Synthesis of 2-Amino-5-sulfanyl-1,3,4-thiadiazole Derivatives and Evaluation of Their Antidepressant and Anxiolytic Activity

Francesca Clerici* and Donato Pocar

Istituto di Chimica Organica, Facoltà di Farmacia, Università di Milano, Via Venezian 21, 20133 Milano, Italy

Maddalena Guido,† Antonella Loche, and Vincenzo Perlini

G. E. T., Laboratori di ricerca, Via Ariosto 17, 18038 Sanremo (Im), Italy

Mario Brufani

Dip. Scienze Biochimiche A. Rossi-Fanelli, Università La Sapienza, Via degli Apuli 9, 00040 Roma, Italy

Received July 18, 2000

Recently a series of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives bearing different substituents were synthesized and screened pharmacologically in order to evaluate their central nervous system activity. The purpose of this study was to evaluate the effects of the title compounds on CNS activity by varying the substituents in the thiadiazole moiety. It was found that some of these compounds possess marked antidepressant and anxiolytic properties comparable in efficiency to the reference drugs Imipramine and Diazepam. The most potent compound **3k** was further investigated to complete its pharmacological profile with respect to undesired side effects. Behavioral results showed that **3k** is a very promising compound, characterized by a mixed antidepressant-anxiolytic activity accompanied by a therapeutic dose range that is essentially 2 orders of magnitude less than that at which side effects such as sedation and amnesia are evident.

Introduction

During recent years there has been intense investigation of different classes of thiadiazole compounds, many of which were found to be pharmacologically active. Of significance are the antihypertensive and anticonvulsant effects.[1−3] A series of 2-amino-5-sulfanyl-1,3,4-thiadiazoles exemplified by the general structure in Figure 1 had originally been synthesized in our laboratories as aldehyde dehydrogenase inhibitors, but subsequent observation of some effects on the central nervous system (CNS) made it essential to evaluate these compounds for antidepressant and anxiolytic activity.[4] This paper deals with the synthesis, pharmacological screening, and preclinical evidence concerning the differences between the therapeutic dose range and that at which side effects are seen, for this class of compound.

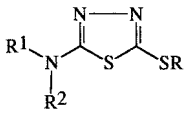
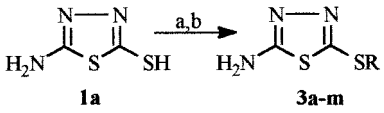


Figure 1.

Scheme 1. Synthesis of Thiadiazoles **3a**−**m**



1a **3a-m**

Reaction conditions
a) KOH 85%, H_2O; b) RCl (**2a-m**)

2,3	R
a	Me
b	n-Pr
c	i-Pr
d	benzyl
e	4-NO_2-benzyl
f	4-CH_3O-benzyl
g	4-Cl-benzyl
h	4-CH_3-benzyl
k	3-CH_3O-benzyl
i	3-Cl-benzyl
j	3-NO_2-benzyl
l	3-CH_3-benzyl
m	3,4,5-$(CH_3O)_3$-benzyl

Results and Discussion

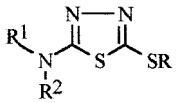
Chemistry. For this study we synthesized three classes of thiadiazole derivatives shown in Table 1. The first class is represented by compounds **3a**−**m** which display a free NH_2 group whereas the second class is represented by compounds **5a**−**n** where the 2-amino group is transformed by acylation into an amido group. The N-methylation of compounds **5a** and **5b** affords **6a** and **6b** which are designated as the third class of compound. Compounds **3a**−**m** were synthesized starting from the available 5-sulfanyl-1,3,4-thiadiazol-2-ylamine **1a** which was suspended in KOH solution and then treated with the appropriate alkyl halide **2a**−**m** as shown in Scheme 1. Compounds **5a**−**n** were obtained from the parent **3** through acylation with the appropriate alkanoyl or aroyl halide **4a**−**d** (Scheme 2). Treat-

* Address correspondence to Francesca Clerici. Phone: 00390270630348. Fax: 00390270638473. E-mail: francesca.clerici@mailserver.unimi.it.

† Present address: Homeopharm, Corso Italia 126, 18012 Bordighera (Im), Italy.

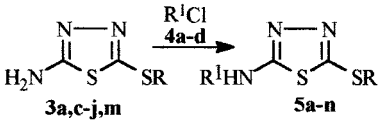
10.1021/jm001027w CCC: $20.00
Published on Web 02/21/2001

Table 1.



