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Hemisynthesis of new thiosemicarbazone derivatives resulting from latex of Moroccan endemic plant: *Euphorbia officinarum*

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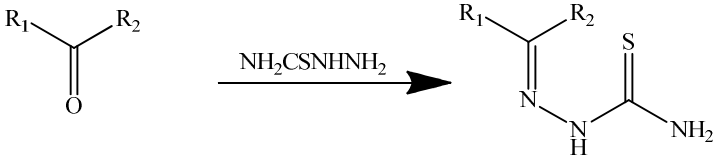
Abstract
The thiosemicarbazone derivatives were prepared by introducing new functions on triterpenic skeletons. The product hemisynthezised from latex of Moroccan endemic plant: *Euphorbia officinarum*, were obtained with good yield and high regioselectivity

Keywords: latex, *Euphorbia officinarum*, triterpenes, thiosemicarbazones.

1. Introduction
For a long time, heterocyclic compounds are known for their interesting pharmacological activities. The chemistry of these compounds didn't stop developing on the synthetic plant since it knew these last years a considerable flight bound to the numerous uses of these derivatives in various domains.
In particular, the thiosemicarbazones and their derivatives were evaluated for inhibitor activity against *Trypanosoma rhodesiense* [1]. Following these studies, a considerable number of their derivatives showed a very interesting pharmacological activities, such as antibacterial [2], antiviral [3], phytotoxic [4, 5] and antiparasitic [4-6]. Some others appeared antineoplastic [7] and antimalarial [8]. Thiosemicarbazones compounds have also showed a selective inhibition of herpes simplex virus [9]. An effect against human immunodeficiency virus (HIV) was also reported [10]. Recently, we have reported a very simple method for preparing thiosemicarbazones [11] using thiosemicarbazide condensation of several ketones (Scheme 1).



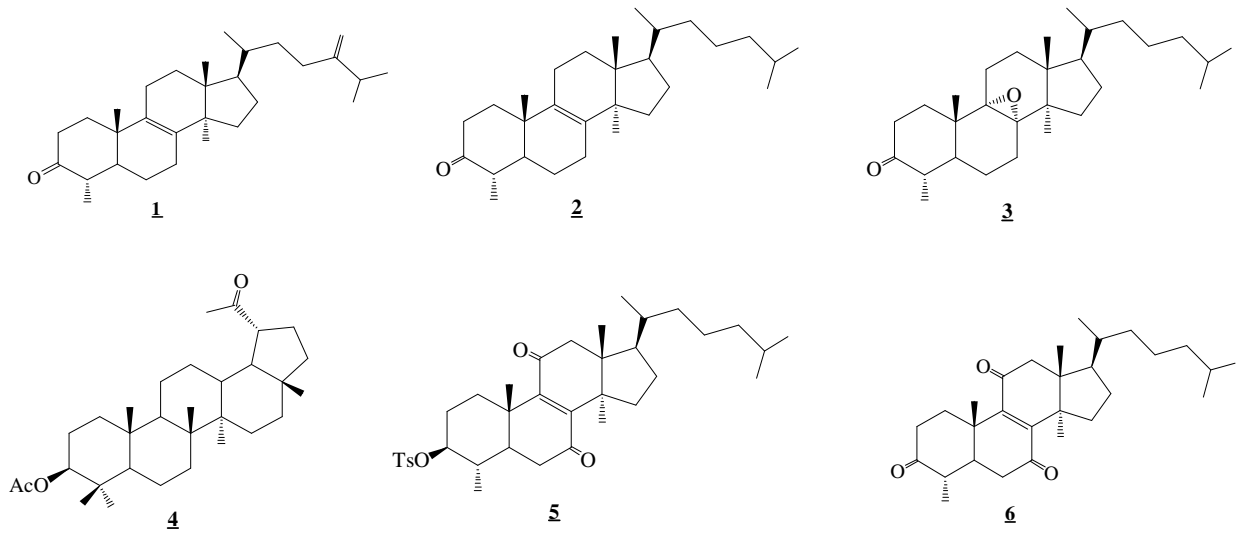
Scheme 1. General pathway for thiosemicarbazone's synthesis [11].

In order to synthesize similar compounds, we have undertaken the following work. Thus, treatment with thiosemicarbazide (TSC) [11, 12] of hemisynthesized mono-, di- and tricarbonyl compounds (Scheme 2), resulting from *Euphorbia officinarum* [13-15], using oxidation by chromic anhydride [16, 17] yielded new thiosemicarbazones derivatives with good yield and high regioselectivity.

