal cytocidal concentration (MCC) were established in accordance with the following definitions: MIC, the minimal concentration of drug for which the growth rate has a value of 1. MCC, the minimal concentration of drug for which the growth rate is 0.

Growth rates were measured at 24 and 48 h of contact among drugs and protozoa. Table 1 shows the percentage of growth and reduction for each compound tested. Growth of 100% is assigned to controls.

Compound **2** showed the highest activity, slightly lower than the standard at 48 h of contact. MCC of **2** at 48 h was between 12.5 and 5 μg/ml and MIC less than 5 μg/ml while for metronidazole 5 μg/ml and less than 5 μg/ml resulted respectively.

Compound **4** is the other product which shows an important activity at 100 μg/ml, indicating that the nitrophentaheterocycle is not an essential feature to show trichomonacidal activity (compare **3** and **4** activities in Table 1).

Surprisingly, the starting heterocycles **8a** and **8b** showed an important activity at 100 μg/ml, which was kept at 10 μg/ml, while their derivatives **3**, **4**, and **5** were not active at this dose. Then, in order to confirm the inactivity of **6**, this compound was newly tested and showed no activity as described[9].

Trypanocidal Activity

In order to assess the efficacy of compounds **2-5**, **6**, **8a**, and **8b**, against the strain[10] of *Trypanosoma (S.) cruzi*, two types of tests were carried out:

i) *In vitro* test to determine the trypanocidal activity on *T. cruzi*[11].

ii) Chemoprophylactic tests, based on previous study of compounds activity on infected stored blood, and posterior inoculation in receptive animals[12]. Parasitaemia by *Brener's* method[13] and mortality rates were followed in the animals.

Nifurtimox (**1**) was used as reference drug in both experiments.

Table 3. Mortality percentage[a]

Experiment[b]	1					2					
Compound	Control	1	2	3	5	Control	G.V.	4	6	8a	8b
Days p.i.											
30	100	33	33	100	50	33	0	33	0	33	0
60	100	50	33	100	66	50	0	33	0	33	0

[a] Dose **1-8b** 1000 μg/ml, G.V. 250 μg/ml.

[b] Two sets of experiments were carried out. Experiment 1 corresponds to fig. 1, Nifurtimox (**1**) as standard, and experiment 2 corresponds to fig. 2, Gentian Violet (G.V.) as standard. Lots of six mice for each compound were used.

In Table 2 results of *in vitro* tests are expressed as growth rate and percentage of growth and reduction of parasite. Except for compounds **5** and **6** all compounds show a high activity at 100 μg/ml which disappears at 10 μg/ml in all cases. Complex forms **2a**, **2b**, and **2c** were tested, too. No difference in their activity and that of **2** was found.

In chemoprophylactic assays Nifurtimox (**1**) and gentian violet (G.V.) were used as standards. Only mice inoculated

with **2**, **8b** and gentian violet treated blood showed no parasitaemia along 30 days after inoculation (Fig. 1 and 2). However, all compounds tested as well as Nifurtimox reduced the parasitaemia levels. Activity of **2** was confirmed by another experiment (Fig. 3) in which the minimal effective concentration is found between 1000 and 500 µg/ml whilst that of gentian violet is 250 µg/ml.

When mortality percentages of animals were seen (Table 3) again compounds **2** and **8b** appeared as the most active compounds since a survival rate for mice greater than even Nifurtimox was obtained. In spite of mice inoculated with **6** treated blood showed some parasitaemia at day 13 *p.i.* (Fig. 2) no mortality was observed.

These tests allow compounds **2**, **3**, **4**, **8a**, and **8b** to be considered as moderately active on epimastigote form, while compounds **2** and **8b** are clearly more active on tripomastigote form, even more than Nifurtimox (**1**).