no.	R	R^1	R^2	no.	R	R^1	R^2	no.	R	R^1	R^2
1a	H	H	H	**3i**	3-Cl-benzyl	H	H	**5g**	benzyl	MeCO	H
3a	Me	H	H	**3j**	3-NO_2-benzyl	H	H	**5h**	3,4,5-$(MeO)_3$-benzyl	MeCO	H
3b	*n*-Pr	H	H	**3l**	3-Me-benzyl	H	H	**5k**	3-MeO-benzyl	MeCO	H
3c	*i*-Pr	H	H	**3m**	3,4,5-$(MeO)_3$-benzyl	H	H	**5i**	3-Cl-benzyl	MeCO	H
3d	benzyl	H	H	**5a**	Me	MeCO	H	**5j**	3-NO_2-benzyl	MeCO	H
3e	4-NO_2-benzyl	H	H	**5b**	*i*-Pr	MeCO	H	**5l**	benzyl	EtCO	H
3f	4-MeO-benzyl	H	H	**5c**	4-NO_2-benzyl	MeCO	H	**5m**	benzyl	HexylCO	H
3g	4-Cl-benzyl	H	H	**5d**	4-MeO-benzyl	MeCO	H	**5n**	benzyl	PhenylCO	H
3h	4-Me-benzyl	H	H	**5e**	4-Cl-benzyl	MeCO	H	**6a**	Me	MeCO	Me
3k	3-MeO-benzyl	H	H	**5f**	4-Me-benzyl	MeCO	H	**6b**	*i*-Pr	MeCO	Me

Scheme 2. Synthesis of Thiadiazoles **5a**−**n**



3a,c-j,m **5a-n**

4	R^1
a	**MeCO**
b	**EtCO**
c	**HexylCO**
d	**PhenylCO**

Scheme 3. Synthesis of Thiadiazoles **6a**,**b**

5a,b **6a,b**

Table 2. Despair Test in Mice

compd	immobility time max % decrease	compd	immobility time max % decrease
3a	23 (50 mg/kg)	**5c**	16 (0.25 mg/kg)
3b	no effect	**5d**	10 (0.5 mg/kg)
3c	17 (10 mg/kg)	**5e**	no effect
3d	35 (10 mg/kg)	**5f**	no effect
3e	no effect	**5g**	22 (0.06 mg/kg)
3f	16 (0.3 mg/kg)	**5h**	14 (0.1 mg/kg)
3g	15 (0.1 mg/kg)	**5k**	no effect
3h	no effect	**5i**	no effect
3k	21 (0.1 mg/kg)	**5j**	no effect
3i	no effect	**5l**	no effect
3j	no effect	**5m**	no effect
3l	17 (5 mg/kg)	**5n**	no effect
3m	no effect	**6a**	no effect
5b	19 (1 mg/kg)	**6b**	17 (0.25 mg/kg)

ment of **5a** and **5b** with methyl iodide produced the desired N-alkylated compounds **6a**,**b** (Scheme 3). All compounds were characterized by their analytical and spectroscopic data.

Antidepressant and Anxiolytic Activity. A considerable number of compounds were synthesized (Table 1), a main objective being to vary the substituents on the benzyl group attached to sulfur. Compounds prepared in this study were tested for their antidepressant and anxiolytic activity. Table 2 gives the results of the primary screening by the mice despair test. The simple methyl compound (entry **3a**) was substantially less active than compound **3b**, and further lengthening of the alkyl chain did not have a significant effect although substitution with a bulky *iso*-propyl group (entry **3c**) did result in a slight increase in activity. The introduction of a substituted benzyl group gave rise to compounds that displayed a better profile in terms of effective dose level and range of activity. Compound **3d**, in which the aromatic ring is unsubstituted, varied the duration of immobility in the mice over a range of dose levels (range 5−15 mg/kg), exhibiting a U-shaped dose−response curve, which indicated a maximal effect at the 10 mg/kg dose level. Introduction of various groups at the para position gave compounds less potent than the unsubstituted **3d**. Both electron-withdrawing as well as electron-donating groups showed similar results (**3e**−**h**). In particular, compound **3e** and **3h** were ineffective, whereas **3f** and **3g** induced a slight reduction of the duration of immobility in comparison with compound **3d**. Substitution at the meta position, both with electron-withdrawing as well electron-donating groups, gave rise to compounds with lower activity, except for the methoxy-substituted compound **3k** that showed activity between 0.1 and 1 mg/kg. Evidently, the introduction of a methoxy substituent resulted in a significant increase in potency. Having three methoxy groups (entry **3m**) on the phenyl ring has probably made compound **3m** too bulky to bind at the active site because it was inactive up to 20 mg/kg. We further investigated the effect of acylation of the 2-amino group (entry **5a**−**n**). Generally less active compounds were obtained except in the case of **5g** which was active in the 0.06−0.25 mg/kg range. The trisubstituted amides **6a**,**b** did not show significant activity. Compounds **3d**, **3k**, and **5g** were selected on the basis of both their potency and their broad window of activity. These were then screened in the rat using the despair test and the social interaction test. Compound **3k** demonstrated a wide range of activity in the primary screening, and this finding was confirmed in the rat despair test as illustrated in Figures 2 and 3. The dose range was 0.1−1 mg/kg ip. This compound was more efficient than imipramine in reducing duration of immobility in the rat despair test, both after acute and repeated treatment. In fact, acute administration of 0.5 mg/kg and subchronic treatment with 0.25 mg/kg (once a day for 1 week) was as effective as 20 mg/kg of imipramine. The effects of compound **3d** are reported in the Tables 3 and 4. This thiadiazole derivative was active in a dose range of 5−20 mg/kg after acute ip administration and 1.25−5 mg/kg after repeated treatment for one week. No