2. Experimental Section
General procedure of thiosemicarbazide condensation
To a solution of equimolecular quantity of substrate and thiosemicarbazide dissolved in ethanol, several drops of HCl (c) were added. The reactional mixture was heated at reflux during 5 h and then evaporated under reduced pressure. The residue obtained was chromatographied on silica gel column with hexane and ethyl acetate as eluents.

4α,14α-Dimethyl-5α-ergosta-8,24-dien-3-one thiosemicarbazone (7). White powder; Yield: 96 %; m.p. 215-216 °C (hexane, ethyl acetate) ; m/z = 497 ($M^{+\cdot}$) ; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.79, 7.17, 6.41 (3H, NH and NH_2), 4.61 (H^a-30), 4.66 (H^b-30, s), 0.68 (H-18, s), 0.94 (H-19, s), (H-21, d, J = 6.2 Hz), 0.98 (H-26, d, J = 2 Hz), 0.99 (H-27, d, J = 2 Hz), 1.15 (H-29, d, J = 6.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 36.4 (C-1), 37.3 (C-2), 156.9 (C-3), 44.5 (C-4), 49.3 (C-5), 21.8 (C-6), 28.0 (C-7), 132.4 (C-8), 135.7 (C-9), 35.8 (C-10), 21.5 (C-11), 25.5 (C-12), 44.5 (C-13), 49.6 (C-14),

30.8 (C-15), 30.5 (C-16), 50.3 (C-17), 15.8 (C-18), 17.4 (C-19), 36.3 (C-20), 18.6 (C-21), 36.1 (C-22), 31.1 (C-23), 155.4 (C-24), 33.6 (C-25), 21.7 (C-26), 21.9 (C-27), 24.5 (C-28), 12.2 (C-29), 106.1 (C-30), 179.2 (C=S).



Scheme 2. Principal carbonyl compounds hemisynthesized from *E. officinarum* latex

4α,14α-Dimethyl-5α-cholest-8-en-3-one thiosemicarbazone (8). White powder; Yield: 94 %; m.p. 212-213°C (hexane, ethyl acetate); m/z = 485 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.74, 7.25, 6.33 (3H, NH and NH_2), 0.70 (H-18, s), 0.96 (H-19, s), 0.88 (H-21, d, J = 6 Hz), 0.85 (H-26, d, J = 2 Hz), 0.86 (H-27, d, J = 2 Hz), 0.87 (H-28, s), 1.20 (H-29, d, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 36.5 (C-1), 37.3 (C-2), 159.3 (C-3), 50.6 (C-4), 49.9 (C-5), 21.7 (C-6), 28.1 (C-7), 132.3 (C-8), 135.7 (C-9), 36.4 (C-10), 21.5 (C-11), 25.4 (C-12), 44.5 (C-13), 49.7 (C-14), 30.9 (C-15), 29.8 (C-16), 46.1 (C-17), 15.8 (C-18), 18.1 (C-19), 36.1 (C-20), 18.6 (C-21), 36.6 (C-22), 24.3 (C-23), 32.4 (C-24), 34.6 (C-25), 21.7 (C-26), 21.9 (C-27), 24.2 (C-28), 12.2 (C-29), 106.1 (C-30), 179.2 (C=S).

4α,14α-Dimethyl-5α-cholesta-7,9-dien-3-one thiosemicarbazone (9). White powder; Yield: 74 %; m.p. 205-206°C (hexane, ethyl acetate); m/z = 483 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.81, 7.17, 6.20 (3H, NH and NH_2); 5.42 (H-7, J = 6.6 Hz), 5.38 (H-11, J = 6.1Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 159.1 (C-3), 44.53 (C-4), 49.7 (C-5), 23.1 (C-6), 118.5 (C-7), 142.5 (C-8), 143.6 (C-9), 36.4 (C-10), 119.1 (C-11), 25.4 (C-12), 39.62 (C-13), 30.9 (C-14), 15.6 (C-15), 29.8 (C-16), 49.9 (C-17), 15.7 (C-18), 18.25 (C-19), 36.5 (C-20), 18.5 (C-21), 36.6 (C-22), 24.3 (C-23), 38.8 (C-24), 28.1 (C-25), 22.8 (C-26), 22 5 (C-27), 24.5 (C-28), 12.5 (C-29), 179.7 (C=S).

