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Neurotropic activity of aldehyde and ketone thiosemicarbazones with a heterocyclic component

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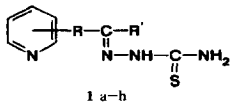
thiosemicarbazone / neurotropic activity

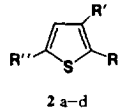
Introduction

Thiosemicarbazones (TSCs) of the heteroaromatic aldehydes and ketones are widely known as carcinostatic and antimicrobial agents. Recently, it has been found that the silyl-substituted furfural TSCs possess neurotropic activity [1]. TSCs of arylidene and aryl aldehydes and ketones have shown anticonvulsant activity in maximal electroshock seizure tests [2–4]. The aim of this study was to evaluate the effect of replacement of the benzene ring with a heterocycle, and to determine the influence of heterocycles and some substituent structures on the neurotropic activity of TSCs.



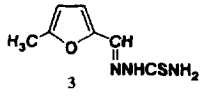
1 a–h

Compound	Isomer	R	R'
1a	2-Pyridyl	-	CH_3
1b	3-Pyridyl	-	CH_3
1c	4-Pyridyl	-	CH_3
1d	4-Pyridyl	CH=CH	H
1e	2-Pyridyl	CH=CH	CH_3
1f	3-Pyridyl	CH=CH	CH_3
1g	4-Pyridyl	CH=CH	CH_3
1h	2-Pyridyl	$CH(OH)CH_2$	CH_3



2 a–d

Compound	R	R'	R''
2a	$C(CH_3)=NNHCSNH_2$	H	CH_3
2b	CH_3	$C(CH_3)=NNHCSNH_2$	CH_3
2c	$C(CH_3)=NNHCSNH_2$	CH_3 H	Br
2d	Br	$C(CH_3)=NNHCSNH_2$	Br



3

Chemistry

TSCs **1–3** were prepared by the reaction of aldehydes or ketones with a thiosemicarbazide.

The ^{1}H-NMR spectroscopic study of TSCs **1–3** in DMSO-d_6 solution showed that the formation of the *E*-isomer was preferable for all the compounds (tables I and II). This conclusion is true for the pyridine derivatives, as is proved by the relative upfield δNH shift (10–11 ppm) of the NH proton (table I). The *Z*-isomers of TSCs of pyridine and 1-isoquinoline aldehydes and pyridazinyl ketones usually have rather downfield shifts (14–16 ppm) [5, 6]. The value of δNH for **1f–h** (11–11.5 ppm) is dictated by the intramolecular hydrogen bonding with the nitrogen heteroatom. Moreover, there is an extraordinary deshielding of pyridine ring H^3 protons of the 2-substituted pyridine derivatives and a downfield shift of OH group proton in compound **1h** typical of *E*-isomers. The small value of $\Delta\delta H^3$ for both *E*- and *Z*-isomers of compound **1h** is an exception. The substituent R structure is responsible for this small value. A 2D-NOESY study of compounds **1b,f** and **2c** confirms these conclusions. The methyl group of **1b** shows cross peaks of different intensity to NH, H^2, and H^4 protons, and the methyl group of **2c** shows different cross peaks to NH and H^3 protons. The spectra of TSCs **1f–h**, which contain the pyridyl moiety, had signals due to the second isomers. The second isomers of compounds **1f–h** were present in a smaller quantity and have the *Z*-configuration. In 2D-NOESY of an *E*- and *Z*-isomer mixture of **1f**, only the methyl group of major isomer shows a cross peak with the NH proton. The *E*/*Z* isomer ratio is found by integrating the CH_3 group protons; these ratios were 5:1, 12:1 and 2:1 for **1f–h**. The compounds **1d–g** with C=C double bonds have *trans* configurations.

Table I. ^{1}H-NMR chemical shifts (ppm, DMSO-d_6) of the pyridine series thiosemicarbazones **1a–h**.

