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A competitive reactivity study on the oxidative cyclization of thiosemicarbazones into 1,3,4-thiadiazoles

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Dedicated to Prof. Girolamo Cirrincione on the occasion of his retirement

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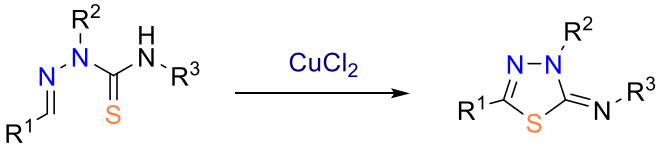
Abstract

In order to obtain useful insights on the mechanism of formation of 2(3*H*)-imino-1,3,4-thiadiazoles by oxidative cyclization of aldehyde thiosemicarbazones with Cu(II) or Fe(III) salts, a competitive reactivity study was performed on a suitable set of diversely substituted substrates, by means of HPLC techniques. This approach enabled to exploit Hammett's equation without performing otherwise difficult-to-run kinetic experiments. The results presented herein support the hypothesis that the formation of the thiadiazole ring is induced by the attack of the oxidizing Lewis acid metal cation onto the imine-like nitrogen atom of the thiosemicarbazone substrate. Beyond mechanistic interpretation, the paper particularly focuses onto the methodological issues implied.

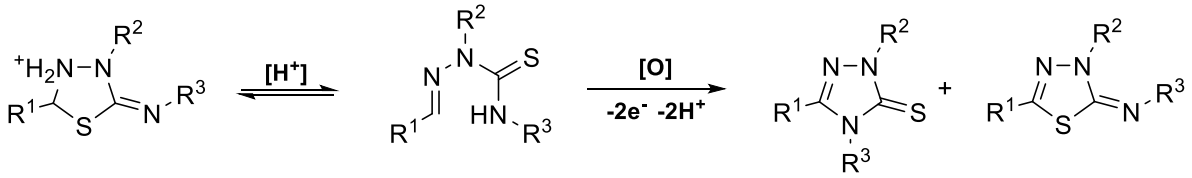


$$\left(\frac{k_1}{k_2}\right) = \frac{\ln\left[1 + \frac{|P_1|(t)}{|S_1|(t)}\right]}{\ln\left[1 + \frac{|P_2|(t)}{|S_2|(t)}\right]}$$

Keywords: Copper(II) chloride, oxidative cyclization, thiosemicarbazones, 1,3,4-thiadiazole

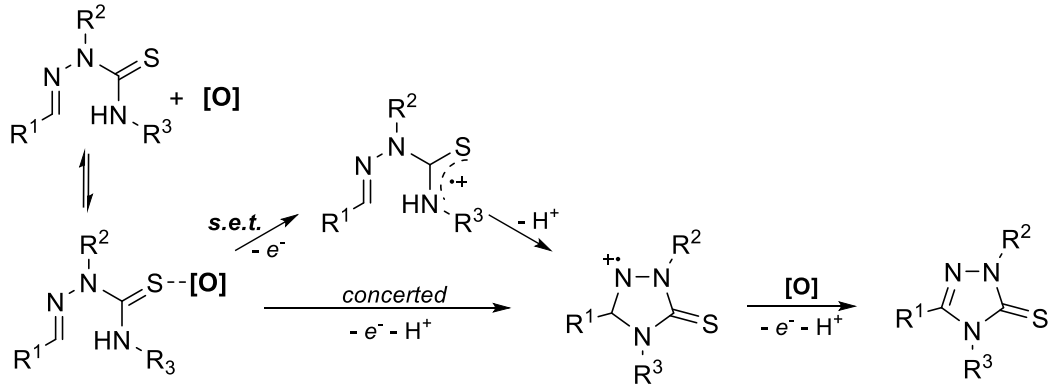
Introduction

The synthesis of the 1,3,4-thiadiazole ring has seen quite some interest, because this framework is present in various bioactive molecules,[1-4] useful in particular as antimicrobial[5-11] and antitumor[12-15] agents. A typical synthetic approach is constituted by the cyclization of ketone thiosemicarbazones, which easily affords C(2)-saturated and spiro-type 1,3,4-thiadiazolidine derivatives.[16-19] On the other hand, along with acid-catalyzed cyclization, aldehyde thiosemicarbazones are also known to undergo an oxidative cyclization reaction,[20-22] which may lead to the corresponding 3*H*-1,2,4-triazole-3-thione and 2(3*H*)-imino-1,3,4-thiadiazole derivatives (Scheme 1), in different amounts depending on the substrate, the oxidizing agent and the reaction conditions used. In particular, iron(III) chloride,[23] copper(II) perchlorate,[24-25] potassium ferricyanide and *tris*-(*p*-bromophenyl)aminium hexachloro-antimoniate (TPBA)[26] have been proven to be effective agents to perform the reaction.



