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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Structure–activity relationship study of thiosemicarbazones on an African trypanosome: *Trypanosoma brucei brucei*

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Abstract To explore the structure–activity relationships of thiosemicarbazones on African trypanosome: *Trypanosoma brucei brucei*, a series of thirty-five thiosemicarbazones **(1–35)** have been synthesized and characterized by their ^{1}H NMR, ^{13}C NMR, and FT-IR spectra. All compounds were tested for trypanocidal activity using the method "Lilit alamar blue". The comparison of trypanocidal power of thiosemicarbazones was performed considering their structures. This study that was done using acetophenone thiosemicarbazone **(1)** as basic model, showed that: (a) the presence of lipophilic substituents in *para* position on benzene ring, (b) substitution of benzene ring and (c) substitution of hydrogen of thioamide function by a phenyl, strongly influence trypanocidal activity. The various modifications to basic structure **(1)** allowed the synthesis of 1-(4-chlorophenyl) ethylidene-4-phenyl-thiosemicarbazide **(34)**. With a trypanocidal activity of 3.97 μM, this compound is the most active of the series.

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Introduction

Thiosemicarbazones are derivatives of aldehydes or ketones. Many thiosemicarbazones are crystalline solids used for identification of the corresponding aldehydes or ketones (Williamson, 1999). However, these small molecules (compared to peptides) have interesting biological activities. Thiosemicarbnazones are DNA replication and many proteases inhibitors'. This inhibitory activity justifies the level of interest given to them in the fight against microbial and parasitic diseases. Molecules used to treat these diseases have costs out of reach of poor and in some cases have high risk of toxicity (Aguirre *et al.*, 2004).Thiosemicarbazones have many biological activities such as: antiviral (Garcia *et al.*, 2003), antibacterial (Sau *et al.,* 2003; Rebolledo *et al.,* 2003; Kasuga *et al.,* 2003), antimalarial (Klayman *et al.*, 1984), antitumor (Quiroga *et al.,* 1998, Perez *et al.*, 1999; Easmon *et al.*, 2001; Hall *et al.,* 2000; Kovala-Demertzi *et al.,* 2002; Afrasiabi *et al.,* 2003, 2004), anticonvulsant (Pandeya *et al.*, 1998, 1999). However, the cost of thiosemicarbazones synthesis is not expensive. In addition, thiosemicarbazones are important intermediates in drugs synthesis, formation of metal complexes and heterocycles such as thiadiazolines preparation (Chapleo *et al*., 1988; Sau *et al*., 2003).

Unlike American trypanosome, for which many studies information regarding were performed on thiosemicarbazone derivatives (Du *et al.*, 2002; Fuiji *et al.*, 2005), much remains to be discovered on the structure–activity relationship thiosemicarbazones of African trypanosome. Yet

it is a protozoan parasite that affects humans as well as animals. While *Trypanosoma brucei brucei* decimate livestock, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* species are responsible for human African trypanosomiasis, an endemic disease in sub-Saharan Africa, with 7139 estimated cases and at-risk population of 60 million people (WHO, 2012).

The aim of this work is to study the structure–activity relationships of thiosemicarbazones of ketones and aldehydes on *Trypanosoma brucei brucei* in order to develop new active molecules in a safer chemotherapeutic approach.

Experimental

Chemistry

Ketones, aldehydes, and thiosemicarbazide hydrochloride used for synthesis of thiosemicarbazones are sold by Acros Organics and Aldrich. TLC analyses were performed using silica gel-precoated plates. The solvent used is dichloromethane/ethyl acetate (v/v 2/1). Thiosemicarbazones were purified by recrystallization in ethanol. Compounds purity was confirmed by LC/MS in mode APCI on column C18. The melting points were taken on a fusionometer *eletrothermal 1A 9000*. The spectrometric data were recorded with the following instruments: IR, Perkin Elmer FT-IR 286; ^{1}H NMR and ^{13}C NMR, Bruker 400. To synthesize thiosemicarbazones: a mixture of ketone or aldehyde (20 mmol dissolved in 100 mL of ethanol) and thiosemicarbazide (20 mmol dissolved in 20 ml of 1 N hydrochloric acid) is stirred until the thiosemicarbazone precipitates. The precipitate is filtered, dried, and recrystallized in ethanol (96°) to give thiosemicarbazone crystals (Fig. 1).

Antitrypanosomal activity (Lilit, Alamar Blue TM)

The test is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the "Lilit Alamar Blue" method (Baltz *et al.,* 1985; Hirumi, 1994; Räz *et al.,* 1997). The stock solutions of thiosemicarbazones have been prepared from a standard solution an initial concentration (10 mg/mL in DMSO). The trypanosomes are grown in a medium containing 10 % of heat-inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensions were adjusted to 5×10^{-4} tryp/mL. In each well, 50 µL of different dilutions of the stock solution were added to 50 µL of suspension of trypanosomes. The plates were then incubated at 37 °C for 72 h in an atmosphere with 5 % CO_2. Then, 10 µL of dye "Alamar BlueTM" is added to each well and incubated for 4 h. The dye "Alamar Blue TM" is a reagent for detecting enzymatic activity. The wells in which the concentration of compound is insufficient to inhibit the proliferation of trypanosomes are stained. The CMI is the concentration of unstained wells in which there is the lowest amount of thiosemicarbazone. The plate reading is made by comparison with control wells on a fluorescence plate reader using an excitation wavelength of 530 nm and an emission wavelength of 590 nm.