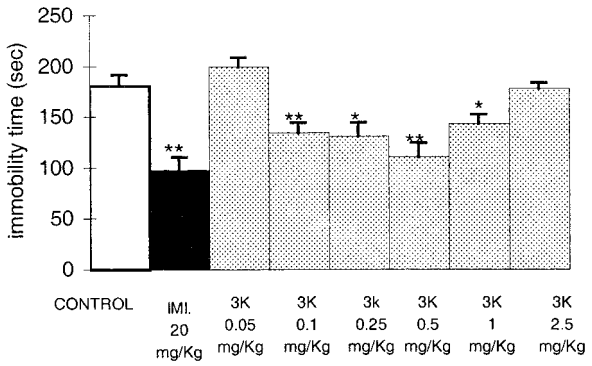


Figure 2. Rat despair test: effects of **3k** after acute i.p. administration. *$p < 0.05$. **$p < 0.01$. $n = 7-8$ rats/group.

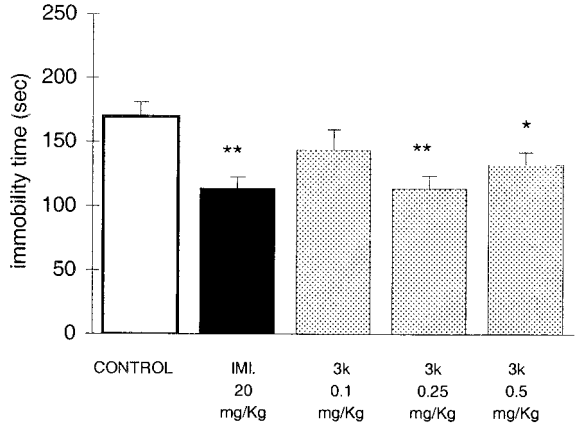


Figure 3. Rat despair test: effects of **3k** after subchronic administration. *$p < 0.05$. **$p < 0.01$. $n = 7-8$ rats/group.

Table 3. Antidepressant Effect of Compound **3d**: Acute Administration in the Rat

mg/kg ip	immobility time % decrease
5	33**
10	10
20	24**
Imipramine 20	45**

**$p < 0.01$. $n = 8$ mice/dose.

Table 4. Antidepressant Effect of Compound **3d**: Subchronic Administration in the Rat

mg/kg ip	immobility time % decrease
1.25	23**
2.5	31**
5	28**
Imipramine 20	56**

**$p < 0.01$. $n = 8$ mice/dose.

tolerance to the antidepressant effect was found after repeated administration of compounds **3k** and **3d**. Compound **5g** seemed promising on the basis of the primary screening in the mouse, but the subsequent evaluation in the rat despair test showed a narrow window of activity, so it was not investigated further. Anxiolytic activity was evaluated by the social interaction test in the rat; Diazepam, administered ip 1 h before the test at 1.25 mg/kg, was adopted as the reference drug. These results are shown in the Figures 4 and 5. The test compounds were both effective in enhancing the time spent in social interaction, and compound **3k** was more efficient than Diazepam after

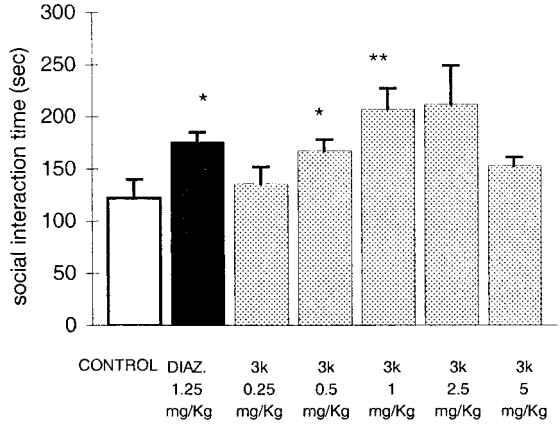


Figure 4. Rat social interaction test: effects of **3k** after acute i.p. administration. *$p < 0.05$. **$p < 0.01$. $n = 6$ pairs of rats/group.

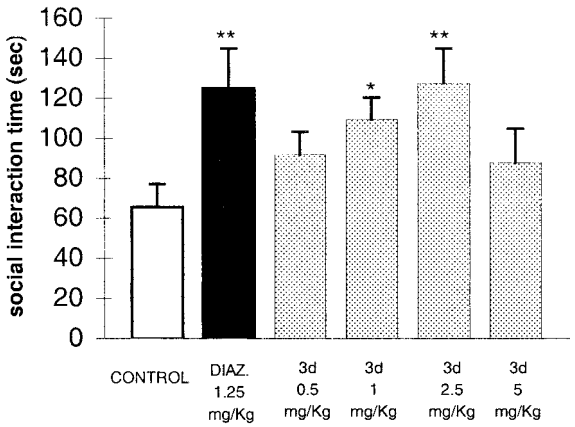


Figure 5. Rat social interaction test: effects of **3d** after acute i.p. administration. *$p < 0.05$. **$p < 0.01$. $n = 5$ pairs of rats/group.