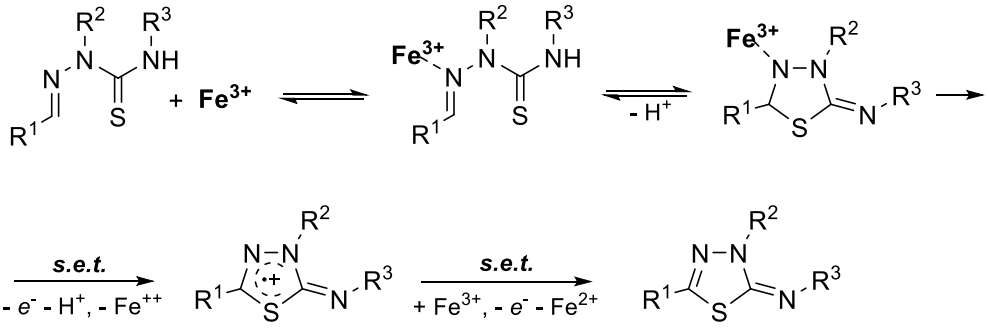
Scheme 1. Cyclizations of thiosemicarbazones.

These oxidative cyclization processes have been subjected to thorough mechanistic investigation. In particular, the mechanism of formation of the 1,2,4-triazole ring has been quite well understood. It has been convincingly demonstrated that one-electron oxidizing agents attack the thioureido-like moiety of the organic molecule.[23-26] In the rate-determining step the imine-type C atom performs a nucleophilic attack onto the N(4) atom. Depending on the conditions, the latter step may be either concerted or subsequent to a single-electron-transfer (*s.e.t.*) process from the substrate to the oxidant (Scheme 2). Hence, a cyclic N-radical-cation intermediate species is formed, which rapidly undergoes a second *s.e.t.* to afford the final product. It is worth stressing here that analysis of kinetic data for 2-methyl-4-aryl- and 2,4-diaryl-thiosemicarbazones of benzaldehydes by means of Hammett's equation has played a fundamental role in understanding the reaction course.



Scheme 2. Mechanism for the oxidative cyclization of thiosemicarbazones into 1,2,4-triazoles.

Unfortunately, the mechanism of formation of the corresponding 1,3,4-thiadiazole product could not be studied in such detail, because of lack of experimental data. Indeed, the latter reaction usually occurs when the oxidizing agent is a metal cation able to act as an effective Lewis acid, namely with $FeCl_3$ and $CuCl_2$, and is not observed with outer-sphere one-electron oxidants such as ferricyanide or TPBA.[23-26] Moreover, the formation of the thiadiazole is rarely observed for 2,4-diaryl-thiosemicarbazones; conversely, it is favored whenever the aldehyde residue bears electron-withdrawing substituents. The only kinetic data available are relevant to the reaction of 2-methyl-4-aryl derivatives of benzaldehydes with $FeCl_3$.[23] In this case, Hammett's sensitivity constant ρ values found for the substitution on either phenyl ring are significantly different from the ones relevant to the formation of the triazole ring. On the grounds of this information, it was suggested that the mechanism leading to the thiadiazole ring might involve an electrophilic attack of the metal cation onto the N(1) imine-like atom (Scheme 3). Indeed, Brønsted-acid-catalyzed cyclization of N(2)-unsubstituted thiosemicarbazones into the relevant 1,3,4-thiadiazolidin-2-imines is a well-known process.[27-28]



Scheme 3. Mechanism for the oxidative cyclization of thiosemicarbazones to 1,3,4-thiadiazoles with $FeCl_3$.

In order to support this mechanistic hypothesis, further kinetic investigations were necessary, in particular by using $CuCl_2$ as the oxidizing agent. However, on the grounds of some qualitative preliminary evidence collected by us, there were some critical issues to deal with. For instance, the reaction of benzaldehyde 2-methyl-4-phenyl-thiosemicarbazone occurs very quickly, even using a stoichiometric amount of the oxidant in methanol at 25 °C (less than 2 min), affording a mixture of the relevant triazole (65%) and thiadiazole (32%) derivatives respectively. On the other hand, it was already known that in the reaction of the same thiosemicarbazone with cupric perchlorate (which affords the triazole product only, with much slower kinetics), a non-linear Michaelis-Menten-like dependence of the reaction rate on the concentration of the oxidant occurs.[24-25] These circumstances pose severe difficulties in setting up the kinetic experiments needed to obtain the apparent kinetic law, and the substituent-dependent apparent kinetic constants required for Hammett's correlations as well. Of course, these pieces of information are strict requirements for the construction of any sensible mechanistic hypothesis. In the present work we will show how such difficulties can be reasonably overcome by smartly revising the competitive reactivity method,[29] which allows to bypass the need of performing classical kinetic experiments.