Toxicity test against *Artemia salina* (Sleetand Brendel)

Encysted eggs of *Artemia salina* (10 mg) were incubated in 100 mL of sea water at pH 7–8. After 48 h of incubation, larvae are collected with a Pasteur pipette. We dissolve the samples with 1 % DMSO (dimethylsulfoxide). Then, a series of ten solutions of thiosemicarbazones at varying and progressive concentration were prepared. A defined number of larvae were introduced into each solution. All solutions and even control solutions containing no active substance are left to stir for 24 h. The number of surviving larvae in each solution that is counting after 24 h of incubation is used to evaluate toxicity of solution. In case of death in control medium, the data is corrected by Abbott's formula: % death = ((test − control)/control)) × 100. The LC_{50} value is determined by linear regression.

Results and discussion

Chemistry

Thirty-five thiosemicarbazones were synthesized with yields going from 45 to 95 %.The use of a polar protic solvent such as ethanol, is good for the reaction. Yields are generally greater than 70 % except with the 2′-chloroacetophenone-thiosemicarbazone (45 %). This is generally the case of 2-substituted aryl ketones (Du *et al.,* 2002). The physical and spectrometric data of these

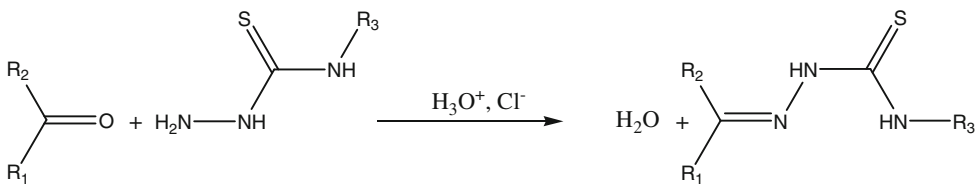


Fig. 1 Synthesis of thiosemicarbazones **(1–35)**

compounds are reported in Table 1. Thin layer chromatography (TLC) shows that thiosemicarbazones have *Rf* ranging from 0.53 to 0.86.

The spectrometric data are in conformity with the structures suggested for the products. Thus, IR spectra of thiosemicarbazones show bands in range of 3,455–3,139 cm^{-1} due to the stretching vibration of NH. The C=N stretching band appears at 1,588 or 1,587 cm^{-1}. In the ^{1}H NMR spectra of the most deshielded proton, which is linked to the central nitrogen atom appears as a broadened singlet between 8.9 and 11.70 ppm. In ^{13}C NMR spectra, thiosemicarbazones C=N bond is indicated by chemical shifts between 145 and 149 ppm while the chemical shift of the C=S bond appears between 176 and 180 ppm.

Biology

Trypanocidal activity of thiosemicarbazones

The synthesized compounds were tested for their trypanocidal activity on *Trypanosoma brucei brucei*. The test results are reported in Table 2. The thiosemicarbazone of acetophenone **(1)** have an IC_{50} value of 211.88 ± 7.38 μM. Presence of a chlorine atom in *ortho* position in the thiosemicarbazone of 2-chloroacetophenone (**5** IC_{50} = 199.37 ± 0.83 μM) causes an increase in trypanocidal activity. Thiosemicarbazone of 4-chloroacetophenone **(6** IC_{50} = 11.06 ± 2.28 μM) showed trypanocidal activity 18 times that of thiosemicarbazone of 2-chloroacetophenone. With bromine in *meta* position, Trypanocidal activity of 3-bromoacetophenone-thiosemicarbazone **(7)** is 70.40 ± 0.25 μM. This activity is enhanced with a bromine atom in *para* position in thiosemicarbazone of 4-bromoacetophenone **(8)** with a trypanocidal activity of 26.89 ± 10.86 μM. The presence of a methoxy group in *para* position (**2** IC_{50} = 91.14 ± 7.47 μM) induced an increase of trypanocidal activity compared to acetophenone thiosemicarbazone. However, the presence of several methoxy groups on the aryl group decreases the trypanocidal activity (**3**, **4**). For these thiosemicarbazones of acetophenone, the presence of halogen or methoxy substituents in *para* position increases the trypanocidal activity which is quite important in the case of chlorine.

The thiosemicarbazones synthesized from benzaldehyde and its derivatives appear to be less active with IC_{50} values greater than 100 μM with the exception of compounds **23** (IC_{50} = 45.58 ± 7.38 μM) and **13** (IC_{50} = 82.64 ± 5 μM). The thiosemicarbazone **13** with a chlorine atom in *para* position is more active than the thiosemicarbazone **12** which has a chlorine atom in *meta* position. The latter is itself more active than compound **11** which has a chlorine atom in *ortho*.

The activities of benzaldehyde thiosemicarbazones are lower than those of acetophenone thiosemicarbazones. This means that the methyl group of the acetophenone thiosemicarbazones plays an important role in trypanocidal activity. Its replacement with an ethyl group in the 4′-methoxypropiophenone-thiosemicarbazone (**16** IC_{50} = 132.40 ± 0.67 μM) reduced the trypanocidal activity compared to that of 4-methoxyacetophenone-thiosemicarbazone (**2** IC_{50} = 91.14 ± 7.47 μM).