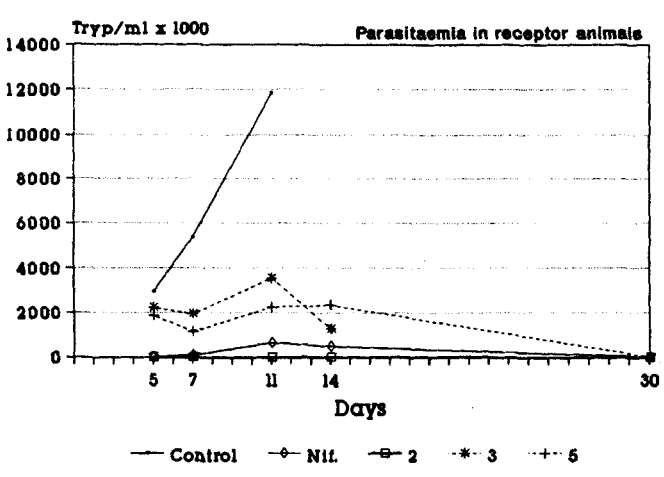


Figure 1. Parasitaemia levels in mice inoculated with blood trypomastigote forms of *Trypanosoma cruzi* previously incubated at 4°C with 1 mg/ml of **1**, **2**, **3**, or **5**.

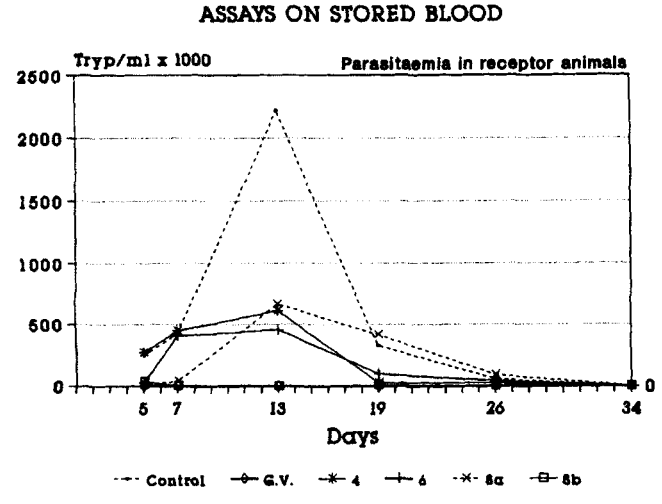


Figure 2. Parasitaemia levels in mice inoculated with blood trypomastigote forms of *Trypanosoma cruzi* previously incubated at 4°C with 1 mg/ml of g.v. **4**, **6**, **8a**, or **8b**.

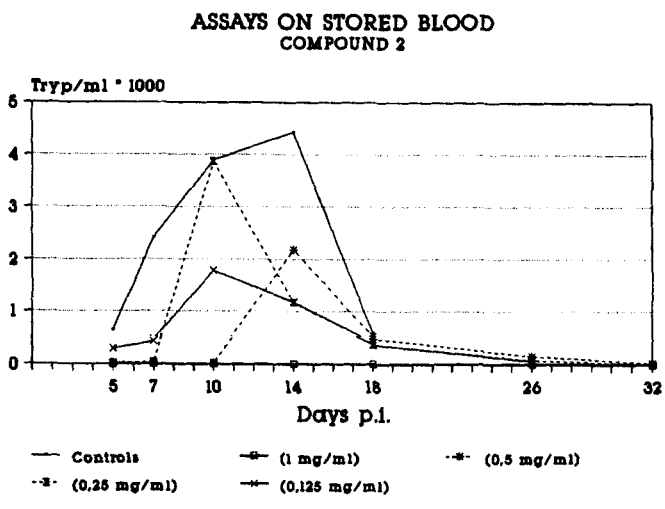


Figure 3. Parasitaemia levels in mice inoculated with blood trypomastigote forms of *Trypanosoma cruzi* previously incubated at 4°C with different doses of **2**.

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Experimental Part

Chemistry

Melting points: Kofler apparatus, uncorrected.- NMR spectra: Bruker AM-200, 200.13 MHz for ^{1}H and at 50.32 MHz for ^{13}C.- ^{1}H and ^{13}C chemical shifts (δ) are given from int. TMS with an accuracy of ± 0.1 ppm, respectively.- UV and IR spectra: spectrophotometers Perkin Elmer 50-SE and 257, respectively.