acute adiminstration of 1 mg/kg ip 1 h before the test. It was observed that there is a particular dose−response relationship among the compounds under investigation in that they share a window of activity, on either side of which higher or lower doses are much less effective. This behavior is described by a U-shaped curve in the despair test (see Figure 2) and by a bell-shaped curve in the social interaction test (see Figure 4). These kinds of dose−response curve are well-documented for other compounds having neuropharmacological properties: $5HT_{1a}$ agonists such as ipsapirone and gepirone share this characteristic in the rat despair test, and an inverted U-shaped dose−response curve also typifies the effects of some $5HT_3$ antagonists in the rat social interaction test.[5]

Side Effects. To obtain information about undesired side effects, the lead compound **3k** was screened further in the spontaneous motor activity, ethanol potentiation, and rotarod tests in the mouse. These tests gave information about sedation and ataxia, both of which are undesired side effects of the benzodiazepines and of other drugs acting on the CNS. Moreover the amnesia-inducing potential was examined by using a passive avoidance test in the rat. As the data in Table 5 demonstrate, the test compound showed only weak activity in the above-mentioned test models. Compound **3k** decreased the motor activity of the mouse in a dose-dependent manner, and the active doses ranged from

Table 5. Side Effects of Compound **3k** in Mice

dose mg/kg ip	motor activity % decrease	rotarod ED_{50} mg/kg ip	ethanol potentiation ED_{50} mg/kg ip
300	19	534 (516−556)	162.3 (133.6−197.0)
400	26*		
500	46**		
600	68**		

*$p < 0.05$. **$p < 0.01$. $n = 8$ mice/dose.

Table 6. Effects of **3k** on Step-Through Latencies of a Passive Avoidance in the Rat

dose mg/kg	S1 mean ± E.S.M.	S2 mean ± E.S.M.
0	5.6 ± 2	166 ± 13.9
0.5	4.7 ± 6.0	146 ± 22.8
1	5.6 ± 3.1	135 ± 23.4
2	7.2 ± 2.7	180 ± 0

$n = 10$ rats per group.

400 to 600 mg/kg ip. The ED_{50} for the rotarod test was 536 mg/kg (516−556) and for the ethanol potentiation test 162 mg/kg (134−197). The results in the passive avoidance test are reported in Table 6. Compound **3k**, administered ip 1 h before training, had no significant effects on either the S1 or S2 latencies at any of the doses tested.

Conclusion

The mixed antidepressant-anxiolytic activity and the particular U-shaped dose−response relationship are a common feature of the compounds that were tested and suggest that their mechanism of action may be related to the serotoninergic (5-HT) system, whose relevance in the pathophysiology of various psychiatric disorders is well-documented. Compound **3k** shows a remarkable psychopharmacological profile in animal behavioral tests that are designed to detect antidepressant and anxiolytic activity yet does not possess many of the adverse properties associated with psychopharmacological treatment, such as sedation and ataxia, alcohol interaction, or the potential for amnesia. In fact, compound **3k** shows an excellent therapeutic index, being antidepressant and anxiolytic at doses well below those able to induce motor disfunction and ethanol potentiation. Moreover, anxiolytic doses were devoid of any amnesic effect on a passive avoidance task in the rat, suggesting a low potential to cause memory disturbance, a troublesome side effect of traditional anxiolytic therapy.

Experimental Section

Chemistry. Melting points were determined on a Buchi 510 capillary melting point apparatus and are uncorrected. Elemental analyses were within ±0.4% of theoretical values. ^{1}H NMR spectra were recorded on a Varian EM-360L (60 MHz); $CDCl_3$ or DMSO-d_6 was used as solvent; chemical shifts are reported as δ (ppm). TLC was performed on silica gel plates. Compounds **3a**−**d**,**f**,**g**,**i**,**k**,**l**,**m** and **5a**,**g**,**l**,**n**[6−12] are known.

5-Methylsulfanyl-1,3,4-thiadiazol-2-ylamine (3a). 5-Sulfanyl-1,3,4-thiadiazol-2-ylamine (**1a**) (0.1 mol, 13.31 g) was suspended in water (9 mL), and 0.1 mol of KOH (85% solution) was added under stirring at room temperature. After a few minutes (5−10 min), the solution was brought to 0 °C in an ice bath, and MeI (0.1 mol) was dropped in with vigorous stirring. The reaction mixture was checked by TLC (Et_2O as eluant). When reaction was complete, water (30 mL) was added, and from the crude mixture a white precipitate of **3a** formed slowly. This was filtered, washed with water, and crystallized from EtOAc (55% yield): mp 180 °C. ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3H, SCH_3); 6.9 (s, 2H, NH_2). Anal. ($C_3H_5N_3S_2$) C, H, N.

Compounds **3b**−**m** also were prepared using the general procedure described for **3a**.

5-Propylsulfanyl-1,3,4-thiadiazol-2-ylamine (3b). Yield 57%; mp 117−119 °C (EtOAc). ^{1}H NMR (DMSO) δ 1.2 (t, 3H, CH_3); 1.9 (m, 2H, CH_2); 3.3 (t, 2H, SCH_2); 7.3 (s, 2H, NH_2). Anal. $C_5H_9N_3S_2$ (175.27) C, H, N.