Results and Discussion

Theory and methodological issues. The competitive reactivity method consists in allowing two substrates S_1 and S_2 (differing in the substitution pattern at R^1, R^2 and/or R^3) to react in the same sample, and then

analyzing the reaction mixture after a period of time short enough to ensure that none of them has been transformed to completion.[26] A basic requirement is that the two substrates react according to the same mechanism and follow the same apparent kinetic law. In our specific case, this implies that the dependence of the reaction rate on the concentration of the oxidizing agent has exactly the same mathematical form for both reactants. Then, assuming as a first approximation that no by products or intermediates are ever present in appreciable amounts in the system during the reaction course, and indicating as P_1 and P_2 the relevant products, the following differential kinetic expressions can be written:

$$d|P_1|/dt = - d|S_1|/dt = k_1 \cdot f([O]) \cdot |S_1| \quad \text{and} \quad d|P_2|/dt = - d|S_2|/dt = k_2 \cdot f([O]) \cdot |S_2|$$

where $f([O])$ is a suitable function of the concentration of the oxidizing agent, which must have exactly the same form for both substrates; k_1 and k_2 are the kinetic constants, the ratio of which has to be determined. The previous expressions can be easily rearranged as:

$$- d \ln|S_1| = k_1 \cdot f([O]) \cdot dt \quad \text{and} \quad - d \ln|S_2| = k_2 \cdot f([O]) \cdot dt$$

Consequently:

$$(1/k_1) \cdot d \ln|S_1| = (1/k_2) \cdot d \ln|S_2|$$

The latter differential equation can be integrated at any value of the time t as:

$$(1/k_1) \cdot [\ln|S_1|(t) - \ln|S_1|_{t=0}] = (1/k_2) \cdot [\ln|S_2|(t) - \ln|S_2|_{t=0}]$$

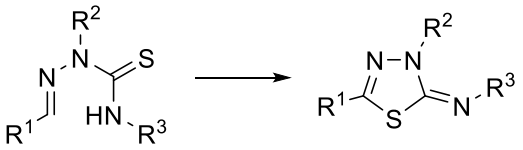
Under the aforementioned hypothesis that the concentrations of any possible intermediate or by-products are negligible, we can assume: $|S_1|_{t=0} = |S_1|(t)+|P_1|(t)$ and $|S_2|_{t=0} = |S_2|(t)+|P_2|(t)$. Then, after few trivial algebraic passages, the previous expression can be further transformed as:

$$\left(\frac{k_1}{k_2}\right) = \frac{\ln\left[1 + \frac{|P_1|(t)}{|S_1|(t)}\right]}{\ln\left[1 + \frac{|P_2|(t)}{|S_2|(t)}\right]}$$

Thus, by stopping the reaction at any time t before its completion (i.e. before either of the reactants has been completely consumed), one can evaluate the ratio between the kinetic constants of the two substrates (k_1/k_2) by simply measuring the concentrations of the products and the residual reactants. The latter information can be easily accessed by means of any analytical method, in particular HPLC. Noticeably, the application of this approach poses no condition on either the initial concentrations $|S_1|_{t=0}$ and $|S_2|_{t=0}$ (which can be put equal or different as well), or the analytical form of the function $f([O])$ (i.e. the reaction order in the oxidant).

Competitive reactivity data. The method of competitive reactivity illustrated above was applied to study the oxidative cyclization of a set of suitably substituted thiosemicarbazones **1a-m** into the relevant 1,3,4-thiadiazole derivatives **2a-m** (Scheme 4), carried out at 0 °C in a dichloromethane/methanol mixture (10:1 v/v) and in the presence of a 20-fold mole/mole excess of anhydrous $CuCl_2$. These peculiar reaction conditions were designed in order to ensure that the reaction is slow enough that it can be stopped after few minutes and neither of the reactants has been completely consumed. Of course, we are operating under pseudo-first-

order conditions with respect to the oxidant (see later). It is worth noting that the simultaneous formation of the possible 1,2,4-triazole derivative by-products is negligible in our case. The results obtained, namely the kinetic constant ratios towards compound **1a**, are collected in Table 1.