Compound	*Heterocycle-H*					*CH=CH*[a]	*CH_3*	*N-H*	*NH_2*
	H-2	*H-3*	*H-4*	*H-5*	*H-6*				
1a	–	8.30	7.73	7.28	8.47	–	2.35	10.2	8.01; 8.29
1b	8.98	–	8.21	7.29	8.45	–	2.26	10.1	7.95; 8.18
1c	8.47	7.80	–	7.80	8.47	–	2.25	10.3	8.00; 8.30
1d	8.56	7.50	–	7.50	8.56	7.02; 7.22	7.93[b]	11.5	7.80; 8.18
1e	–	8.40	7.70	7.20	8.45	7.04; 7.26	2.11	10.3	7.65; 8.25
E-**1f**	8.72	–	7.98	7.41	8.48	7.00; 7.15	2.35	10.3	7.82; 8.30
Z-**1f**	8.88	–	8.21	7.47	8.52	7.00; 7.14	2.23	11.0	7.79; 8.17
E-**1g**	8.56	7.48	–	7.48	8.56	7.08; 7.14	2.18	10.4	7.90; 8.38
Z-**1g**	8.60	7.70	–	7.70	8.60	7.08; 7.14	2.20	11.2	7.83; 8.22
E-**1h**	–	7.42	7.68	7.15	8.48	–	1.92	10.0	7.52; 8.03
Z-**1h**	–	7.45	7.78	7.24	8.59	–	1.88	10.5	7.43; 7.92

[a] ^3J = 16.0 ± 0.5 Hz; [b]CH=N (doublet, J = 8.1 Hz).

Table II. ^{1}H-NMR chemical shifts (ppm, DMSO-d_6[a]) of the thienyl and furyl thiosemicarbazones **2a–d**, **3**.

Compound	*Heterocycle-H*		*5-CH_3*[c]	*$C(CH_3)$=N*	*NH*	*NH_2*
	3-H[b]	*4-H*				
E-**2a**	7.09	6.64	2.41	2.27	10.1	7.1; 8.0
2b	–	6.88	2.28 2.40[d]	2.16	10.0	7.3; 8.1
Z-**2c**	7.28	7.14	–	2.24	10.3	7.4; 8.2
2d	–	7.35	–	2.29	10.4	7.4; 8.3
3	6.78	6.19	2.28	7.85[e]	11.3	7.5; 8.0

[a]$CDCl_3$ + DMSO-d_6 (1:4); [b] $^3J_{3H\text{-}4H}$ = 3.8 (**2a**), 4.0 (**2c**) and 3.2 Hz (**3**); [c] $^4J_{(4H)\text{-}(5\text{-}CH)}$ = 0.9–1.0 Hz; [d]2-CH_3; [e]HC=N.

Results and discussion

The compounds examined may be arranged into three groups. One includes TSCs of 2-, 3- and 4-acetylpyridine (**1a–c**), another includes TSCs of ketones containing pyridylvinyl and pyridyl(hydroxy)ethyl groups (**1d–h**) and the third TSCs of thienyl methyl ketones (**2a–d**) and 5-methyl-2-furanaldehyde (**3**). The experimental data summarized in table III ascertain that the depriming activity in the 'rota rod', 'tube' and 'traction' tests is the highest for the first group of compounds with the acetyl group in the 2 and 4 positions (**1a, c**). It is lower to some degree in the case of the 3-isomer (**1b**). Compound **1a** stands out for its acute toxicity. The toxicity is considerably lower in the case of the 3- and 4-isomers (**1b, c**). The insertion of the CH=CH group between the pyridine ring and thiosemicarbazono group in position 2 of the ring also increases the LD_{50} value. The protective index (PI = LD_{50}/ED_{50}) of the depriming activity of compound **1a** is 1.57, and so its therapeutic action is only pronounced at the subtoxic doses. The PI significantly increases when the acetylthiosemicarbazone group is transferred to the 3- or 4-positions, where its value is 3.9 and 31.7, respectively. Analogous correlations between the activity and the position of acetyl group in pyridine ring have been observed in hypothermal tests. The depriming activity of TSC **1d–h** of the second group is sometimes lower. The comparison of pharmacological action of compounds **1e–g** shows with certain probability that the depriming activity of **1e** is higher than that of the 3- and 4-isomers. For all compounds of this group the analogous hypothermal activity has been observed.