5-Isopropylsulfanyl-1,3,4-thiadiazol-2-ylamine (3c). Yield 65%. mp 150−151 °C (CH_2Cl_2). ^{1}H NMR (DMSO) δ 1.3 (d, 6H, $(CH_3)_2C$); 3.6 (m, 1H, CH═); 7.1 (s, 2H, NH_2). Anal. $C_5H_9N_3S_2$ (175.27) C, H, N.

5-Benzylsulfanyl-1,3,4-thiadiazol-2-ylamine (3d). Yield 83%; mp 158−160 °C (MeOH). ^{1}H NMR (DMSO) δ 4.35 (s, 2H, SCH_2); 3.45 (s, 2H, NH_2); 7.4 (s, 5H, arom.). Anal. $C_9H_9N_3S_2$ (223.31) C, H, N.

5-(4-Nitro-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3e). Yield 76%; mp 171−172 °C (EtOH). ^{1}H NMR (DMSO) δ 4.4 (s, 2H, SCH_2); 7.0 (bs, 2H, NH_2). Anal. $C_9H_8N_4O_2S_2$ (268.31) C, H, N.

5-(4-Methoxy-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3f).Yield 85%; mp 151−153 °C (EtOAc). ^{1}H NMR (DMSO) δ 3.7 (s, 3H, OCH_3); 4.3 (s, 2H, SCH_2); 6.8−7.6 (m, 6H, arom. + NH_2). Anal. $C_{10}H_{11}N_3OS_2$ (253.34) C, H, N.

5-(4-Chloro-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3g). Yield 68%; mp 168−169 °C (EtOAc). ^{1}H NMR (DMSO) δ 4.3 (s, 2H, SCH_2); 6.85 (s, 2H, NH_2) 7.38 (s, 4H, arom.). Anal. $C_9H_8ClN_3S_2$ (257.76) C, H, N.

5-(4-Methyl-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3h). Yield 89%; mp 181−182 °C (EtOH). ^{1}H NMR (DMSO) δ 2.3 (s, 3H, CH_3); 4.25 (s, 2H, SCH_2); 6.55 (s, 2H, NH_2); 7.55 (dd, 4H, arom.). Anal. $C_{10}H_{11}N_3S_2$ (237.34) C, H, N.

5-(3-Methoxy-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3k). Yield 79%; mp 148−149 °C (EtOAc). ^{1}H NMR (DMSO) δ 3.8 (s, 3H, OCH_3); 4.35 (s, 2H, SCH_2); 7.15 (m, 6H, arom. + NH_2). Anal. $C_{10}H_{11}N_3OS_2$ (253.34) C, H, N.

5-(3-Chloro-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3i). Yield 74%; mp 138−139 °C (EtOAc). ^{1}H NMR (DMSO) δ 4.4 (s, 2H, SCH_2); 7.2−7.75 (m, 6H, arom. + NH_2). Anal. $C_8H_8ClN_3S_2$ (257.74) C, H, N.

5-(3-Nitro-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3j). Yield 61%; mp 195−196 °C (MeOH). ^{1}H NMR (DMSO) δ 4.5 (s, 2H, SCH_2); 7.4 (s, NH_2); 7.4−8.5 (m, 4H, arom.). Anal. $C_9H_8N_4O_2S_2$ (264.30) C, H, N.

5-(3-Methyl-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3l). Yield 79%; mp 128−129 °C ($EtOH/H_2O$). ^{1}H NMR (DMSO) δ 2.35 (s, 3H, CH_3); 4.35 (s, 2H, SCH_2); 7.3 (s, NH_2); 7.4−7.5 (m, 4H, arom). Anal. $C_{10}H_{11}N_3S_2$ (237.34) C, H, N.

5-(3,4,5-Trimethoxy-benzylsulfanyl)-1,3,4-thiadiazol-2-ylamine (3m). Yield 91%; mp 180−181 °C (EtOH). ^{1}H NMR (DMSO) δ 3.7 (s, 3H, 4-OCH_3); 3.8 (s, 6H, 3,5-OCH_3); 4.35 (s, 2H, SCH_2−); 6.75 (s, 2H, arom.); 7.4 (s, 2H, NH_2). Anal. $C_{12}H_{15}N_3O_3S_2$ (313.39) C, H, N.

***N*-[5-Methylsulfanyl-1,3,4-thiadiazol-2-yl]-acetamide (5a).** 5-Methylsulfanyl-1,3,4-thiadiazol-2-ylamine **(3a)** (0.022 mol) was suspended in acetic anhydride (0.024 mol, 2.28 mL), and acetic acid (9 mL) was added under stirring. The reaction mixture was heated (35−40 °C) until homogeneity was observed. After a few minutes (10−15 min), the solution was brought to room temperature and crystallization of **5a** was observed after the addition of some water (11 mL). Filtration of the precipitate and recrystallization from EtOH afforded compounds **5a** (yield 97%): mp 215−216 °C. ^{1}H NMR (DMSO) δ 2.2 (s, 3H, $COCH_3$); 2.7 (s, 3H, SCH_3); 3.3 (bs, 1H, NH). Anal. $C_5H_7N_3OS_2$ (189.25) C, H, N.