3,5-Diamino-4-(5'-nitrofurylidene)-4H-thiadiazine 1,1-Dioxide (**2**)

Compound **6** (1 g, 6.2 mmol) was dissolved in 40 ml of hot aqueous acetic acid (3:1) and a solution of 5-nitrofurfural (0.87 g, 6.2 mmol) in methanol (10 ml) was added. The mixture was stirred at room temp. for 4 h. The solid was filtered, washed with water and dried. Compound **2** (1.42 g, 80% yield) was obtained without recrystallization. M.p. 248°C (dec.).- IR (nujol): ν = 3460; 3350; 3260; 3060 (NH_2); 1640 (C=N); 1520; 1350 (NO_2); 1280; 1180-1080 cm^{-1} (SO_2).- ^{1}H-NMR (DMSO-d_6: δ (ppm) = 8.30-8.00 (b.s, 4H, NH_2), 7.77 (d, 1H, ^{3}J = 4.1 Hz, H-4'), 7.34 (s, 1H, =CH), 7.30 (d, 1H, ^{3}J = 4.1 Hz, H-3').

Complex of **2** *with DMF* (**2a**)

Compound **2** was dissolved in an excess of anhydrous *N,N*-dimethylformamide (DMF) at room temp., ethyl ether was added until the solution became turbid. On cooling a yellow solid appeared corresponding to the 1:1 complex of **2** and DMF (95% yield). M.p. 276-278°C.- IR (KBr): $\bar{\nu}$ = 3300; 3180; 3100 (NH_2); 1660 (C=N); 1520; 1350 (NO_2); 1270; 1180-1080 cm^{-1} (SO_2).- UV (MeOH): λ max (log ε) = 284 (4.04), 316 (4.02), 240 (sh) nm (4.01).- ^{1}H-NMR (DMSO-d_6): δ (ppm) = 8.30 (b.s, 2H, NH_2), 8.10 (b.s, 2H, NH_2), 7.95 (s, 1H, HC=O, DMF), 7.80 (d, 1H, ^{3}J = 4.0 Hz, H-4'), 7.36 (s, 1H, HC=), 7.30 (d, ^{3}J = 4.0 Hz, 1H, H-3'), 2.87 (s, 3H, NCH_3), 2.70 (s, 3H, NCH_3).- $C_{11}H_{14}N_6O_6S$ (358.4) Calcd. C 36.9 H 3.94 N 23.5 S 9.0 Found C 36.5 H 3.85 N 23.5 S 9.2.

Complex of **2** *with pyridine* (**2b**)

Compound **2** was dissolved in anhydrous pyridine at room temp. The solution was evaporated to dryness under vacuum and the residue recrystal-

lized from pyridine/ethyl ether to give a white solid, m.p. > 250° (dec.).- IR (KBr): $\tilde{\nu}$ = 3320; 3180 (NH_2); 1660 (C=N); 1530; 1350 (NO_2); 1280; 1180-1140 cm^{-1} (SO_2).- ^{1}H-NMR (DMSO-d_6): δ (ppm) = 8.50 (dd, 2H, ^3J = 4.3 Hz, ^4J = 1.9 Hz, H_α*), 8.20 (b.s, 2H, NH_2), 8.00 (b.s, 2H, NH_2), 7.73 (tt, 1H, ^3J = 7.5 Hz, ^4J = 1.9 Hz, H_γ*), 7.71 (d, 1H, ^3J = 4.0 Hz, H-4'), 7.32 (ddd, 2H, ^4J = 1.5 Hz, ^3J = 4.3 Hz, ^3J = 7.5 Hz, H_β*), 7.30 (s, 1H, HC=), 7.23 (d, ^3J = 4.0 Hz, 1H, H-3').- $C_{13}H_{12}N_6O_5S$ (362.4) Calcd. C 42.9 H 3.32 N 23.1 S 8.8 Found C 43.0 H 3.44 N 23.0 S 8.9.