The substitution of the pyridine ring for the thienyl and furyl structures significantly decreases the toxicity of the compounds. Comparison of the TSCs of **2a** and **2b** with **2c** and **2d** shows that the toxicity of the compounds with bromine atoms instead of methyl groups is higher by 1.5–6.5 times.

Table III. Acute toxicity and depriming activity of the thiosemicarbazones of pyridine, thiophene and furan series.

Compound	LD_{50} *(mg/kg)*	ED_{50} *(mg/kg)*			
		Rota rod	*Tube*	*Traction*	*Hypothermia*
1a	7.1 (5.0–9.3)	4.5 (2.6–6.4)	4.1 (2.7–5.5)	2.8 (1.6–4.2)	3.6 (2.5–4.6)
1b	112.0 (79–147)	28.2 (18.3–37.2)	25.8 (16.8–35.7)	43.5 (16.1–52.4)	32.5 (21.9–45.5)
1c	178.0 (136–230)	5.6 (3.9–7.4)	3.5 (2.5–4.6)	4.1 (2.7–5.5)	4.5 (3.1–6.0)
1d	178 (112–253)	81.5 (56.7–111.0)	44.7 (31.3–59.6)	65.0 (43.8–88.6)	51.5 (36.2–69.2)
1e	141.0 (92–209)	20.5 (14.6–28.8)	17.8 (13.6–23.0)	17.8 (13.6–23.0)	25.8 (16.8–35.7)
1f	224 (144–285)	32.5 (21.9–45.0)	22.4 (14.4–28.5)	41.0 (26.8–55.2)	44.7 (31.3–59.6)
1g	178 (136–230)	44.7 (31.3–59.6)	35.5 (24.9–46.1)	56.4 (38.7–74.3)	51.5 (36.2–69.2)
1h	141.0 (92–209)	41.0 (26.8–55.2)	22.4 (14.4–28.5)	51.5 (36.2–69.5)	41.0 (21.1–62.2)
2a	515 (362–692)	34.6 (12.0–66.2)	22.4 (14.4–28.5)	69.0 (24.2–130.3)	44.7 (31.3–59.6)
2b	708 (501–925)	109.0 (46–205.8)	89.0 (63.0–119.7)	282.0 (183–372)	258.0 (168–357)
2c	355 (249–461)	2.8 (1.8–3.7)	2.8 (1.6–4.2)	2.8 (1.6–4.2)	4.4 (1.6–8.2)
2d	129 (84–179)	56.4 (34.2–81.4)	28.2 (18.3–37.2)	65.0 (43.8–88.6)	44.7 (31.3–59.6)
3	564 (387–743)	70.8 (43.0–102.0)	69.0 (24.2–130.3)	112.0 (64.8–163.9)	103.0 (67.4–138.0)

The highest depriming activity and PI value were found for TSC **2c** in the third group. The replacement of 5-Br by 5-CH_3 considerably decreased its activity.

The location of the side chain at the thienyl ring appears to be decisive. The activity of **2b** is substantially lower (by a factor of 12.3) than that of **2a**. The significant decrease in the depriming activity is caused by the substitution of the thienyl ring of compound **2a** for furan (**3**).

The depriming activity of the examined compounds is testified by their ability to prolong the hexobarbital and ethanol anaesthesia. As seen from table IV, the TSC derivatives of the pyridine prolong anaesthesia the most. The degree of strengthening also depends on the location of the side chain on the heterocycle. In both groups (**1a–h**) of substances, the derivatives of acetylpyridine and pyridylbutenone TSCs substituted at position 2 exhibit the best anaesthesia-prolongation properties. The activity of 3- and 4-isomers is lower in both cases. In the case of hexobarbital the duration of anaesthesia appears to be lower for compounds containing the bromine atom (**2c**, **2d**) than that for the corresponding methyl derivatives (**2a**, **2b**). In contrast, TSCs of the methylthienyl methyl ketones do not exert the significant activity towards ethanol anaesthesia, but the corresponding TSCs of the bromothienyl methyl ketones prolong the duration of ethanol anaesthesia by a factor of 1.6. The furyl derivative **3** does not prolong this type of anaesthesia.