Also prepared using the general procedure described for **5a** were **5b**−**j**.

***N*-[5-Isopropylsulfanyl-1,3,4-thiadiazol-2-yl]-acetamide (5b)**. From **3c**. Yield 98%; mp 187−189 °C (CH_3COCH_3/EtOH). ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 6H, $C(CH_3)_2$); 2.5 (s, 3H, $COCH_3$); 3.85 (m, 1H, CH═); 13.2 (bs, 1H, NH). Anal. $C_7H_{11}N_3OS_2$ (217.30) C, H, N.

***N*-[5-(4-Nitro-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5c)**. From **3e.** Yield 87%; mp 263−265 °C (EtOH). ^{1}H NMR (DMSO) δ 2.2 (s, 3H, $COCH_3$); 3.35 (bs, 1H, NH); 4.65 (s, 2H, SCH_2); 7.6−8.4 (m, 4H, arom.). Anal. $C_{11}H_{10}N_4O_3S_2$ (310.35) C, H, N.

***N*-[5-(4-Methoxy-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5d)**. From **3f**. Yield 77%; mp 177−179 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.5 (s, 3H, $COCH_3$); 3.8 (s, 3H, OCH_3); 4.4 (s, 2H, SCH_2); 6.8−7.5 (dd, 4H, arom.); 13.4 (bs, 1H, NH). Anal. $C_{12}H_{13}N_3O_2S_2$ (295.37) C, H, N.

***N*-[5-(4-Chloro-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5e)**. From **3g**. Yield 86%; mp 213-215 °C (EtOH). ^{1}H NMR (DMSO) δ 2.2 (s, 3H, CH_3CO); 4.5 (s, 2H, SCH_2); 7.45 (dd, 4H, arom.); 12.5 (bs, 1H, NH). Anal. $C_{11}H_{10}ClN_3OS_2$ (299.79) C, H, N.

***N*-[5-(4-Methyl-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5f)**. From **3h**. Yield 85%; mp 212−213 °C (EtOH). ^{1}H NMR (DMSO) δ 2.2 (s, 3H, CH_3CO); 2.3 (s, 3H, CH_3); 4.5 (s, 2H, SCH_2); 7.25 (dd, 4H, arom.); 13.0 (bs, 1H, NH). Anal. $C_{12}H_{13}N_3OS_2$ (279.37) C, H, N.

***N*-[5-Benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5g)**. From **3d**. Yield 96%; mp 170 °C (H_2O). ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 3H, CH_3CO); 4.4 (s, 2H, SCH_2); 7.35 (m, 5H, arom); 13.4 (bs, 1H, NH). Anal. $C_{11}H_{11}N_3OS_2$ (265.35) C, H, N.

***N*-[5-(3,4,5-Trimethoxybenzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5h)**. From **3m**. Yield 88%; mp 200−201 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 2.5 (s, 3H, CH_3CO); 3.9 (s, 9H, 3,4,5-OCH_3); 4.45 (s, 2H, SCH_2); 6.7 (s, 2H, arom.); 13.45 (s, 1H, NH). Anal. $C_{14}H_{17}N_3O_4S_2$ (355.43) C, H, N.

***N*-[5-(3-Methoxy-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5k)**. From **3k**. Yield 82%; mp 172−173 °C (EtOAc). ^{1}H NMR (DMSO) δ 2.25 (s, 3H, CH_3CO); 3.8 (s, 3H, OCH_3); 4.45 (s, 2H, SCH_2); 7.5−7.7 (m, 4H, arom.); 11.0−13.0 (bs, 1H, NH). Anal. $C_{12}H_{13}N_3O_2S_2$ (295.37) C, H, N.

***N*-[5-(3-Chloro-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5i)**. From **3i**. Yield 93%; mp 184−186 °C (EtOAc). ^{1}H NMR (DMSO) δ 2.25 (s, 3H, CH_3CO); 2.8−3.6 (bs, 1H, NH); 4.5 (s, 2H, SCH_2); 7.4−7.5 (m, 4H, arom.). Anal. $C_{11}H_{10}ClN_3OS_2$ (299.79) C, H, N.

***N*-[5-(3-Nitro-benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (5j)**. From **3j**. Yield 69%; mp 223−224 °C (EtOH). ^{1}H NMR (DMSO) δ 2.2 (s, 3H, CH_3CO); 3.2−4.0 (bs, 1H, NH); 4.7 (s, 2H, SCH_2); 7.6−8.5 (m, 4H, arom.). Anal. $C_{11}H_{10}N_4O_3S_2$ (310.35) C, H, N.

***N*-[5-Benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-propionamide (5l)**. From **3d** and propionic anhydride. Yield 65%; mp 162−163 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, CH_3); 2.8 (q, 2H, CH_2CO); 4.5 (s, 2H, SCH_2); 7.4 (m, 5H, arom.); 13.2 (s, 1H, NH). Anal. $C_{12}H_{13}N_3OS_2$ (279.37) C, H, N.