The compounds with the halogen substituent in *para* position are more active than those that have it in *meta* position and they are themselves more active than thiosemicarbazones with the halogen in *ortho* position.

It is interesting to note that the 4′-(*N*,*N*-dimethylamino)-cinnamaldehyde thiosemicarbazone (**29** IC_{50} = 17.71 ± 3.46 μM) which has the dimethylamino group in *para* position is much more active than cinnamaldehyde thiosemicarbazone (IC_{50} = 27 76.28 ± 7.37 μM). The reduction of the exocyclic double bond C=C of cinnamaldehyde thiosemicarbazone decreases trypanocidal activity for the 3-phenylpropionaldehyde-thiosemicarbazone (**28** = 260.21 ± 10.78 μM). The exocyclic double bond C=C plays an important role in trypanocidal activity.

The trypanocidal activity is quite important in the case of acetonaphthone-thiosemicarbazone (**9** IC_{50} = 9.62 ± 2.67 μM) in which two aromatic nuclei are joined. This activity decreases when the two nuclei are separated by one carbon atom. This is the case of benzophenone-thiosemicarbazone (**31** IC_{50} = 68.34 ± 0.04 μM). The trypanocidal activity decreases even more when the two nuclei are separated by three carbon atoms in the case of 1,3-diphenylacetone-thiosemicarbazone (**32** IC_{50} = 146.80 ± 7.24 μM).

We also note that substitution of methyl group of acetophenone thiosemicarbazone (**1** IC_{50} = 211.88 ± 7.38 μM) by a phenyl in the case of benzophenone-thiosemicarbazone (**31** IC_{50} = 68.34 ± 0.04 μM) induced an increase in trypanocidal activity in accordance with the work of Fujii (2005). Similarly, the substitution of hydrogen by a phenyl group on the thioamide function on thiosemicarbazones **6**, **7**, and **12** leads to a sharp increase in trypanocidal activity for compounds **34** (IC_{50} = 3.97 ± 0.06 μM) and **35** (IC_{50} = 5.19 ± 0.06 μM) derivatives of **7** and **12**, respectively. Again, the compound with the chlorine in *para* **(34)** is more active than that with bromine in *para* **(35)**. The thiosemicarbazone **33**, derived from **6** is insoluble.

The substitution of the aromatic ring of the acetophenone thiosemicarbazone (**1** IC_{50} = 211.88 ± 7.38 μM) by the adamantyl group results in a significant increase in trypanocidal activity in the case of 1-methyladamantylcetone (**26** IC_{50} = 8.38 ± 0.71 μM).

Table 1 Chemical structure, yield, and melting point of synthesized compounds (**1–35**)

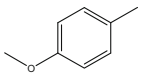
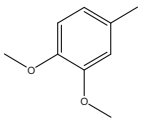
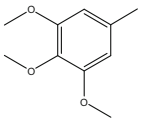
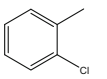
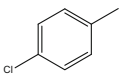
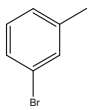
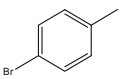
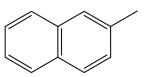
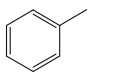
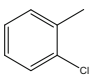
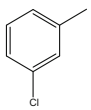
Compounds	R_1	R_2	R_3	Yield (%)	*Rf*	M. P (°C)
1	$-CH_3$		H	85	0.79	120–121
2	$-CH_3$		H	89	0.82	180–181
3	$-CH_3$		H	82	0.75	224–225
4	$-CH_3$		H	74	0.62	215–216
5	$-CH_3$		H	45	0.88	157–158
6	$-CH_3$		H	70	0.82	194–196
7	$-CH_3$		H	75	0.86	172–173
8	$-CH_3$		H	84	0.80	198–199
9	$-CH_3$		H	89	0.83	182–183
10	H		H	65	0.80	162–163
11	H		H	75	0.88	208–210
12	H		H	81	0.82	195–196

Table 1 continued

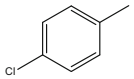
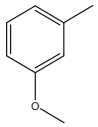
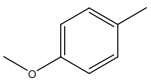
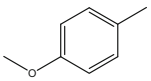
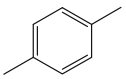
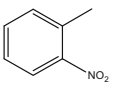
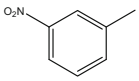
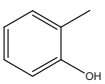
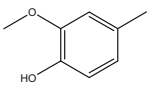
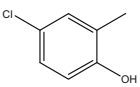
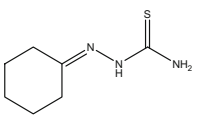
Compounds	R_1	R_2	R_3	Yield (%)	*Rf*	M. P (°C)
13	H		H	78	0.82	212–213
14	H		H	85	0.78	192–193
15	H		H	83	0.73	167–168
16	$-CH_2-CH_3$		H	78	0.80	126–127
17	H		H	65	0.85	169–170
18	H		H	55	0.87	180–181
19	H		H	90	0.78	254–255
20	H		H	95	0.67	235–236
21	H		H	92	0.64	229–230
22	H		H	64	0.69	194–195
23	H		H	81	0.70	278–279
24				54	0.71	160–161