Table IV. Neurotropic activity of thiosemicarbazones of the pyridine, thiophene and furan series.

Compound	*(M + m) % (control 100%)*					*Porsolt's test*		*Retrograde amnesia*[a]	*Passive avoidance response*[a]
	Hypoxia	*Hexo-barbital anaesthesia*	*Ethanol anaesthesia*	*Amphetamine locomotor activity*	*Pentylene-tetrazole action*	*Latent time (s)*	*Immobiliza-tion time (s)*		
1a	173.0*	> 300*	291.2*	19.1*	67.8*	129.8	98.2	50.0	18.3
1b	188.2*	104.5	243.1*	10.0*	145.2*	243.1*	95.2	50.0	82.5*
1c	115.8	155.5*	256.1*	13.0*	78.0*	256.1*	71.1*	50.0	39.2
1d	119.3	126.3	215.8*	15.8	101.8*	109.6	67.2*	66.7*	36.6
1e	137.8*	303.9*	259.9*	7.4*	184.1*	250.9*	84.2*	66.7*	58.0*
1f	130.0*	152.7*	230.0*	88.9	132.9*	230.0*	81.4	16.7	6.6
1g	128.5*	162.5*	247.0*	8.0*	139.2*	247.0*	89.4	66.7*	103.3*
1h	117.3	208.5*	271.7*	3.3*	108.0	135.3*	116.0	100.0*	77.5*
2a	139.9*	164.5*	121.7	11.2*	131.0	99.3	120.0	50.0	14.2
2b	118.8	146.6*	108.5	44.0*	105.8	111.0	109.5	66.7	80.0*
2c	179.1*	132.5*	162.5*	12.1*	107.8	89.8	115.0	50.0	32.5
2d	141.6*	139.5*	150.0*	19.7*	92.2	88.2	111.3	66.7	35.8
3	187.7*	92.9	97.5	26.1*	139.9*	84.7*	115.4	33.3	5.0

[a]Control 3.6 s; 16.6%. *Differences are statistically significant *vs* control at $P < 0.05$.

All the examined substances were shown to lack any protective activity at the dose of 5 mg/kg. As regards pentylenetetrazole-induced convulsions, a good activity was observed for the compounds **1b**,**d–h**, **2a–c** and **3**. Opposite results were observed for the TSCs of aryl alkyl ketones [4]. These compounds protect against seizures induced under maximal electroshock but not in the pentylenetetrazole seizure test even at a dose of 50 mg/kg. It is necessary to note that all the examined TSCs of the pyridyl and thienyl series exhibit a pronounced action on central dopaminergic structures and the depressing action on the pharmacological effects of amphetamine, particularly on the locomotor activity, which was diminished up to 3–25% *vs* control.

The action of the examined substances on the memory processes is both interesting and peculiar. Some of the studied TSCs improve the learning at the dose of 5 mg/kg and diminish the retrograde amnesia caused by electroshock by 50–100%. TSCs **1g** and **1h** have the highest activity in this test. The influence on memory processes is correlated with the antihypoxic activity of the examined substances. These data give evidence for the fact that, in the spectrum of the neurotropic activity of the substituted pyridine TSCs, the significant role belongs to the components of nootropic activity. These results are ascertained by the data of the immobilization duration in the Porsolt's test, demonstrating that the substances of the second group (the TSCs of the substituted pyridine butenones) and compound **1c** prolong the latent period of the primary active swimming and shorten the duration of immobilization period. Thus, the stimulating antidepressive properties of the mentioned substances are confirmed.

Conclusion

The structure of heterocycle and the location of the side chains are responsible for the depriming activity as well as for the toxicity of the studied compounds.

The spectrum of the pharmacological activity of TSCs of the pyridine derivatives differs from that of the thiophene- and furan-containing compounds in the specific action towards hexobarbital and ethanol anaesthesia, the convulsions caused by pentylenetetrazole, the memory processes, the antihypoxic properties, the duration of immobilization and the action on the central dopaminergic structures. TSCs of the thienyl bromo derivative and TSCs of 4-acetylpyridine possess the highest depriming activity.