1a-m **2a-m**

	R^1	R^2	R^3
a	C_6H_5-	CH_3-	C_6H_5-
b	*p*Cl-C_6H_4-	CH_3-	C_6H_5-
c	*m*Br-C_6H_4-	CH_3-	C_6H_5-
d	*p*CF_3-C_6H_4-	CH_3-	C_6H_5-
e	*p*CN-C_6H_4-	CH_3-	C_6H_5-
f	*p*NO_2-C_6H_4-	CH_3-	C_6H_5-
g	*p*NO_2-C_6H_4-	CH_3-	*p*CH_3O-C_6H_4-
h	*p*NO_2-C_6H_4-	CH_3-	*p*CH_3-C_6H_4-
i	*p*NO_2-C_6H_4-	CH_3-	*p*Cl-C_6H_4-
j	*p*NO_2-C_6H_4-	CH_3-	*m*Cl-C_6H_4-
k	*p*NO_2-C_6H_4-	CH_3-	*p*NO_2-C_6H_4-
l	*p*NO_2-C_6H_4-	C_6H_5-	C_6H_5-
m	*p*NO_2-C_6H_4-	*p*CH_3O-C_6H_4-	C_6H_5-

Scheme 4. Structure of thiosemicarbazones **1a-m** and 1,3,4-thiadiazoles **2a-m**.

Table 1. Competitive reactivity data for thiosemicarbazones **1a-m**

substrate	$k_X/k_{(1a)}$ [a]	substrate	$k_X/k_{(1a)}$ [a]
1a	1		
1b	0.701	**1h**	0.272
1c	0.533	**1i**	0.187
1d	0.498	**1j**	0.141
1e	0.352	**1k**	0.082
1f	0.286	**1l**	0.069
1g	0.300	**1m**	0.272

[a] All data are affected by a ± 6% uncertainty.

Experimental data, of course, are liable to be analyzed by means of Hammett's equation. In details, taking into account the set of substrates **1a-f**, one considers the electronic requirements on the imine-like C atom (the relevant plot is depicted in Figure 1). Linear regression analysis leads to: Log (k_X/k_H) = (-0.66 ± 0.05) σ [r = 0.989; n = 6]. The apparent negative ρ value obtained can be compared with the one of -0.80 (with σ^+ constants) found for the analogous reaction with $FeCl_3$ in methanol.[23] Conversely, from substrates **1f-k** one

can consider the electronic requirements on the thioureido-like moiety (with the presence throughout of a *p*-nitro group on the phenyl ring at the imine moiety; the relevant plot is depicted in Figure 2). The observed substituent effect is peculiar. In fact, electron-donating substituents seem to have scarce impact on the reaction course; by contrast, electron-withdrawing substituents decrease the reactivity, with a quantitative effect nearly as large as in the previous case. Again, the latter finding can be compared with the analogous result obtained for the reaction of 2-methyl-4-aryl-thiosemicarbazones of unsubstituted benzaldehyde with $FeCl_3$, for which a ρ value of -0.87 was found.[23] Finally, comparison between substrates **1f**, **1l** and **1m** enables to evaluate the effects exerted by possible substituents bound at the N(2) atom, which is not directly involved in the ring-closure process. It is worth mentioning that N(2)-unsubstituted substrates (i.e. R^2 = H) afford the corresponding thiadiazole product exclusively, when reacted with an oxidizing agent.[30-33] Data reported herein indicate that replacement of a methyl group with a phenyl group as R^2 largely decreases the reaction rate (*ca.* 4 times). However, the presence of a further electron-donating group (namely *p*-CH_3O-, substrate **1m**) has a favorable effect on reactivity (making the reaction *ca.* 4 times faster).

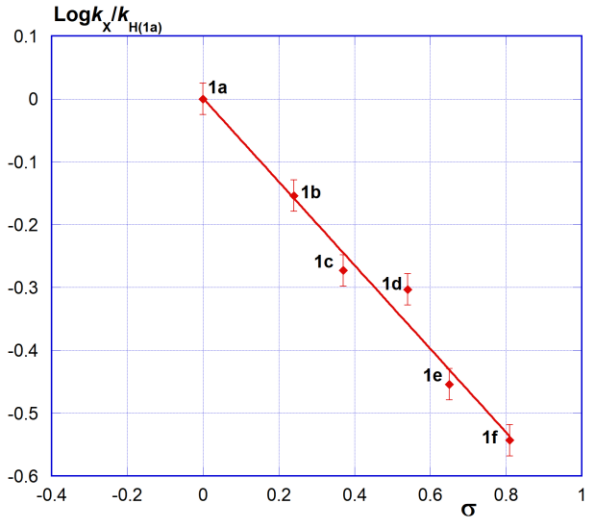


Figure 1. Hammett plot for thiosemicarbazones **1a-f**.