Complex of **2** *with HMPT* (**2c**)

A solution of **2** and hexamethylphosphotriamide was heated at 90°C for 1 h. On cooling and by addition of dry ethyl ether a dark compound precipitated. M.p. > 300°C (dec.).- IR (KBr): ν = 3300, 3150 (NH_2); 1660 (C=N); 1480 (NCH_3); 1525; 1350 (NO_2); 1260 (P=O); 1310-1280; 1180-1140 cm^{-1} (SO_2).- ^{1}H-NMR (DMSO-d_6): δ (ppm) = 8.30 (b.s, 2H, NH_2), 8.10 (b.s, 2H, NH_2), 7.79 (d, 1H, ^3J = 4.0 Hz, H-4'), 7.36 (s, 1H, HC=), 7.32 (d, 1H, ^3J = 4.0 Hz, H-3'), 2.45, 2.56 (s, 9H, NCH_3, HMPT).- $C_{14}H_{25}N_8O_6SP$ (464.5) Calcd. C 36.2 H 5.42 N 24.1 S 6.90 Found C 36.1 H 5.65 N 24.0 S 7.0.

2,6-Diphenethyl-2,3,5,6-tetrahydro-3,5-dioxo-4(5'-nitrofurylidene)-1,2,6-thiadiazine 1,1-dioxide (**3**)

To a solution of **8a** (3 mmol) and 4-nitrofurfural (3.3 mmol) in dry toluene (100 ml) some drops of piperidine were added and the mixture was refluxed for 2 h. The water produced was removed with a dean-star. On cooling, the solid was filtered and recrystallized from ethanol, 55% yield, m.p. 142-144°C.- IR (nujol): $\tilde{\nu}$ = 1740; 1680 (C=O); 1540; 1360 (NO_2); 1250; 1170 cm^{-1} (SO_2).- UV (MeOH): λ max (log ε) = 269 (3.80); 353 nm (4.20).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 8.03 (dd, 1H, ^4J = 0.7 Hz, ^5J = 0.6 Hz, HC=), 7.91 (dd, 1H, ^3J = 4.0 Hz, ^5J = 0.6 Hz, H-4'), 7.42 (dd, 1H, ^3J = 4.0 Hz, ^4J = 0.7 Hz, H-3'), 7.25 (s, 10 H, C_6H_5), 4.16 (t, 4H, ^3J = 7.5 Hz, CH_2N), 3.10 (t, ^3J = 7.5 Hz, $C_6H_5CH_2$).- $C_{24}H_{21}N_3O_7S$ (493.5) Calcd. C 58.2 H 4.27 N 8.5 S 6.5 Found C 58.3 H 4.43 N 8.5 S 6.2.

2,6-Diphenethyl-2,3,5,6-tetrahydro-3,5-dioxo-4-(thienylidene)-1,2,6-thiadiazine 1,1-dioxide (**4**)

From 3 mmol of **8a** and 3.3 mmol of thienylcarbaldehyde, and working as above, compound **4** was obtained in 73% yield. M.p. 114-115°C (ethyl acetate).- IR (nujol): $\tilde{\nu}$ = 1700; 1670 (C=O); 1290; 1180 cm^{-1} (SO_2).- UV (MeOH): λ max (log ε) = 318 (sh) (4.01); 365 nm (4.48).- ^{1}H-NMR ($CDCl_3$): δ (ppm) = 8.40 (s, 1H, HC=), 7.76 (dd, 1H, ^3J = 5.1 Hz, ^3J = 1.2 Hz, H-5'), 7.66 (dd, ^3J = 3.6 Hz, ^3J = 1.2 Hz, H-3'), 7.13 (s, 10 H, C_6H_5), 7.09 (m, 1H, H-4'), 4.06 (t, 4H, ^3J = 7.5 Hz, CH_2N), 2.96 (t, 4H, ^3J = 7.5 Hz, $C_6H_5CH_2$).- $C_{24}H_{22}N_2O_4S_2$ (466.6) Calcd. C 61.8 H 4.75 N 6.05 Found C 62.1 H 4.74 N 6.1 S 13.7.

