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New 1,3,4-Thiadiazolines Hemisynthesized from Moroccan Endemic Plants: *Euphorbia officinarum* latex

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Abstract
Heterocycles are inextricably woven into the life processes. The vital interest of the pharmaceutical and agrochemical industries in heterocycles is often connected with their natural occurrence. The synthesis of novel heterocyclic derivatives and investigation of their chemical and biological behaviour have gained more importance in recent decades. Heterocyclic chemistry is an integral part of the chemical sciences and constitutes a considerable part of the modern researches that are occurring presently throughout the world. Hemisynthesis of 1,3,4-thiadiazolic triterpenes resulting from *Euphorbia officinarum* latex require initially preparation of their precursors, the thiosemicarbazones and gave thiadiazolic compounds characterized by new spiro carbons.

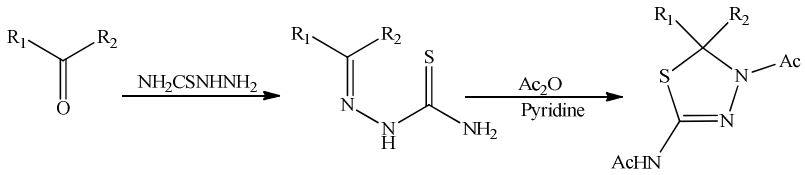
Keywords: *Euphorbia officinarum*; triterpenes; thiosemicarbazones; thiadiazolines

Introduction
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 varied domains. Medicinal chemistry had its beginning when chemists, pharmacist and physicians isolated and purified active principles of plants and animals tissues and taken from micro-organism and their fermentation products. Some of these chemicals have been associated with therapeutic properties: Medicinal chemistry which has leaned on the classical fields of chemistry, especially organic chemistry, biology and some area of physics. A limited number of natural and synthetic products and serve directly as therapeutic agents although lack of specificity frequently limits their application in human and veterinary medicines and in analogous pesticidal and other uses in agriculture. The study of heterocyclic systems is of great interest both from the theoretical and practical point of view. Heterocycles also play an important role in the design and discovery of new pharmacologically active compounds. By dissecting the structure of these products chemically, one arrives at its therapeutically significant molecular sections, the pharmacophores, the portion that can be deleted are of no interest as components of drug action; they are regarded as the result of the biosynthetic efforts on the parent organism to construct materials for its own metabolic or defensive purposes.

Thiadiazolines possess a wide range of biological properties and they act as antihypertensives [1], antihelmintics [2], antitumour [3], antibacterial, analgesic and anticancer [4-7].

The thiadiazolic compounds are associated with diverse biological activities. Like wise 1,3,4-thiadiazoles nucleus which incorporate toxiphoric -N=C-S- linkage possesses fungicidal [8], insecticidal [9], antimicrobial [10] and [11] properties.

Recently, we have reported a very simple method for preparing thiadiazolines by cyclization under acetylating conditions of the corresponding thiosemicarbazones of several ketones (Scheme 1).



Scheme 1: General pathway for thiadiazolines's synthesis

2. Materials and methods

2.1.General procedure of condensation on thiosemicarbazide

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

For spectral data of thiosemicarbazone derivatives, the precursors of thiadiazolines **7-12** see reference [12].

2.2.Preparation of 1,3,4-thiadiazolines

(0.25 mmol) of thiosemicarbazone was dissolved in 1mL of pyridine and 1mL of acetic anhydride. The mixture was heated at reflux during 1h with magnetic stirring, and then evaporated under reduced pressure. The residue obtained was purified on silica gel column using a mixture of hexane/ethyl acetate 90/10 as eluent.

4α,14α-Dimethyl-5α-ergosta-8,24-dien-3-one thiadiazoline (7). Yield: 70 %; m.p. 175-176°C (hexane); m/z = 581 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 7.82 (NH, s), 2.15, 2.17 (CO<u>CH3</u>, 2s), 0.71 (H-18, s), 1.12 (H-19, s), 0.95 (H-21, d, J = 6.2 Hz), 0.78 (H-26, d, J = 2 Hz), 0.88 (H-27, d, J = 2 Hz), 1.05 (H-29, d, J = 6.6 Hz), 4.69 (H^a-30), 4.74 (H^b-30, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) : 36.4 (C-1), 37.3(C-2), 91.8 (C-3), 50.6 (C-4), 50.4 (C-5), 21.8 (C-6), 28.0 (C-7), 132.7 (C-8), 134.9 (C-9), 35.9 (C-10), 21.5 (C-11), 25.3 (C-12), 44.6 (C-13), 47.0 (C-14), 30.8 (C-15), 31.1 (C-16), 49.9 (C-17), 15.7 (C-18), 17.4 (C-19), 36.7 (C-20), 18.6 (C-21), 36.3 (C-22), 31.6 (C-23), 156.9 (C-24), 33.9 (C-25), 21.7 (C-26), 21.9 (C-27), 24.5 (C-28), 15.2 (C-29), 106.0 (C-30), 143.7 (C=N), 168.6, 169.6 (<u>CO</u>CH_3), 23.4, 25.1 (CO<u>CH3</u>).