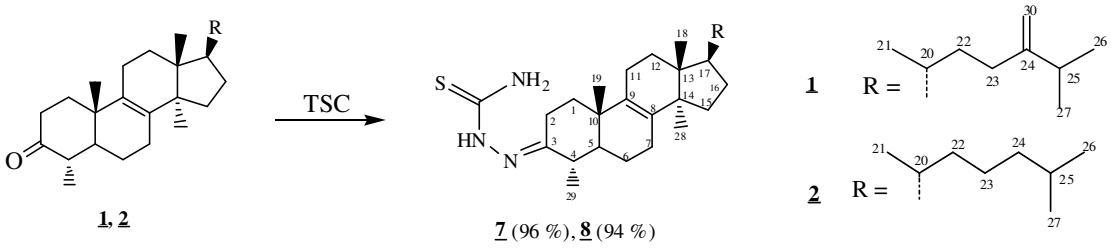
3β-Acetoxy-28-norlup-20-one thiosemicarbazone (10). White powder; Yield: 76 %; m.p. 214-215°C (hexane, ethyl acetate); m/z = 543 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 4.45 (H-3, dd, J_1 = 11 Hz, J_2 = 5 Hz), 8.63, 7.20, 6.65 (3H, NH and NH_2), 2.03 (CO<u>CH3</u>), 0.68 (H-5, d, J = 9 Hz), 2.38 (H-19, ddd, J_1 = 11.3 Hz, J_2 = 11.5 Hz, J_3 = 5.6 Hz), 1.35 (H-23, s), 0.77 (H-24, s), 0.83 (H-25, s), 0.98 (H-26, s), 0.97 (H-28, s), 1.90 (H-29, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 37.6 (C-1), 38.0 (C-2), 81.1 (C-3), 38.8 (C-4), 55.6 (C-5), 18.2 (C-6), 34.5 (C-7), 40.7 (C-8), 50.3 (C-9), 37.0 (C-10), 20.8 (C-11), 25.0 (C-12), 37.9 (C-13), 42.7 (C-14), 27.3 (C-15), 35.5 (C-16), 42.9 (C-17), 42.8 (C-18), 47.9 (C-19), 158.4 (C-20), 29.7 (C-21), 39.9 (C-22), 27.8 (C-23), 15.3 (C-24), 16.0 (C-25), 15.9 (C-26), 14.5 (C-27), 17.9 (C-28), 19.0 (C-29), 178.8 (C=S), 21.1 (CO<u>CH3</u>), 171.2 (<u>CO</u>CH3).

3β-Tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione-7-thiosemicarbazone (11). White powder; Yield: 74 %; m.p. 215-216°C (hexane, ethyl acetate); m/z = 669.84 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 4.11 (H-3, ddd, J_1= 11Hz, J_2 =11Hz, J_3 = 3 Hz), 8.83, 7.10, 6.59 (3H, NH and NH_2), 7.80 (H-2', d, J = 8.1Hz), 7.34 (H-3', d, J = 7.8 Hz), 2.43 (H-5'), 0.69 (H-18, s), 1.25 (H-19, s), 1.15 (H-28, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 33.1 (C-1), 27.2 (C-2), 86.2 (C-3), 51.4 (C-4), 48.9 (C-5), 39.4 (C-6), 152.3 (C-7), 149.0 (C-8), 146.3 (C-9), 36.9 (C-10), 200.1 (C-11), 45.2 (C-12), 48.4 (C-13), 47.5 (C-14), 32.5 (C-15), 27.9 (C-16), 49.5 (C-17), 16.5 (C-18), 18.4 (C-19), 36.2 (C-20), 18.1 (C-21), 34.8 (C-22), 27.4 (C-23), 39.5 (C-24), 31.8 (C-25), 21.1 (C-26), 22.5 (C-27), 15.3 (C-28), 144.7 (C-1'), 134.5 (C-2'), 129. (C-3'), 127.00 (C-4'), 21.6 (CH_3-5'), 179.7 (C=S).