2,6-Dicyclohexyl-2,3,5,6-tetrahydro-3,5-dioxo-4-(thienylidene)-1,2,6-thiadiazine 1,1-dioxide (**5**)

Working as above from 3 mmol of **8b** and 3.3 mmol of thienylcarbaldehyde, compound **5** was obtained in 65% yield. M.p. 213°C (ethyl acetate).- IR (nujol): ν = 1675; 1640 (C=O); 1330; 1170 cm^{-1} (SO_2).- UV (MeOH): λ max (log ε) = 358 nm (4.03).- ^{1}H-NMR (DMSO-d_6): δ (ppm) = 8.40 (s, 1H, HC=), 7.86 (d, 1H, ^3J = 5.2 Hz, H-5'), 7.77 (d, 1H, ^3J = 3.6 Hz, H-3'), 7.23 (m, 1H, H-4'), 4.57 (tt, 1H, $^3J_{a,a}$ = 12.5 Hz, $^3J_{a,e}$ = 3.6 Hz, H-1a*), 4.36 (tt, 1H, $^3J_{a,a}$ = 12.5 Hz, $^3J_{a,e}$ = 3.6 Hz, H-1a''*), 2.30-1.25 (m, 20 H, C_6H_{11}*).- $C_{20}H_{26}N_2O_4S_2$ (422.6) Calcd. C 56.8 H 6.20 N 6.6 S 15.2 Found C 56.7 H 6.37 N 6.7 S 15.0.

* Signals corresponding to pyridine.
* Cyclohexyl moiety.

N,N-Diphenethylsulfamide (**7a**)

A mixture of sulfamide (5 g, 52 mmol) and β-phenylethylamine (13.3 g, 110 mmol) was heated at 100-105°C under stirring for 3 h. On cooling, 2 N HCl (20 ml) was added to remove the amine in excess. The precipitate which appeared was filtered and recrystallized from ethanol to give white plates (66%). M.p. 96°C.- IR (nujol): $\tilde{\nu}$ = 3270 cm^{-1} (NH).- ^{1}H-NMR (DMSO-d_6): δ (ppm) = 7.25 (m, 10 H, C_6H_5), 4.17 (t, 2H, ^3J = 6.7 Hz, NH), 3.19 (q, 4H, $^3J_{NH,CH2}$ = $^3J_{CH2,CH2}$ = 6.7 Hz, CH_2N), 2.80 (t, 4H, ^3J = 6.7 Hz, $C_5H_6CH_2$).- $C_{16}H_{20}N_2O_2S$ (196.4) Calcd. C 63.1 H 6.63 N 9.2 S 10.5 Found C 62.8 H 6.66 N 10.6.

2,6-Diphenethyl-2,3,5,6-tetrahydro-3,5-dioxo-1,2,6-thiadiazine1,1-Dioxide (**8a**)

To a solution of *N,N'*-diphenethylsulfamide (**7a**) (3.88 g, 12.7 mmol) in dry toluene (50 ml) malonyl chloride (1.8 g, 12.7 mmol) in dry toluene (10 ml) was added. The reaction mixture was kept at 70°C for 4 h. The solvent was removed under vacuum and the solid residue was crystallized from ethanol to give 3.8 g of white needles (78% yield). M.p. 94-95°C.- IR (nujol): $\tilde{\nu}$ = 1730 cm^{-1} (C=O).- UV (MeOH) λ max: 272 nm.- ^{1}H-NMR (Cl_3CD): δ (ppm) = 7.30 (m, 10 H, C_6H_5), 4.10 (t, 4H, ^3J = 7.5 Hz, CH_2N), 3.90 (s, 2H, CH_2), 2.90 (t, 4H, ^3J = 7.5 Hz, $C_6H_5CH_2$).- $C_{19}H_{20}N_2O_4S$ (208.5) Calcd. C 61.3 H 5.40 N 7.5 S 8.6 Found C 61.1 H 5.56 N 7.6 S 8.8.

Biological Methods

Trichomonacidal in vitro activity

Cultures of a recently isolated strain of *Trichonomas vaginalis* (S) were carried out in TYM medium without agar and supplemented with heat 10% inactivated equine serum. The experiments were made using 24 wells NUNCLON plates, containing 100,000 protozoa/ml in a final volume of 2 ml. The compounds to be tested were dissolved in ethylenglycol/PBS (1:4) and added to the cultures at predeterminated doses in a volume of 0.1 ml, six h after re-seeding (h zero). Counting of viable protozoa were assessed at 0, 24, and 48 h after incubation with the tested compound at 37°C. Counting was made by using the *Neubauer* chamber. For each experiment there were six control wells, containing only the solvent. Metronidazole, used as reference drug, was added at 5 and 2.5 μg/ml to a group of wells. Compounds were assayed at 400, 100, 25, 12.5, and 5 μg/ml. The tests were carried out in triplicate.