Heptanoic Acid *N*-[5-Benzylsulfanyl)-1,3,4-thiadiazol-2-yl]amide (5m). From **3d** and heptanoic anhydride. Yield 71%; mp 123−124 °C (EtOH). ^{1}H NMR ($CDCl_3$) 0.7−2.0 (m, 10H, $(CH_2)_5$); 2.75 (t, 3H, CH_3); 4.5 (s, 2H, $C_6H_5CH_2$): 7.5 (m, 5H, arom.); 13.3 (s, 1H, NH). Anal. $C_{16}H_{11}N_3OS_2$ (335.48) C, H, N.

***N*-[5-Benzylsulfanyl)-1,3,4-thiadiazol-2-yl]-benzamide (5n)**. 2-Amino-5-benzylsulfanyl-1,3,4,thiadiazole (**3d**) (0.2 mol, 26.62 g) was dissolved in distilled water (25 mL) with stirring, and pure benzoyl chloride was dropped into the solution while mantaining the temperature at 0−5 °C. After 30 min, water (125 mL) was added, and a white precipitate was formed. Filtration of the precipitate and recrystallization from EtOH afforded compound **5n** (yield 67%): mp 193−194 °C. ^{1}H NMR ($CDCl_3$) δ 4.4 (s, 2H, SCH_2); 7.2−8.5 (m, 10H, arom.); 12.8 (bs, 1H, NH). Anal. $C_{16}H_{13}N_3OS_2$ (327.42) C, H, N.

***N*-Methyl-*N*-[5-methylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (6a)**. To a solution of compound **5a** (0.2 mol) in DMF (50 mL) was added K_2CO_3 (1 g, 0.007 mol) under stirring, and then methyl iodide (0.2 mol, 28.4 g) was added dropwise. After 30 min, water (150 mL) was added, and a white precipitate was formed. This was removed by filtration then recrystallized from EtOH, affording compound **6a** (yield 41%): mp 72−74 °C. ^{1}H NMR ($CDCl_3$) δ 2.4 (s, 3H, $COCH_3$); 2.75 (s, 3H, SCH_3); 3.8 (s, 3H, NCH_3). Anal. $C_6H_9N_3OS_2$ (203.28) C, H, N.

***N*-Methyl-*N*-[5-isopropylsulfanyl)-1,3,4-thiadiazol-2-yl]-acetamide (6b)**. From **5b** by the same procedure described for **6a**. Yield 51%; mp 44−45 °C (EtOH). ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 6H, $CH(CH_3)_2$); 2.4 (s, 3H, $COCH_3$); 3.8 (s, 3H, NCH_3). Anal. $C_8H_{13}N_3OS_2$ (231.33) C, H, N.

Pharmacology. Animals. Male albino Swiss mice 25−30 g were used in the despair test and in the experimental procedures adopted for side effects detection.

Male Sprague−Dawley rats 200−250 g were used in the despair test and social interaction test.

Despair Test. As described by Porsolt et al.[13,14] the animals were forced to swim inside a plexiglass cylinder containing water, and the total duration of immobility during a 5 min test was recorded. Antidepressants decrease the duration of immobility.

Test compounds were injected intraperitoneally 1 h before evaluation of the mice, while acute administration in the rat was performed in three ip injections 24, 5, and 1 h before the test and repeated administration once a day for 1 week.

Social Interaction Test. The method was based on that described by File et al.[15] The test arena consisted of a white open-topped box (55 × 55 × 30 cm^3) with a 100 W lamp 50 cm above the box floor. The behavior of pairs of rats was observed over a 10 min test period, and the time spent in social interaction (following, sniffing, crawling, tumbling, boxing, grooming) was recorded. Such increases in social interaction are considered to be predictive of anxiolytic activity. The compounds were administered ip 1 h before the test.

Spontaneous Motor Activity. Each mouse was placed in a cage (40 × 40 × 20 cm^3) designed for horizontal motor activity detection (Activity Cages-U.Basile) 1 h after treatment and allowed to explore its environment. The movements were recorded every 5 min for a total period of 15 min.

Ethanol Potentiation Test. Mice were treated with the test compound and 1 h later with ethanol 2.5 g/kg ip. This dose of ethanol did not induce lateral position in the control animals. The number of animals that were in the lateral position after receiving ethanol in each group was then determined.