The highest toxicity is specific for 2-isomers of pyridine-containing TSCs.

The obtained results show that, in contrast to TSC of arylidene and aryl ketones, a good anticonvulsant activity in the pentylenetetrazole test occurs even at the dose of 5 mg/kg in the pyridine series.

All these data show that the compound examined exert an exceptional neurotropic activity. The search for compounds with depriming activity involved 2-pyridyl and 2-thienyl aldehyde and ketone TSCs. One useful substituent for the heteryl ring may be bromine. For improving memory processes, the structures of pyridyl-substituted aliphatic aldehyde and ketone TSC need to be determined.

Experimental protocols

Chemistry

^{1}H-NMR spectra were registered on a Bruker WM-90/DS, 90 MHz spectrometer in Me_2SO-d_6 using TMS as an internal standard. TLC (silica gel plates Silufol UV-254, hexane/ethylacetate 2:5 system) and GLC methods were used to follow the reactions. Chromatographic columns were loaded with 10% of SE-301 and 2.5% of Reoplex 400 or 5% OV-17 on Chromosorb WAW. The control of the product purity was accomplished by HPLC. Elementary analysis was performed using a Carlo Erba instrument (Model 1108). All compounds were analyzed for C, H, N, S and the analytical values were within ±0.4% with respect to the calculated ones.

*Synthesis of thiosemicarbazones **1–3** (general procedure)*
Thiosemicarbazide (4.5 mmol) was added to a solution of 4.5–5 mmol aldehyde or ketone in ethanol or a water/ethanol mixture. The mixture was refluxed for 1–27 h (table V). When the reaction was over, the reaction mixture was cooled and filtered, the resulting precipitate washed with ethanol and dried at ambient temperature. The reaction yields and melting points are summarized in table V. 3-(4-Pyridyl)acrolein, pyridylvinyl and thienyl ketones were synthesized according to the literature [10–12].

Table V. Thiosemicarbazones of heteryl-substituted aldehydes and ketones.

Compound	*Reaction time (h)*	*Yield (%)*	*Mp (°C) (literature data)*	*Formula*
1a	6	77	157–159 dec (158–160 [7])	$C_8H_{10}N_4S$
1b	10	82	212–216 (217 [7])	$C_8H_{10}N_4S$
1c	10	82	225–228 dec (218 [7])	$C_8H_{10}N_4S$
1d	1	75	206–208 dec	$C_8H_{10}N_4S$
1e	1	49	167–169	$C_{10}H_{12}N_4S{\cdot}0.5H_2O$
1f	1	90	208–211	$C_{10}H_{12}N_4S$
1g	1	78	218–220	$C_{10}H_{12}N_4S$
1h	1	35	149–150	$C_{10}H_{14}N_4OS$
2a	15	53	163–164 (161–163 [8])	$C_8H_{11}N_3S_2$
2b	18	71	151–153 (157 [7])	$C_9H_{13}N_3S_2$
2c	27	39	201–203 (200–201 [8])	$C_7H_8BrN_3S_2$
2d	27	31	177–181	$C_7H_7Br_2N_3S_2$
3	5	71	163–165 (162–164 [9])	$C_7H_8N_3OS$ $C_7H_9N_3OS$