Trypanocidal activity

In vitro activity was assayed on epimastigote cultures during the exponential phase in LIT medium supplemented with 10% of foetal calf serum. NUNCLON plates were filled with 2.5 ml/well of medium containing 500,000 trypomastigotes/ml. Three wells were used for every dose. Compounds were dissolved in phosphate buffer saline (PBS) with 0.2% dimethyl sulfoxide, a concentration at which no adverse effects attributable to the solvent were noticed. After six days at 28°C, double counting by *Neubauer* chambers were made. Results are expressed as in Trichomonacidal assays.

Chemoprophylactic activity of the compounds was determined on refrigerated stored infected blood obtained from mice by cardiac puncture. Compounds were dissolved in PBS containing 15% of ethylenglycol and given at a concentration of 1 mg/ml. These solutions (0.5 ml) were added to tubes containing 0.5 ml of infected blood with 250,000 trypomastigotes/ml. Tubes were incubated at 4°C on a shaker placed in a dark room. After 24 and 48 h, groups of six NMRI mice were inoculated by intraperitoneal way with 0.5 ml of treated blood.

Table 4. ^{13}C-NMR data[a] of new compounds

Comp.	C-3	C-4	C-5	C-7	C-2′	C-3′	C-4′	C-5′
2[b]	163.1(d) $^3J_{cis}$=6.2	117.7(b.s.)	160.4(d) $^3J_{trans}$=9.6	123.7(d) ^1J=162.6	149.4(t) ^2J=8.8, ^3J=8.8	114.2(d) ^1J=190.7	122.1(d) ^1J=183.4	152.5(d.d) ^2J=9.6, ^3J=5.5
2a*[b]	163.1(d) $^3J_{cis}$=6.2	117.8(b.s.)	160.4(d) $^3J_{trans}$= 9.4	123.7(d) ^1J=162.3	149.5(t) ^2J=8.4, ^3J=8.4	114.2(d) ^1J=187.6	122.1(d) ^1J=183.2	152.5(d.d) ^2J=9.4, ^3J=5.5
2b**[b]	162.1(d) $^3J_{cis}$=5.8	116.9(m.)	159.4(d) $^3J_{trans}$=10.1	122.7(d) ^1J=161.7	148.5(t) ^2J=8.8, ^3J=8.8	113.2(d) ^1J=190.0	121.0 ^1J=180.3	151.5(m)
2c***[b]	162.8(d) $^3J_{cis}$=5.4	117.8(b.s.)	160.2(t) $^3J_{trans}$=10.7	123.4(d) ^1J=161.0	149.5(d) ^2J=8.6	114.0(m)	121.5(d) ^1J=182.3	152.3(m)
3[c,d+]	160.5	123.1	159.3	135.5(d) ^1J=160.7	149.8	112.7(d) ^1J=191.7	124.5(d) ^1J=188.5	153.4
4[c, e+]	161.7	114.6	160.4	128.1(d)	134.4	148.3(d)	144.2(d)	140.2(d)
5[c, e+]	162.7	116.4	161.9	127.9(d)	136.2	147.2(d)	143.4(d)	139.3(d)
8a[c]	162.3	44.2(t) ^1J=137.7	162.3	-	-	-	-	-

[a] Chemical shifts in ppm and J in Hz, numbering in Scheme 1. [b] In DMSO-d_6 at 20.15 MHz. [c] In $CDCl_3$ at 20.15 MHz. [d] Long distance couplings were not measured. [e] Multiplicity from off-resonance spectra. * Signals corresponding to $HCONME_2$: 162.5 (s, J = 192.0), 39.9, 30.9 (t, ^1J = 140). ** Signals corresponding to C_5H_5N: 148.5 (d.m., ^1J = 173.0), 135.6 (d.d., ^1J = 164.0), 123.2 (d). *** Signals corresponding to HMPT: 35.5 (t, ^1J = 136.0). + Data of N-substituentes are the usual for phenethyl and cyclohexyl moieties.

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