4α,14α-Dimethyl-5α-cholest-8-ene-3,7,11-trione-7-thiosemicarbazone (12). White powder ;Yield: 78 %; m.p. 206-207°C (hexane, ethyl acetate); m/z = 511 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.87, 7.08, 6.55 (3H, NH and NH_2), 0.83 (H-18, s), 1.19 (H-19, s), 0.89 (H-21, d, J = 6.46 Hz), 0.86 (H-26, d, J = 2.69 Hz), 0.87 (H-27, d, J = 2.73 Hz), 1.48 (H-28, s), 1.06 (H-29, d, J = 6.61 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 36.1 (C-1), 36.5 (C-2), 211.1 (C-3), 51.7 (C-4), 49.3 (C-5), 39.7 (C-6), 153.1 (C-7), 148.0 (C-8), 146.0 (C-9), 36.7 (C-10), 200.4 (C-11), 44.6 (C-12), 37.8 (C-13), 36.5 (C-14), 28.4 (C-15), 29.8 (C-16), 48.7 (C-17), 17.8 (C-18), 18.9 (C-19), 37.8 (C-20), 17.5 (C-21), 27.7 (C-22), 26.3 (C-23), 27.5 (C-24), 29.1 (C-25), 16.6 (C-26), 17.3 (C-27), 24.2 (C-28), 15.5 (C-29), 180.1 (C=S).

Results and discussion

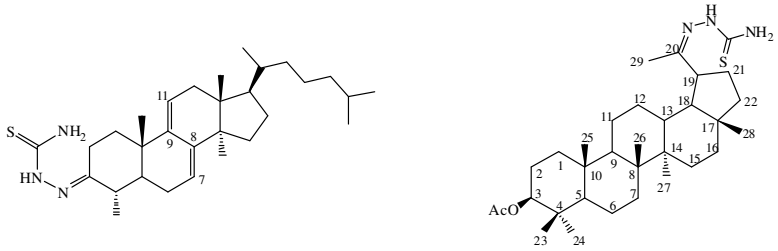
In order to prepare new heterocyclic triterpene derivatives, we were interested to the reactivity of mono-, di- and tricarbonyl triterpenic compounds hemisynthesized from *Euphorbia officinarum* latex using thiosemicarbazide (TSC) condensation. Thus, treatment with equimolecular quantity of compounds **1**, **2** and thiosemicarbazide in the presence of hydrogen chloride in ethanol, yielded respectively, after heating at reflux during 5h, to products **7** and **8** (Scheme 3).



1, **2** **7** (96 %), **8** (94 %)

Scheme 3

The entire newly prepared product **7** and **8** were fully characterized from their spectral data (NMR). Thus, ^{1}H NMR spectra showed more especially three peaks at 6.39, 6.40 and 8.78 ppm corresponding to NH and NH_2 resonance for product **7** whereas the same signals are observed at 6.33, 7.25 and 8.74 ppm for component **8**.
The ^{13}C NMR spectrum revealed particular signals at 156.9 and 179.2 ppm assigned respectively to C3 and C=S groups for product **7**. However, for compound **8,** the signals of C=N and C=S were respectively present at 159.3 and 179.2 ppm.
Treatment of 8α,9α-epoxy-4α,14α-dimethyl-5α-cholestan-3-one **3** and 3α-acetoxy-norlup-20-one **4**, according to the general procedure previously described, allowing us to prepare respectively the new thiosemicarbazones **9** and **10** with a good yield (Scheme 4).



9 (74 %) **10** (76 %)

Scheme 4

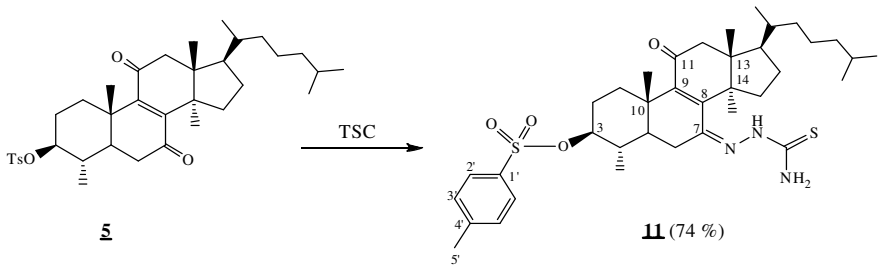
Product **9** is characterized more precisely by the appearance of two new conjugated doublets due to the instability of oxiranic bridge in acid medium of compound **3**.

Table 1. ^{1}H NMR[a] data of compounds **9** and **10** (300 MHz, δ [ppm])

H	**9** δ (ppm)	**10** δ (ppm)
3H(NH and NH_2)	8.81	8.62
	7.20	7.20
	6.38	6.65

[a] Recorded in $CDCl_3$ (δ_H 7.25)

^{13}C NMR spectra reveal C=N and C=S group resonance respectively at 159.1 and 179.7 ppm for compound **9** whereas the same signals are observed at 159.2 and 178.8 ppm for product **10**.
Treatment of compounds **1**, **2**, **3** and **4** proved to be highly chimiospecific leading respectively to single condensated products **7**, **8**, **9** and **10** with a good yield.
In order to understand the regio- and periselectivity behaviour condensation toward triterpene derivatives, we have studied condensation of thiosemicarbazide on di- and tricarbonyl compounds following the same procedure as before. Thus, treatment by an equimolecular quantity of thiosemicarbazide on 3β-tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione (**5**) gave the sole product **11** with a good yield (Scheme 5).