Table 1 continued

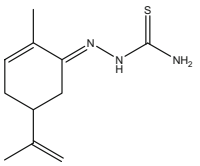
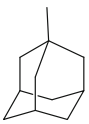
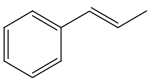
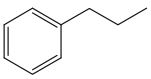
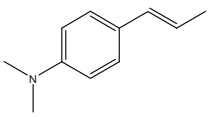
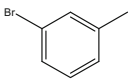
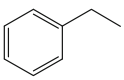
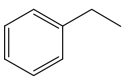
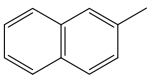
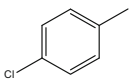
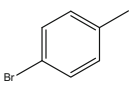
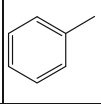
Compounds	R_1	R_2	R_3	Yield (%)	*Rf*	M. P (°C)
25				65	0.91	111–112
26	$-CH_3$		H	58	0.90	214–215
27	H		H	70	0.82	139–140
28	H		H	73	0.75	114–115
29	H		H	53	0.88	215–216
30	H		H	87	0.84	202–203
31			H	45	0.98	169–170
32			H	88	0.90	161–162
33	$-CH_3$			85	–	198–200
34	$-CH_3$			90	–	155–156
35	$-CH_3$			80	–	154–155

Table 2 Trypanocidal activity of synthesized compounds

Compounds	Trypanocidal activity	Compounds	Trypanocidal activity	Compounds	Trypanocidal activity
1	211.88 ± 7.38	**13**	82.64 ± 5	**25**	39.71 ± 10.20
2	91.14 ± 7.47	**14**	–	**26**	8.38 ± 0.71
3	–	**15**	258.80 ± 6.21	**27**	76.28 ± 7.37
4	–	**16**	132.40 ± 0.67	**28**	260.21 ± 10.78
5	199.37 ± 0.83	**17**	123.6 ± 7.92	**29**	17.71 ± 3.46
6	11.06 ± 2.28	**18**	221.50 ± 7.04	**30**	135.85 ± 32
7	70.40 ± 0.25	**19**	–	**31**	68.34 ± 0.04
8	26.89 ± 10.86	**20**	–	**32**	146.80 ± 7.24
9	9.62 ± 2.67	**21**	170.35 ± 9.27	**33**	–
10	443.54 ± 5.85	**22**	292.53 ± 7.57	**34**	3.97 ± 0.06
11	220 ± 7.68	**23**	45.58 ± 7.38	**35**	5.19 ± 0.06
12	163.64 ± 7.40	**24**	–		

According to the work of Du and Fujii (2002; 2005), thiosemicarbazones that have IC_{50} values between 10 and 100 μM are considered as moderate trypanocidal. The thiosemicarbazones which have IC_{50} values below 10 μM can be considered as active molecules. Indeed, Du found that macrophage cells are generally sensitive to concentrations above 10 μM thiosemicarbazones. Thus, in addition to inhibiting property of the parasite, they would preserve the human macrophage host cell (Du *et al.,* 2002; Fujii *et al.,* 2005). On this basis, thiosemicarbazones **9** ($IC_{50} = 9.62 \pm 2.67$ μM), **26** ($IC_{50} = 8.38 \pm 0.71$ μM), **34** ($IC_{50} = 3.97 \pm 0.06$ μM), and **35** ($IC_{50} = 5.19 \pm 0$, 06 μM) were selected as good trypanocidal.

The toxicity of thiosemicarbazones **9** (9.62 ± 2.67 μM), **26** (8.38 ± 0.71 μM), **34** (3.97 ± 0.06 μM), and **35** ($IC_{50} = 5.19 \pm 0.06$ μM) was evaluated on *Artemia salina* larvae.

The analysis of the toxicity of *Artemia salina* larvae also allows us to determine the selectivity of different molecules by calculating the selectivity index (SI = larvae LC_{50}/parasite IC_{50}). If the IS value is greater than the unit, the tested compound is considered to be selective about the parasites. However, if IS value is less than unity, the compound in question is more cytotoxic than trypanocidal (Tiuman *et al.,* 2005). The selectivity index has been calculated for thiosemicarbazones that with IC_{50} value less than 100 μM. Table 3 summarizes the results.

The results of this table show that all thiosemicarbazones tested are selective on the parasite *Trypanosoma brucei brucei* in addition to their interesting trypanocidal power ($IC_{50} < 10$ μM). In addition, Lipinski in 1997 brought together some of the properties that he deemed important to study the pharmacokinetics and drug development. A molecule according to three criteria would be a good pharmacokinetic and be a good drug candidate (Lipinski *et al.,* 1997). It is interesting to note that the

Table 3 Selectivity index calculated for active thiosemicarbazones

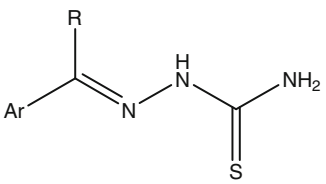
Compounds	LC_{50} larve	IC_{50} parasite	Selectivity index
Thiosemicarbazones			
9	480.80	9.62	49.98
26	119.33	8.38	14.24
34	438.30	3.97	110.40
35	134.40	5.19	25.90

Table 4 Thiosemicarbazones and Lipinski's criteria

Compounds	Mol. wt.	C log *P*	No. of H bond donors	No. of H bond acceptors	No. of criteria met
Rule	<500	<5	<5	<10	3 at least
9	243.32	3.57	3	3	All
26	251.39	3.39	3	3	All
34	319.42	4.87	3	3	All
35	348.26	5.02	3	3	3

calculated values in Table 4 show that the four most trypanocidal thiosemicarbazones meet the criteria of Lipinski. These thiosemicarbazones are good drug candidates. Table 4 summarizes the results.