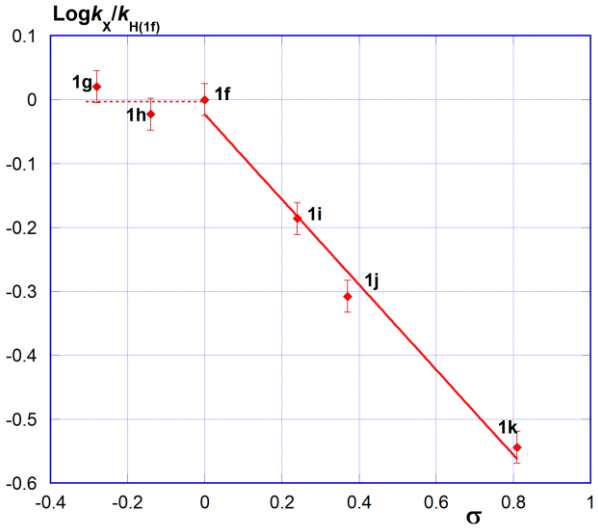
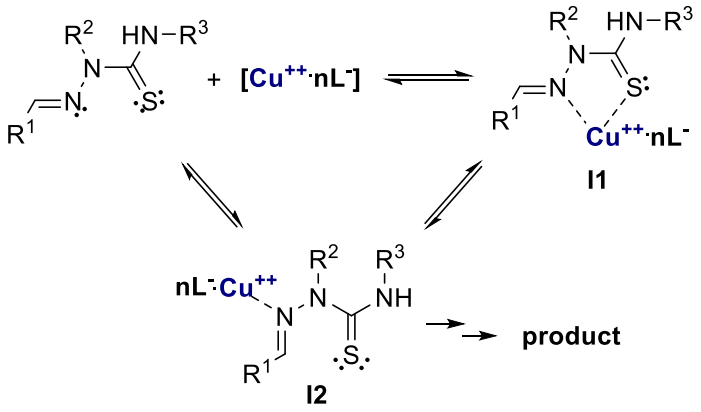


Figure 2. Hammett plot for thiosemicarbazones **1f-k**.

Mechanistic considerations. The whole of the results shown hereinabove seems consistent with the mechanistic hypothesis shown in Scheme 3. As a first observation, the peculiar non-linear Hammett's plot found for substrates **1f-k** indicates the occurrence of a multi-step mechanism. Indeed, the cupric ion (possibly bearing some counter-anion ligands in its first coordination sphere) can form chelate complexes with thiosemicarbazones (Scheme 5), by coordinating the S atom and the imine-like N atom.[34-36] Of course, the chelate complex (indicated as I1 in Scheme 5) must undergo partial disruption to afford a monodentate complex (I2), for the reaction to occur. Under these circumstances, it can be reasonably expected that the functional dependence of the apparent kinetic constant on the concentration of the cupric salt should follow a hyperbolic Michaelis-Menten-like trend, similar to the one observed in the case of cupric perchlorate.[24] Therefore, the use of a large excess of the oxidizing agent (pseudo-first order conditions, as mentioned hereinabove) is aimed at reasonably ensuring that the boundary conditions required for the application of the competitive reactivity method are fulfilled.



Scheme 5. Coordination of thiosemicarbazones by Cu(II) species.

The partial disruption of the chelate complex I1 into the reactive monodentate form I2 is clearly favored by the presence of electron-withdrawing substituents on the residue R^3, whereas the subsequent ring-closure nucleophilic attack of the S atom on the imine-like C atom is favored by electron-donating groups. Therefore, on passing from strong electron-donating to strong electron-withdrawing substituents, the rate-determining step of the reaction mechanism shifts from the formation of I2 to the ring closure step. Even the role played by the groups bound to the benzaldehyde residue R^1 is peculiar. In fact, electron-withdrawing substituents disfavor the electrophilic attack of the cupric ion on the imine-like N atom, bur favor the subsequent ring closure step. As a consequence, the apparent ρ values observed (-0.87 for $CuCl_2$, to be compared to the value of -0.80 with σ^+ for $FeCl_3$) are indeed the algebraic sum of the actual ρ values for the two separate steps, thus resulting in being much lower than those observed for the possible formation of the triazole ring[23-26] (usually ranging between -2 and -3). At this purpose, it is interesting to notice that the apparent increase in the relative amount of thiadiazole product along the series **1a-f**, observed in the case of the reaction with $FeCl_3$, is indeed due to the fact that electron-withdrawing substituents on R^1 slow down the formation rate of both the triazole and the thiadiazole products, but the effect on the former one is much stronger. Moreover, a strong support for the mechanistic hypothesis reported on Scheme 3 is provided by considering the effect of the residue R^2. In fact, its ability to provide electron density to the thioureido S atom strongly affects the reaction course. By changing R^2 from a methyl to a conjugating phenyl group, this largely decreases the ability of the N atom to support the nucleophilic attack of the S atom in the ring-closure step, which is restored when the same phenyl