Biological evaluation

The neurotropic activity of TSC of pyridine, thienyl and furyl aldehydes and ketones were studied on BALB/c, Icr:Icl and CBA male mice weighing 18–24 g in the autumn/winter. During the experiment mice received granulated food of full value and water *ad libitum*. Ambient temperature in the laboratory and in the animal colony was maintained at 19–22°C. The test substances were administrated intraperitoneally (ip) at the dose of 5 mg/kg, 1 h prior the assay (the aqueous suspensions prepared with Tween-80, 1–2 drops of 0.6% solution). Control animals received injections of equal amounts of distilled water with addition of Tween-80. The action of TSC on the central nervous system was assessed by observing the effects on coordination and muscle tone using the 'rota rod', 'traction' and 'tube' tests [13–15]. Rectum temperature was registered with electrothermometer. Anticonvulsant activity was measured in the maximal electroshock seizure [16] and pentylenetetrazole [17, 18] (1% solution, iv, 0.01 ml/s) seizure tests. Effects on the duration of ethanol (59 ml/kg, ip) and hexobarbital (70 mg/kg, iv) induced sleeping time, the average life span in hypoxia test, learning and memory processes were evaluated in a passive avoidance response (PAR) test and retrograde amnesia (RA) was evaluated by applying maximal electroshock through corneal electrodes immediately after the learning session [19, 20]. A forced swimming test was carried out by the method described by Porsolt [21]. The influence of amphetamine-induced (10 mg/kg, sc) locomotor activity and rectal temperature was tested after 0.5 and 1 h following the amphetamine administration [22]. Statistical evaluation of the experimental results, and the values of mean lethal doses (LD_{50}) and the mean effective doses (ED_{50}) was carried out. The results obtained during sleeping time after administration of hexobarbital and ethanol, amphetamine locomotor activity, pentylenetetrazole-induced convulsions and hypoxia tests were expressed as the mean values ± SEM and analyzed by a Student's *t*-test. The criterion for statistical significances was $P < 0.05$.

References

1 Lukevics E, Demicheva L, Erchak N, Germane S (1993) *Appl Organomet Chem* 7, 543–551
2 Dimmock JR, Smith DC, Brenner JM *et al* (1986) *Eur J Med Chem* 21, 187–192
3 Dimmock JR, Jonnalagadda SS, Hussein S *et al* (1990) *Eur J Med Chem* 25, 581–588
4 Dimmock JR, McColl JM, Wonko SL, Thayer RS, Hancock DS (1991) *Eur J Med Chem* 26, 529–534
5 Antonini I, Claudi F, Franchetti P, Grifantini M, Martelli S (1977) *J Med Chem* 20, 447–449
6 Easmon J, Heimisch G, Holzer W (1989) *Heterocycles* 29, 1399–1408
7 Anderson FE, Duca CJ, Siudi JV (1951) *J Am Chem Soc* 73, 4967–4968
8 Compaigne E, Monroe PA, Archer WL (1953) *J Am Chem Soc* 75, 988–989
9 Mndzhoyan AL, Afrikyan VG, Khorenyan GA, Vasileva TN, Zhuruli LD, Karagezyan SG (1962) *Izv Akad Nauk Arm SSR, Ser Khim* 15, 391–397
10 Leitis LJ, Shymanska MV, Rubina KI, Jansone DP, Goldberg YSH (1980) *Izv Akad Nauk Latv SSR, Ser Khim* N4, 469–472 (in Russian)
11 Kuchkova KJ, Popa DP (1986) *Izv Akad Nauk Mold SSR, Ser Biol and Khim* N1, 77–78
12 Minnis W (1932) *In: Organic Synthesis*, Vol 12 (Whitmore FC, ed) John Wiley and Sons, New York, 62–63
13 Germane SK, Eberlinsh OE, Kozhukhov AN (1987) *In: Scientific and Practical Aspects of Biological Research of New Drugs* (E Lukevics, ed) Zinatne Publisher, Riga, 86–99
14 Dunham NW, Miya TS (1957) *J Am Pharm Assoc Sci* 46, 208–209
15 Kuribara H, Higuchi Y, Todokoro S (1977) *Jpn J Pharmacol* 27, 117–126
16 Goodman LS, Grawal M, Brown WC, Swinyard EA (1953) *Pharmacol Exp Ther* 108, 168–176
17 Swinyard EA (1969) *Epilepsia* 10, 107–119
18 Jenney EH, Pfeiffer CC (1956) *Ann NY Acad Sci* 64, 679–689
19 Sweeney JE, Bachman ES, Coyle JT (1990) *Psychopharmacology* 102, 191–200
20 DeNoble VJ, DeNoble KF, Spencer KR *et al* (1990) *Pharm Biochem Behav* 36, 957–961
21 Porsolt RD, Bertin A, Jaffre M (1977) *Arch Int Pharmacodyn* 229, 327–336
22 Snyder SH (1970) *In: Principles of Psychopharmacology* (Clark WG, ed) Plenum Press, New York, 115–125