To study the structure–activity relationship, Du (2002) and Fujii (2005) have synthesized several thiosemicarbazones such as:



Ar: substitued phenyl or not
R: Alkyl

They tested their trypanocidal activity in cysteine protease of *T. cruzi*. It appears that: The establishment of one

or more halogen atoms or methoxy group on the aromatic ring Ar, the lengthening of the alkyl radical R (R = hydrogen, methyl, ethyl, and butyl) considerably increase the trypanocidal activity. The nature and number of substituents also seem to have an impact, as well as congestion induced by the radical R (*t*-butyl). Our results are similar to those of Du and Fujii, especially the replacement of hydrogen (benzaldehyde) by a methyl (acetophenone), the introduction of halogen or methoxy group on the benzene ring. However, some differences (nature and position of the halogen on the aromatic ring) could be explained by the fact that the trypanosome species tested are not the same.

Conclusion

This study that is a first of its kind on *Trypanosoma brucei brucei*, unlike those made on *Trypanosoma cruzi* showed an increase in trypanocidal activity when moving the halogen (Cl or Br) in *ortho* position to *para* position of benzene ring of acetophenone-thiosemicarbazone. It reveals that the presence of lipophilic substituents in *para* position is beneficial for the activity. The replacement of the methyl group of acetophenone-thiosemicarbazone by hydrogen decreases the trypanocidal activity. This reduction increases with the ethyl group. As against the phenyl substituent instead of methyl increases the activity. When the phenyl ring of acetophenone-thiosemicarbazone is substituted by naphthyl group, it follows a significant increase in the trypanocidal activity. It is the same when it is replaced by the adamantyl group. The substitution of a hydrogen atom on thiosemicarbazones thioamide function leads to a sharp increase in the trypanocidal activity. All these changes have led to the synthesis of 1-(4-Chlorophenyl) ethylidene-4-phenyl-thiosemicarbazide **(34)**. With trypanocidal activity of 3.97 μM, this compound is the most active of the series.

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Appendix: Spectral data of thiosemicarbazones

Acetophenone-thiosemicarbazone (**1**)

IR ν (KBr cm^{-1}): 3408, 3233, 3145 (NH), 1587 (C=N).

^{1}H NMR δ ($CDCl_3$ ppm): 2.3 (3H, s, CH_3), 7.39–7.52 (several signals: 5H of ArH and 1H of NH_2), 6.54 (1H, s, NH_2); 8.87 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 12.28 (CH_3) from 124.31 to 135.24 (Aromatic C), 146.75 (C=N), 177.21 (C=S).

4-Methoxyacetophenone-thiosemicarbazone (**2**)

IR ν (KBr cm^{-1}): 3400, 3247, 3162 (NH), 1588 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.26 (3H, s, CH_3), 3.78 (3H, s, O–CH_3), 7.39–7.52 (several signals, 4H of ArH and 1H of NH_2) 8.19 (1H, s, NH_2) 10.10 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 13.24 (CH_3) 54.59 (O–CH_3) from 112.94 to 129.45 and 159.61 (Aromatic C), 147.21 (C=N), 178.02 (C=S).

3′,4′-Dimethoxyacetophenone-thiosemicarbazone (**3**)

IR ν (KBr cm^{-1}): 3376, 3267, 3155 (NH), 1588 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.27 (3H, s, CH_3), 3.78 (3H, s, O–CH_3), 3.82 (3H, s, O–CH_3), 6.92–7.51 (3H, several signals, ArH), 7.88 (1H, s, NH_2), 8.22 (1H, s, NH_2), 10.06 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 12.95 (CH_3) 54.45 (O–CH_3) 54.65 (O–CH_3) from 108.60 to 129.19 and 149.12, 147.49 (Aromatic C), 147.19 (C=N), 177.52 (C=S).

2′,3′,4′-Trimethoxyacetophenone-thiosemicarbazone (**4**)

IR ν (KBr cm^{-1}): 3341, 3264, 3173 (NH), 1585 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.30 (3H, s, CH_3), 3.68 (3H, s, O–CH_3), 3.84 (6H, s, 2-OCH_3) 7, 12 (2H, s, ArH), 7.92 (1H, s, NH_2), 8.27 (1H, s, NH_2), 10.10 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 12.25 (CH_3) 53.98 (2 O–CH_3) 57.91 (O–CH_3), 102.24 to 136.70 and 150.51 (Aromatic C), 146.12 (C=N), 176.60 (C=S).