group bears the strong electron-donating *p*-methoxy substituent. It is worth noting that for 2-N-unsubstituted substrates (i.e. if R^2 = H) the Lewis acid catalyzed ring-closure step, depicted on Scheme 1, is likely eased by facile proton loss from the thioureido-like moiety. This, in turn, largely favors the formation of the thiadiazole product over the possible 1,2,4-triazole. Conversely, replacing the H atom as R^2 with a methyl group (and thus hampering proton loss), the nucleophilic character of the S atom is largely decreased, and the formation of the thiadiazole product disfavored.

As a final remark, it is interesting to notice the effect of the counter-anion present in the copper salt. As we mentioned previously, copper perchlorate reacts much more slowly and affords the triazole derivative as the sole product.[24] This peculiar difference in behavior with the chloride salt may be explained, in our opinion, by the different coordination ability of the salt anions.[37-39] As a weak donor the perchlorate anion weakly interacts with cupric ion in methanol solution; conversely, chloride strongly interacts with Cu^{++} (particularly in a poorly ionizing medium such as dichloromethane), which in turn switches to a tetrahedral coordination geometry. Under the latter circumstances, the rate of ligand exchange on the first coordination shell of the oxidizing cation largely varies. In particular, in the presence of the weak perchlorate ligand, the possible chelate complex I1 of Scheme 5 is relatively stable; conversely, the presence of a stronger ligand such as Cl^- favors its disruption and evolution to products.

Conclusions

By a smartly exploiting the competitive reactivity method, we gained valuable mechanistic information on the oxidative cyclization of aldehyde thiosemicarbazones into the relevant 2(3*H*)-imino-1,3,4-thiadiazoles. In particular, this methodology enabled us to apply Hammett's equation without performing proper kinetic experiments. Data obtained, in particular comparison of the susceptivity constants ρ with the ones obtained for the formation of the corresponding 3*H*-1,2,4-triazole-3-thione products, support the hypothesis that the formation of the thiadiazole ring proceeds from an electrophilic attack of the oxidizing cation (Fe^{+++} or Cu^{++}) on the imine-like nitrogen atom of the substrate, followed by ring closure and subsequent electron transfer steps. The role played by the substituents on the thiosemicarbazone scaffold and by the oxidizing salt has also been suitably rationalized. Considering the synthetic interest towards the 1,3,4-thiadiazole ring, in particular in medicinal chemistry, it is worth noting in our opinion that oxidative cyclization may provide an easy and straightforward alternative synthetic strategy to these compounds, an approach which deserves to be revitalized and subjected to deeper investigation in future studies.

Experimental Section

General. All the commercial reagents needed were used as purchased, without further purification. Dichloromethane was dried by refluxing for 4 h over an excess CaH_2, and then distilled. The synthesis of thiosemicarbazones **1a-f**[26] and **1l-m**[25] was performed as reported in literature. Elemental analyses were performed on a Perkin Elmer 2400 CNHS/O Analyser. FTIR spectra (nujol) were recorded on an Agilent Technologies Cary 630 FTIR spectrometer. NMR spectra were acquired on a Bruker 300 MHz spectrometer. The HPLC analyses were performed on a LC-10AD Shimadzu liquid chromatograph apparatus, equipped with a SPD-10AV Shimadzu UV-vis detector and an ordinary 25 cm C18 HPLC column was used. Elutions were performed with water-acetonitrile mixtures at various ratios, usually ranging from 20:80 v/v to 30:70 v/v. Flow

and eluent conditions had to be adapted case by case; as a general rule, thiosemicarbazones are eluted at shorter retention times (usually 4-10 min) than the relevant 1,3,4-thiadiazole products (usually 8-20 nmn).

General procedure for the preparation of thiosemicarbazones (1g-j). The suitable thiosemicarbazide[23] (10 mmoles) and 4-nitro-benzaldehyde (10 mmoles) were dissolved in dimethylsulfoxide (5 mL) at r.t., few drops of acetic acid were then added, and the mixture kept under magnetic stirring for 4 h. The reaction crude was then mixed with ethanol (25 mL), kept under magnetic stirring for 15 min, and the product finally filtered off (yield 70-95%). The product could be further re-crystallized from ethanol if needed.