Scheme 5

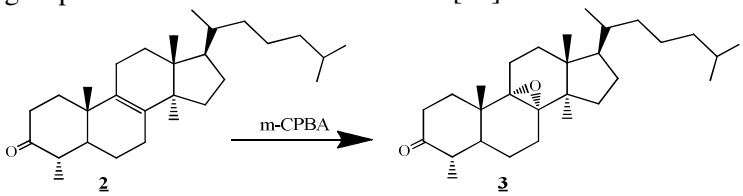
Compound **11** was characterized more especially in its ^{1}H and ^{13}C NMR by new signals after thiosemicarbazide condensation. Table 2 summarises some principal results obtained.

Table 2: ^{1}H and ^{13}C NMR spectroscopic data (at 300 and 75 MHz, respectively) of compound **11**[a]

C	^{1}H δ (mult, J/Hz)	^{13}C δ (ppm)
C-3	4.10 (H-3, ddd, J_1= 11, J_2 = 11 and J_3 = 3 Hz)	86.2
C-7	-	152.3
C-8	-	148.1
C-9	-	146.3
C11	-	200.1
-	8.83, 7.10 and 6.59 (3H, NH and NH2)	179.7 (C=S)

[a] Chemical shifts are expressed in ppm and the coupling constant (J) in Hz.

The thiosemicarbazide condensation was carried out in C7 for compound **11**. This difference of condensation is explained by steric genes of methyl groups in positions 10 and 13 [18].
Our research work has been made to prepare compound **3**. However the reaction with metachloroperbenzoic acid [22] to the endocyclic double bond was assigned by forming the oxirane bridge linking the two C atoms, C8 and C9, and cis to the methyl groups attached to atoms C4 and C14 [18] scheme 6.



Scheme 6: Epoxidation reaction of compound **2**

The stereochemistry of compound **3** has been confirmed by single-crystal X-ray diffraction (figure 1).
Structures of α,β-unsaturated ketones were elucidated through their ^{1}H NMR spectral data, ^{13}C and mass spectrometry.
Referring to work done by Tanaka et al. [19], carbonyl product **5** has the same physico-chemical properties as identified and synthesized acetates from *Euphorbia chamaesyce* species. Therefore, in comparison with the results published by these researchers, we have identified the thiosemicarbazone **11**. Triterpene derivative of departure has two positions that can lead to condensation (positions 7 and 11). For this, through to study of single and dimensional NMR to compound **11** (Figure 2), we could identify all structures of this product.

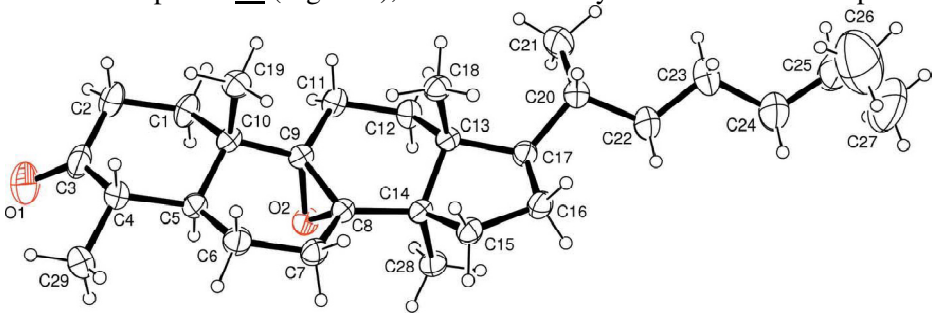


Figure 1: ORTEP drawing of compound **3** [18].

A detailed study of the ^{1}H, ^{13}C NMR and HMBC spectral analyzes allowed to assign the chemical shifts of different carbons of compound **11** structure. We noted particularly in Figure 2, a correlations at two and three bonds: 1.62 ppm (CH, H-5) with 152.3 ppm (C = N, C-7) and 2.45 ppm (CH2, H-6) with 152.3 ppm (C = N, C-7).