2′-Chloroacetophenone-thiosemicarbazone (**5**)

IR ν (KBr cm^{-1}): 3400, 3138 (NH), 1587 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.28 (3H, s, CH_3), 7.38–7.49 (4H, several signals, ArH), 7.62 (1H, s, NH_2) 8, 24 (1H, s, NH_2), 10.34 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 18.46 (CH_3) 127–138 (Aromatic C), 148.44 (C=N), 179.21 (C=S).

4′-Chloroacetophenone-thiosemicarbazone (**6**)

IR ν (KBr cm^{-1}): 3418, 3139 (NH), 1587 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 2.29 (3H, s, CH_3), 7.41–7.96 (4H, several signals, ArH), 8.00 (1H, s, NH_2) 8, 32 (1H, s, NH_2), 10.26 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 14.12 (CH_3) from 128.42 to 136.74 (Aromatic C), 146.80 (C=N), 179.25 (C=S).

3′-Bromoacetophenone-thiosemicarbazone (**7**)

IR ν (KBr cm^{-1}): 3392, 3212, 3144 (NH), 1588 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 2.28 (3H, s, CH_3), 7.34–7.90 and 8.19 (4H, several signals, ArH), 8.11 (1H, s, NH_2), 8.32 (1H, s, NH_2), 10.25 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 13.93 (CH_3) from 122.01 to 140.01 (Aromatic C), 146.27 (C=N), 179.01 (C=S).

4′-Bromoacetophenone-thiosemicarbazone (**8**)

IR v (KBr cm^{-1}): 3410, 3193, 3140 (NH), 1587 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 2.28 (1H, s, CH_3), 7.54–7.90 (4H, several signals, ArH), 8.00 (1H, s, NH_2) 8, 32 (1H, s, NH_2), 10.33 (1H, s, NH). ^{13}C NMR δ (DMSO-d_6 ppm): 13.79 (CH_3) from 122.68 to 136.83 and 146.59 (Aromatic C), 142.12 (C=N), 178.96 (C=S).

Acetonaphthone-thiosemicarbazone (**9**)

IR v (KBr cm^{-1}): 3435, 3193 (NH), 1606 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.4 (3H, s, CH_3), 7.54–7.88 (7H, several signals, ArH), 8.00 (1H, s, NH_2) 8, 38 (1H, s, NH_2), 10.30 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 11.89 (CH_3) from 122.18 to 133.19 (Aromatic C), 145.71 (C=N), 177.05 (C=S).

Benzaldehyde-thiosemicarbazone (**10**)

IR v (KBr cm^{-1}): 3401, 3145 (NH), 1600, 1584 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 7.39–7.79 (5H, several signals, ArH), 8.00 (1H, s, NH_2), 8.06 (1H, s, CH=N); 8.21 (1H, s, NH_2), 11.44 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 127.26–134.15 (Aromatic C), 142.24 (C=N), 177.97 (C=S).

2′-Chlorobenzaldehyde-thiosemicarbazone (**11**)

IR v (KBr cm^{-1}): 3416, 3154 (NH), 1593 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 7.35–7.42 and 8.28 (4H, several signals, ArH), 8.11 (1H, s, NH_2), 8.31 (1H, s, NH_2), 8.47 (1H, s, CH=N), 11.62 (1H, s, NH). ^{13}C NMR δ (DMSO-d_6 ppm): 127.28–133.09 (Aromatic C), 138.11 (C=N), 178.21 (C=S).

3′-Thiosemicarbazone-chlorobenzaldehyde (**12**)

IR v (KBr cm^{-1}): 3392, 3233, 3156 (NH), 1532 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 7.41–7.90 (4H, several signals, ArH), 7.93 (1H, s, CH=N), 8.19 (1H, s, NH_2); 8.26 (1H, s, NH_2), 11.51 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 125.98–136.45 (Aromatic C), 140.48 (C=N), 178.17 (C=S).

4′-Chlorobenzaldehyde-thiosemicarbazone (**13**)

IR v (KBr cm^{-1}): 3437, 3281, 3165 v (NH), 1600, 1525 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 7.43–7.84 (4H, several signals, ArH), 8.03 (1H, s, CH=N), 8.07 (1H, s, NH_2); 8.24 (1H, s, NH), 11.49 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 128.65–134.22 (Aromatic C), 140.85 (C=N), 178.06 (C=S).

3′-Thiosemicarbazone-methoxybenzaldehyde (**14**)

IR v (KBr cm^{-1}): 3396, 3279, 3155 (NH), 1576 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 3.80 (1H, s, OCH_3), 6.94–7.44 (4H, several signals, ArH), 8.02 (1H, s, CH=N); 8.06 (1H, s, NH_2), 8.22 (1H, s, NH_2), 11.43 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 55.25 (OCH_3), 110.91–135.57 and 159.54 (Aromatic C), 142.12 (C=N), 177.94 (C=S).

4′-Methoxybenzaldehyde-thiosemicarbazone (15)

IR v (KBr cm^{-1}): 3404, 3290 (NH), 1599, 1537 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 3.78 (3H, s, O–CH_3) 6.94–7.74 (4H, several signals, ArH), 7.91 (1H, s, NH_2) 8, 01 (1H, s, CH=N), 8.11 (1H, s, NH), 11.32 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 55.91 (OCH_3), 113.29–128.87 and 160.65 (Aromatic C), 142.23 (C=N), 177.59 (C=S).