Rotarod Test. The disruptive effects on motor coordination were assessed using the Rotarod Treadmills mouse test (U.Basile). The animals were placed on a rotating rod (24 rpm) and then observed for 5 min. Each daily session consisted of two trials to evaluate predrug performance (T_1) and postdrug performance (T_2) 1 h after ip administration of the test compound. Animals that fell from the rod three times during the first trial were excluded. The number of mice in each single dose group that fell from the rod is taken to calculate the ataxic properties of the test compound, and the impairment of motor coordination was defined as

$$[(T_1 - T_2)/T_1] \times 100$$

Passive Avoidance Test. The method followed that described by Bammer et al.[16] Briefly, rats were placed individually into the lit compartment of a two-compartment box. When they crossed to the darker compartment they received a light foot-shock (1 mA) and then were immediately removed (S1). After 24 h they were again placed in the lighted compartment, and the time interval before the rats crossed into the dark compartment (step-through) was measured with a cutoff time of 180 s (S2). An increase in the S2 step-through interval compared with S1 would indicate that the rat remembered having received a shock in the dark compartment 24 h previously. A decrease in the S2 latency in rats that had received the test compound compared to control group would indicate an amnesia-inducing effect. The experiment was performed using 10 rats per group. The test compound was administered ip 1 h before S1 at 0.5, 1, or 2 mg/kg.

Statistical Analysis. Data from the despair test, the social interaction test, passive avoidance, and motor activity were

analyzed by the two-tailed Mann−Withney test for independent samples.

ED_{50} values were determined by the Spearman−Kärber method.[17]

References

(1) Chapleo, C. B.; Myers, M.; Myers, P. L.; Saville, J. F.; Smith, A. C. B.; Stillings, M. R.; Tulloch, I. F.; Walter, D. S.; Welbourn, A. P. Substituted 1,3,4-thiadiazoles with anticonvulsivant activity. I. Hydrazines. *J. Med. Chem*. **1986**, *29*, 2273−2280.

(2) Stillings, M. R.; Welbourn, A. P.; Walter, D. S. Substituted 1,3,4-thiadiazoles with anticonvulsivant activity. II. Aminoalkyl derivatives. *J. Med. Chem*. **1986**, *29*, 2280−2284.

(3) Chapleo, C. B.; Myers, P. L.; Smith, A. C. B.; Tulloch, I. F.; Walter, D. S. Substituted 1,3,4-thiadiazoles with anticonvulsivant activity. III. Guanidines. *J. Med. Chem*. **1987**, *30*, 951−954.

(4) Brufani, M.; Loche, A.; Perlini, V.; Pocar, D. (Laboratorio Farmaceutico C. T. S.r.l.) Eur. Pat. Appl. EP 579,129; Preparation of 2-amino-5-[(ar)alkylthio]thiadiazoles as antidepressant. *CA* **120**, 1994, 245119.

(5) Costall, B.; Naylor, R. J.; Tyers, M. B. The psychopharmacology of 5 HT3 receptors. *Pharmacology and Therapeutics* **1990**, *47*, 181−202.

(6) Pala, G. Sintesi di composti solfonilici per alogenazione catalitica. *Il Farmaco* **1958,** *13*, 650−664.

(7) Shams El-Dine S. A.; Hazzaa, A. A. B Synthesis of compounds with potential fungicidal activity. *Pharmazie* **1974**, *29*, 761−763.

(8) Eldawy, M. A.; Shams El-Dine S. A.; El-Brembaly, K. M. Potential broad spectrum anthelmintics. Part 4: design, synthesis and spectral study of imidazo[2,1-*b*]-1,3,4-thiadiazole derivatives. *Pharmazie* **1979**, *34*, 144−147.

(9) Avetisyan, A. Kh.; Ovsepyan, T. R.; Stepanyan, N. O.; Sapondzhyan, L. G. Synthesis and hypoglycemic activity of sulfonamido-1,3,4-thiadiazoles. *Khim.-farm. Zh.* **1981** *15*, 69−72. CA **1981**, 95, 115404.

(10) Eberle, M.; Manning, R. E. (Sandoz Ltd.) Gen. Off. DE 2212245; 2-Amino-5-(phenylalkyl)-1,3,4-thiadiazoles. *CA* **1972**, *78*, 16192.

(11) Iwamoto, H.; Tanaka, A.; Nishikiori, K.; Ikadai, H. (Yamanouchi Pharmaceutical Co., Ltd.) Japan Kokai Tokkio Koho JP 62 93, 284 [87 93, 284] Preparation of [(alkoxyphenyl)alkylthio]-aminoazoles useful in prophylaxis and treatment of hepatopathy. *C. A.* **1987**, *107*, 115597.

(12) Pilgram, K.; Skiles, R. D. Bridgehead nitrogen heterocycles. III. Formation by reaction of α-ureido N−Heterocylic compounds with chlorothioformyl chloride. *J. Org. Chem*. **1973**, *38*, 1578−1582.

(13) Porsolt, R. D.; Bertin, A.; Jalfre, M. Behavioural despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn*. **1977**, *229*, 327−336.

(14) Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur. J. of Pharm*. **1978**, *47*, 379−391.

(15) File, S. E. The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *J. Neurosci. Meth*. **1980**, *2*, 219−238.

(16) Bammer, G.; Chesher, G. B. An analysis of some effects of ethanol on performance in a passive avoidance task. *Psychopharmacology* **1982**, *77*, 66−73.

(17) Cavalli-Sforza, L. Analisi dei Probit. In *Analisi statistica per medici e biologi*, 2th ed.; Boringhieri Ed.; Torino, 1965; pp 173−175.

JM001027W