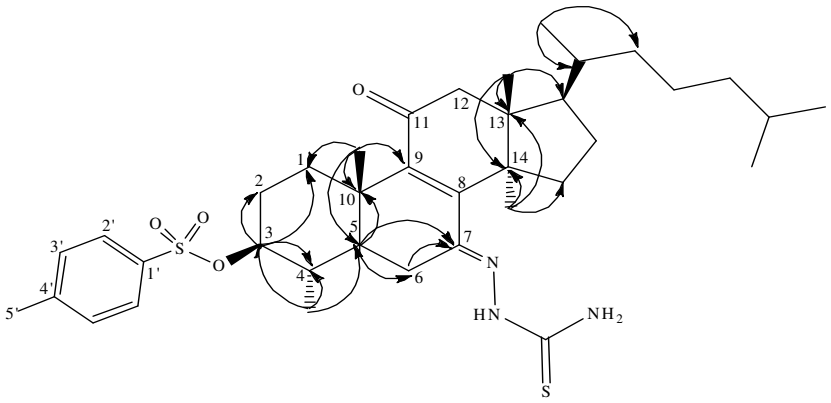


Figure 2: Principal correlations observed

The increase of stoichoimetric quantity of thiosemicarbazide gave the only product **11** with a good yield (Table 4).

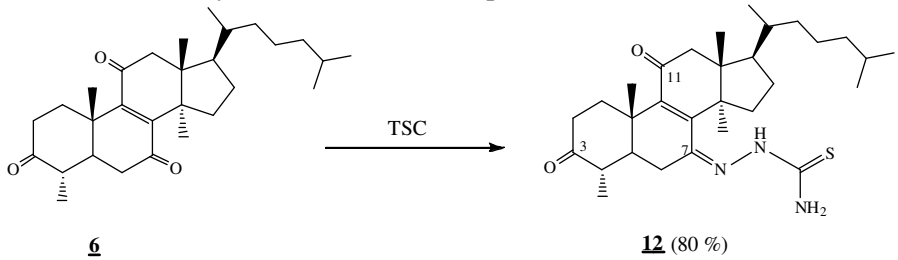
Table 4 : Principal results obtained by treatment of **5** with thiosemicarbazide.

TSC (eq. number)	1[a]	2	3	4
Yield of compound **11** (%)	74	78	78	79

[a] General conditions: **5** = 2.35 mmol; TSC (2.35 mmol); Ethano l = 50 mL; HCl (cc) several drops; Time = 5h; Temperature = 110 °C.

These results allowed us to conclude the regio- and the periselectivity of thiosemicarbazide (TSC) condensation on dicarbonyl compound **5**. For well examining the regio- and periselectivity of this condensation, we treated the tricarbonyl compound **6** under the same conditions as before. All this allowed us to prepare the sole product **12** with a good yield and high regioselectivity (Scheme 6).

The structure elucidation of dicarbonyl compound **12** was based on spectral data including ^{1}H and ^{13}C NMR. Thus, ^{1}H NMR spectrum exhibited more especially three peaks at 8.87, 7.08 and 6.55 ppm due respectively to the resonance of NH and NH_2 groups. While its ^{13}C NMR spectrum showed the disappearance of the only peak due to carbonyl resonance in C7 and the appearance of a new signal at 153.1 ppm characterising C=N group. Table 5 gave some various results obtained by condensation on compound **6** with thiosemicarbazide.



6 **12** (80 %)

Scheme 6

Table 5: Various results obtained by action of TSC on compound **6**.

TSC (eq. number)	1[a]	2	3	4
Yield of compound **12** (%)	80	82	83	83

[a] General conditions: **6** = 1.95 mmol; TSC (1.95 mmol); Ethanol = 60 mL; HCl (cc) several drops; Temperature = 110 °C; Time = 5h.

These results reveal that thiosemicarbazide condensation on tricarbonyl compound **6** was highly regio- and periselective. This regioselectivity is due to steric genes carried by the methyl groups in position 10 and 13[18].

Conclusion

We have described in our study hemisynthesis of new thiosemicarbazones (**7-12**), by condensation of the carbonyl products **1-6** on thiosemicarbazide (TSC). This reaction of condensation was regio- and periselective procedure in the presence of two or three carbonyls in position 3, 7 or 11. However, we have prepared only the monocondensated products with a good yield. This monocondensation was highly regio- and periselective with the tricarbonyl compound **6**. This difference of condensation can be explained by the steric genes of methyl groups in positions 10 and 13 [18].

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