Methoxypropiophenone-4′-thiosemicarbazone (**16**)

IR v (KBr cm^{-1}): 3433, 3278 (NH), 1596 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 1.01 (3H, t, CH_3), 2.83 (2H, q, CH_2), 3.78 (3H, s, O–CH_3), 6.92–7.87 (4H, several signals, ArH), 7.85 (1H, s, NH_2), 8.18 (1H, s, NH_2), 10.19 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 11.01 (CH_3) 19.11 (CH_2) 55.17 (O–CH_3) from 112.96 to 128.06 and 160.17 (Aromatic C) 151, 78 (C=N), 178.70 (C=S).

4′-Methylbenzaldehyde-thiosemicarbazone (**17**)

IR v (KBr cm^{-1}): 3402, 3239, 3156 (NH), 1598 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.31 (1H, s, CH_3), 7.19–7.68 (4H, several signals, ArH), 8.03 (1H, s, CH=N); 7.94 (1H, s, NH_2), 8.18 (1H, s, NH_2), 11.38 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 18.83 (CH_3) from 124.79 to 137.15 (Aromatic C), 139.92 (C=N), 175.36 (C=S).

2′-Methylbenzaldehyde-thiosemicarbazone (**18**)

IR v (KBr cm^{-1}): 3420, 3257, 3156 (NH), 1592 (C=N, C=C).

1H NMR δ (DMSO-d_6 ppm): 2.34 (1H, s, CH_3), 7.19–7.26 and 8.03 (4H, several signals, ArH), 7.83 (1H, s, NH_2), 8.19 (1H, s, NH_2), 8.40 (1H, s, CH=N), 11.34 (1H, s, NH).

^{13C}NMR δ (DMSO-d_6 ppm): 18.83 (CH_3) from 125.96 to 136.75 and 159.54 (Aromatic C), 141.08 (C=N), 177.82 (C=S).

2′-Nitrobenzaldehyde-thiosemicarbazone (**19**)

IR v (KBr cm^{-1}): 3424, 3245, 3159 v (NH), 1539 (C=N, C=C).

^{1}H NMR δ (DMSO-d_6 ppm): 7.63–8.02 and 8.41 (4H, several signals, ArH), 8.11 (1H, s, NH_2), 8.39 (1H, s, NH_2), 8.47 (1H, s, CH=N), 11.74 (1H, s, NH). ^{13}C NMR δ (DMSO-d_6 ppm): 124.44–137.19 (Aromatic C), 148.22 (C=N), 178.45 (C=S).

3′-Thiosemicarbazone-nitrobenzaldehyde (**20**)

IR v (KBr cm^{-1}): 3395, 3242, 3157 (NH), 1604, 1526 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 7.65–8.24 (4H, several signals, ArH), 8.33 (1H, s, NH_2), 8.30 (1H, s, CH=N); 8.63 (1H, s, NH_2), 11.61 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 121.32–139.88 (Aromatic C), 148.33 (C=N), 178.31 (C=S).

2′-Hydroxybenzaldehyde-thiosemicarbazone (**21**)

IR v (KBr cm^{-1}): 3443, 3319, 3172 v (NH), 1539 v (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 6.79–7.90 (4H, several signals, ArH), 7.67 (1H, s, NH_2), 7.92 (1H, s, NH_2) 8, 37 (1H, s, CH=N), 9.86 (OH) 11.37 (1H, s, NH). ^{13C}NMR δ (DMSO-d_6 ppm): 114.91–155.28 (Aromatic C), 138.50 (C=N), 176.55 (C=S).

4′-Hydroxy-3′-thiosemicarbazone-methoxybenzaldehyde (**22**)

IR v (KBr cm^{-1}): 3528 (OH), 3437, 3276, 3154 (NH), 1587 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 3.83 (3H, s, CH_3), 6.79–7.32 (3H, several signals, ArH), 7.95 (1H, s, CH=N); 7.93 (1H, s, NH_2), 8.08 (1H, s, NH_2), 9.41 (1H, s, OH), 11.23 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 55.74 (O–CH_3) from 109.31 to 140.77 and 148.06 (Aromatic C), 142.89 (C=N), 177.38 (C=S).

5′-Chloro-2′-hydroxybenzaldehyde-thiosemicarbazone (**23**)

IR v (KBr cm^{-1}): 3405, 3236, 3165 v (NH), 1598, 1565 v (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 6.86–8.08 (3H, several signals, ArH), 8.15 (2H, s, NH_2), 8.31 (1H, s, CH=N); 10.18 (1H, s, OH), 11.42 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 117.64–130.33 and 155.09 (Aromatic C), 137.37 (C=N), 177.83 (C=S).

Cyclohexanone thiosemicarbazone (**24**)

IR v (KBr cm^{-1}): 3379, 3215, 3144 (NH), 1585 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 1.54–2.39 (10H, several signals, CH_2 hexane), 7.50 (1H, s, NH_2), 7.95 (1H, s, NH_2) 10, 13 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 25.01–34.87 (cyclohexane CH_2), 156.94 (C=N), 178.49 (C=S).