2-Methyl-4-(4-methoxyphenyl)-thiosemicarbazone of 4-nitrobenzaldehyde (1g). mp 218-221 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 3290 (NH), 1575 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 3.84 (s, 3H, CH_3O-), 4.01 (s, 3H, CH_3N<), 6.96, 7.42 (2d, 2H+2H, *J* 9.0 Hz, *p*-CH_3O-C_6H_4-), 7.78 (s, 1H, -CH=N-), 7.86, 8.30 (2d, 2H+2H, *J* 8.85 Hz, *p*-NO_2-C_6H_4-), 9.74 (s, 1H, NH). Elemental analysis for $C_{16}H_{16}N_4O_3S$: calcd. C 55.80, H 4.68, N 16.27; found C 55.76, H 4.71, N 16.24.

2-Methyl-4-(4-methylphenyl)-thiosemicarbazone of 4-nitrobenzaldehyde (1h). mp 205-208 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 3310 (NH), 1582 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 2.37 (s, 3H, CH_3-), 4.00 (s, 3H, CH_3N<), 7.23, 7.43 (2d, 2H+2H, *J* 8.3 Hz, *p*-CH_3-C_6H_4-), 7.77 (s, 1H, -CH=N-), 7.85, 8.29 (2d, 2H+2H, *J* 8.75 Hz, *p*-NO_2-C_6H_4-), 9.82 (s, 1H, NH). Elemental analysis for $C_{16}H_{16}N_4O_2S$: calcd. C 58.52, H 4.91, N 17.06; found C 58.53, H 4.89, N 17.09.

2-Methyl-4-(4-chlorophenyl)-thiosemicarbazone of 4-nitrobenzaldehyde (1i). mp 218-219 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 3330 (NH), 1570 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 4.00 (s, 3H, CH_3N<), 7.39, 7.55 (2d 2H+2H, *J* 8.7 Hz, *p*-Cl-C_6H_4-), 7.80 (s, 1H, -CH=N-), 7.86, 8.31 (2d, 2H+2H, *J* 8.9 Hz, *p*-NO_2-C_6H_4-), 9.87 (s, 1H, NH). Elemental analysis for $C_{15}H_{13}ClN_4O_2S$: calcd. C 51.65, H 3.76, N 16.06; found C 51.69, H 3.78, N 16.04.

2-Methyl-4-(3-chlorophenyl)-thiosemicarbazone of 4-nitrobenzaldehyde (1j). mp 204 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 3280 (NH), 1592 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 4.00 (s, 3H, CH_3N<), 7.22-7.26 (m, 1H) 7.36 (t, 1H, *J* 8.1 Hz) 7.50-7.54 (m, 1H) 7.67-7.70 (m, 1H, *m*-Cl-C_6H_4-), 7.80 (s, 1H, -CH=N-), 7.86, 8.31 (2d, 2H+2H, *J* 8.65 Hz, *p*-NO_2-C_6H_4-), 9.87 (s, 1H, NH). Elemental analysis for $C_{15}H_{13}ClN_4O_2S$: calcd. C 51.65, H 3.76, N 16.06; found C 51.69, H 3.78, N 16.04.

2-Methyl-4-(4-nitrophenyl)-thiosemicarbazone of 4-nitrobenzaldehyde (1k). mp 234-237 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 3265 (NH), 1592 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) 3.95 (s, 3H, CH_3N<), 7.91-8.26 (2d, 2H, *J* 9.0 Hz, *p*-NO_2-C_6H_4-), 8.27 (s, 1H, -CH=N-), 8.30 (s, 4H, *p*-NO_2-C_6H_4-), 10.87 (s, 1H, NH). Elemental analysis for $C_{15}H_{13}N_5O_4S$: calcd. C 50.13, H 3.65, N 17.81; found C 50.13, H 3.70, N 17.84.

General procedure for the oxidative cyclization of thiosemicarbazones 1g-k into the relevant thiadiazole derivatives 2g-k with $CuCl_2$. The thiosemicarbazone (5 mmoles) was dissolved (or suspended) in boiling ethanol (40 mL), after which a solution prepared dissolving cupric chloride trihydrate (2.0 g, 10.6 mmoles) in ethanol (20 mL) was added, and the mixture kept under reflux for 1 h. The mixture was cooled overnight, and part of the crystalline product filtered off. To obtain a further aliquot of product, the reaction mixture was evaporated and the residue dissolved in water (50 mL), after which the turbid suspension obtained was extracted thrice with ethyl acetate (50 mL each). The organic extracts were dried (Na_2SO_4) and concentrated in vacuo, and the final residue subjected to column chromatography on silica gel with a cyclohexane/ethyl acetate mixture (4:1 v/v) as the eluent. Yield (60-90%).