Carvone-thiosemicarbazone (**25**)

IR v (KBr cm^{-1}): 3415, 3259 (NH), 1598 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 1.82 (3H, s, CH_3), 1.9 (3H, s, CH_3) 2, 28 (4H, m, $2CH_2$), 2.68 (1H, q, CH_2–CH_2–CH), 4.8 (2H, s, =CH_2), 5.1 (1H, t, CH=C), 6.3 (1H, s, NH_2), 7.81 (1H, s, NH_2) 10.3 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 19–22 (CH_3) 29–42 (CH_2 CH and carvone) 109–150 (=CH and =CH_2), 148.29 (C=N), 176.16 (C=S).

Methyladamantylcetone-1-thiosemicarbazone (**26**)

^{1}H NMR δ (DMSO-d_6 ppm): 1.62–1.71 (15H, several signals, CH and CH_2), 1.98 (3H, s, CH_3), 7.30 (1H, s, NH_2); 8.10 (1H, s, NH_2), 8.31 (1H, s, CH=N), 9.80 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 11.51 (CH_3) 27.61–36.17 (several signals CH and CH_2) 159.52 (C=N), 178.80 (C=S).

Cinnamaldehyde thiosemicarbazone (**27**)

IR v (KBr cm^{-1}): 3418, 3259, 3156 (NH), 1592 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 6.83–7.02 (2H, several signals, CH=CH), 7.29–7.55 (5H, several signals, ArH), 7.91 (1H, d, CH=N), 7.62 (1H, s, NH_2), 8.18 (1H, s, NH_2), 11.41 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 125.02–140.84 (Aromatic C and CH=CH), 144.72 (C=N), 177.67 (C=S).

4′-(*N,N*-dimethylamino)-cinnamaldehyde thiosemicarbazone (**29**)

IR (KBr cm^{-1}): 3408, 3245, 3140 v (NH), 1598 v (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.94 (6H, s, CH_3–N–CH_3), 6.54–7.38 (6H, several signals, CH=CH and ArH), 7.51 (1H, s, NH_2), 7.85 (1H, d, CH=N), 8.05 (1H, s, NH_2), 11.24 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 40.14 (CH_3–N–CH_3) from 112.00 to 139.81 and 150.66 (Aromatic C), 145.88 (C=N), 177.12 (C=S).

3′-Bromobenzaldehyde-thiosemicarbazone (**30**)

IR v (KBr cm^{-1}): 3391, 3235, 3155 (NH), 1602, 1532 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 7.22–7.48 and 7.74 (4H, several signals, ArH), 7.79 (1H, s, CH=N), 7.84 (1H, s, NH_2), 7.87 (1H, s, NH_2), 11.51 (1H, s, NH). ^{13C}NMR δ (DMSO-d_6 ppm): 122.33–136.65 (Aromatic C), 140.43 (C=N), 178.15 (C=S).

Benzophenone-thiosemicarbazone (**31**)

IR v (KBr cm^{-1}): 3412, 3248, 3153 (NH), 1609 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 7.35–7.47 (10H, several signals, ArH), 7.86 (1H, s, NH_2), 7.87 (1H, s, NH_2) 8, 65 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 127.55–136.29 (Aromatic C), 149.06 (C=N), 177.85 (C=S).

1,3-Diphenylacetone-thiosemicarbazone (**32**)

IR v (KBr cm^{-1}): 3336, 3296, 3228, 3138 (NH), 1601 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 3.48 (1H, s, CH_2), 3.75 (1H, s, CH_2), 7.12–7.25 (10H, several signals, ArH), 7, 43 (1H, s, NH_2), 8.21 (1H, s, NH_2), 10.43 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 34.41 (CH_2) 42.35 (CH_2) 126.53–137.04 (Aromatic C), 152.36 (C=N), 179.03 (C=S).

1-(1-(Naphth-2-yl) ethylidene)-4-phenylthiosemicarbazide (**33**)

IR v (KBr cm^{-1}): 3303, 3237 (NH), 1589, 1520 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.53 (3H, s, CH_3), 7.24–8.28 (12H, several signals, ArH), 10.17 (1H, s, NH-ArH); 10.82 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 14.25 (CH_3) from 124.77 to 139.22 (Aromatic C), 148.77 (C=N), 177.10 (C=S).

1-(4-Chlorophenyl) ethylidene-4-phenyl-thiosemicarbazide (**34**)

IR v (KBr cm^{-1}): 3303, 3256 (NH), 1590, 1520 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.39 (3H, s, CH_3), 7.23–8.07 (9H, several signals, ArH), 10.10 (1H, s, NH-ArH); 10.65 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 14.22 (CH_3) from 125.36 to 139.40 (Aromatic C), 147.54 (C=N), 177.07 (C=S).

1-(4-Bromophenyl) ethylidene-4-phenyl-thiosemicarbazide (**35**)

IR v (KBr cm^{-1}): 3303, 3225 (NH), 1587, 1518 (C=N).

^{1}H NMR δ (DMSO-d_6 ppm): 2.38 (3H, s, CH_3), 7.22–7.90 (9H, several signals, ArH), 10.10 (1H, s, NH-ArH); 10.65 (1H, s, NH).

^{13}C NMR δ (DMSO-d_6 ppm): 14.23 (CH_3) from 122.93 to 139.13 (Aromatic C), 147.67 (C=N), 177.01(C=S).

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