***N*-(3-methyl-5-(4-nitro-phenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-4-methoxy-phenylamine (2g)**. mp 137-138 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 1613, 1590 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 3.80, 3.82 (2s, 3H+3H, CH_3O- and CH_3N<), 6.93, 7.02 (2d, 2H+2H, *J* 8.85 Hz, *p*-CH_3O-C_6H_4-), 7.88, 8.22 (2d, 2H+2H, *J* 9.05 Hz, *p*-NO_2-C_6H_4-). Elemental analysis for $C_{16}H_{14}N_4O_3S$: calcd. C 56.13, H 4.12, N 16.36; found C 55.16, H 4.11, N 16.34.

***N*-(3-methyl-5-(4-nitro-phenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-4-methyl-phenylamine (2h)**. mp 164 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 1613, 1585 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 2.35 (s, 3H, CH_3-), 3.80 (s, 3H, CH_3N<), 6.98, 7.18 (2d,

2H+2H, *J* 8.3 Hz, *p*-CH_3-C_6H_4-), 7.75, 8.23 (2d, 2H+2H, *J* 8.9 Hz, *p*-NO_2-C_6H_4-). Elemental analysis for $C_{16}H_{14}N_4O_2S$: calcd. C 58.88, H 4.32, N 17.17; found C 58.83, H 4.29, N 17.19.

***N*-(3-methyl-5-(4-nitro-phenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-4-chloro-phenylamine (2i)**. mp 170-171 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 1613, 1580 (C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 3.82 (s, 3H, CH_3N<), 7.01, 7.33 (2d 2H+2H, *J* 8.6 Hz, *p*-Cl-C_6H_4-), 7.77, 8.26 (2d, 2H+2H, *J* 9.0 Hz, *p*-NO_2-C_6H_4-). Elemental analysis for $C_{15}H_{11}ClN_4O_2S$: calcd. C 51.95, H 3.20, N 16.16; found C 51.98, H 3.18, N 16.11.

***N*-(3-methyl-5-(4-nitro-phenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-3-chloro-phenylamine (2j)**. mp 117-118 °C. FTIR: $\tilde{\nu}$ (cm^{-1}) 1608,1575(C=N). ^{1}H NMR ($CDCl_3$): δ (ppm) 3.82 (s, 3H, CH_3N<), 6.94-6.99 (m, 1H) 7.7-7.10 (m, 2H) 7.25-7.34 (m, 1H, *m*-Cl-C_6H_4-), 7.79, 8.87 (2d, 2H+2H, *J* 8.8 Hz, *p*-NO_2-C_6H_4-). Elemental analysis for $C_{15}H_{11}ClN_4O_2S$: calcd. C 51.95, H 3.20, N 16.16; found C 51.91, H 3.21, N 16.19.

***N*-(3-methyl-5-(4-nitro-phenyl)-1,3,4-thiadiazol-2(3*H*)-ylidene)-4-nitro-phenylamine (2k)**. mp 221-223 °C. FTIR: ñ (cm^{-1}) 1618, 1572 (C=N). ^{1}H NMR (DMSO-d_6): δ (ppm) 3.90 (s, 3H, CH_3N<), 7.39, 8.34 (2d, 2H+2H, *p*-NO_2-C_6H_4-), 8.06, 8.40 (2d, 2H+2H, *J* 9.0 Hz, *p*-NO_2-C_6H_4-). Elemental analysis for $C_{15}H_{11}N_5O_4S$: calcd. C 50.42, H 3.10, N 19.60; found C 50.43, H 3.10, N 19.59.

Competitive reactivity experiments

A mixture of two different substrates (ca. 10 µmoles each) dissolved in dichloromethane (10 mL) was cooled in an ice bath (0 °C), after which an ice-cooled stock solution of $CuCl_2$ trihydrate 220 mM (1 mL) was added. The mixture was kept under magnetic stirring for ca. 5 min; after which a small aliquot (ca. 0.5 mL) was quickly transferred into a SEP-PAK® C18 mini-column and eluted first with water (5 mL) to eliminate the inorganic salts, and then with acetonitrile (2 mL) to recover the organic substances. The latter eluate was analyzed by HPLC as described above.

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