4α,14α-Dimethyl-5α-cholest-8-en-3-one thiadiazoline (8). Yield: 72 %; m.p. 218-219°C (hexane); m/z = 569 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.75 (NH, s), 2.21, 2.25 (CO<u>CH3</u>, 2s), 0.74 (H-18, s), 1.15 (H-19, s), 0.87 (H-21, d, J = 6 Hz), 0.84 (H-26, d, J = 2 Hz), 0.85 (H-27, d, J = 2 Hz), 0.87 (H-28, s), 1.10 (H-29, d, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) : 36.4 (C-1), 37.4 (C-2), 100.0 (C-3), 51.3 (C-4), 50.7 (C-5), 21.8 (C-6), 28.3 (C-7), 129.0 (C-8), 131.1 (C-9), 36.3 (C-10), 22.0 (C-11), 25.5 (C-12), 44.9 (C-13), 49.5 (C-14), 30.8 (C-15), 29.8 (C-16), 46.5 (C-17), 15.8 (C-18), 18.2 (C-19), 36.0 (C-20), 18.7 (C-21), 35.9 (C-22), 24.3 (C-23), 32.4 (C-24), 34.6 (C-25), 21.7 (C-26), 21.8 (C-27), 24.2 (C-28), 14.2 (C-29), 143.2 (C=N), 170.1, 171.0 (<u>CO</u>CH_3), 22.5, 24.2 (CO<u>CH3</u>).

4α,14α-dimethyl-5α-cholesta-7,9-diene-3-one thiadiazoline (9). Yield: 68 %; m.p. 170-171°C (hexane); m/z = 567 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 8.48 (NH, s), 5.39 (H-7, J = 6.4 Hz), 5.37 (H-11, d, J = 6.6 Hz), 2.15, 2.20 (CO<u>CH3</u>, 2s), 0.68 (H-18, s), 1.10 (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), 1.10 (H-29, d, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 36.8 (C-1), 36.9 (C-2), 91.8 (C-3), 51.4 (C-4), 50.7 (C-5), 21.8 (C-6), 117.8 (C-7), 142.8 (C-8), 143.2 (C-9), 38.2 (C-10), 119.1 (C-11), 26.9 (C-12), 44.1 (C-13), 44.5 (C-14), 31.8 (C-15), 29.7 (C-16), 45.5 (C-17), 15.8 (C-18), 18.2 (C-19), 36.1 (C-20), 18.7 (C-21), 34.8 (C-22), 24.9 (C-23), 30.0 (C-24), 36.6 (C-25), 21.8 (C-26), 21.8 (C-27), 24.1 (C-28), 15.3 (C-29), 143.5 (C=N), 168.5, 169.8 (<u>CO</u>CH_3), 22.4, 23.50(CO<u>CH3</u>).

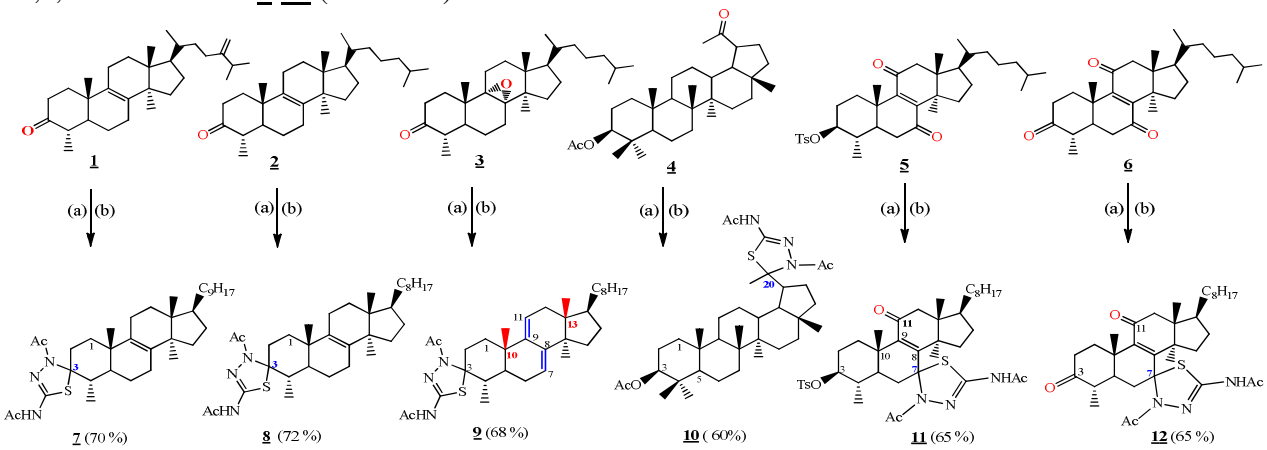
3β-Acetoxy-28-norlup-20-one thiadiazoline (10). Yield : 76 %; m.p. 203-204°C (hexane); m/z = 627 ($M^{+\cdot}$); ^{1}H NMR(300 MHz, $CDCl_3$) δ (ppm): 4.45 (H-3, dd, J_1 = 11 Hz, J_2 = 5 Hz), 8.39 (NH, s), 2.03, 2.14, 2.18 (CO<u>CH3</u>), 0.68 (H-5, d, J = 9 Hz), 2.38 (H-19, ddd, J_1 = 11.3 Hz, J_2 = 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): 37.5 (C-1), 38.1 (C-2), 81.5 (C-3), 38.6 (C-4), 55.4 (C-5), 18.1 (C-6), 34.4 (C-7), 40.8 (C-8), 50.2 (C-9), 37.1 (C-10), 20.5 (C-11), 25.1 (C-12), 37.8 (C-13), 42.6 (C-14), 27.2 (C-15), 35.4 (C-16), 42.8 (C-17), 42.6 (C-18), 47.8 (C-19), 100.5 (C-20), 29.5 (C-21), 39.8 (C-22), 27.8 (C-23), 15.3 (C-24), 16.0 (C-25), 15.9 (C-26), 14.5 (C-27), 17.8 (C-28), 19.1 (C-29), 145.5 (C=N), 21.1, 22.4, 22.5 (CO<u>CH3</u>), 168.2, 169.4, 170.2 (<u>CO</u>CH3).

3β-Tosyloxy-4α,14α-dimethyl-5α-cholest-8-ene-7,11-dione-7-thiadiazoline (11). Yield: 74 %; m.p. 221-222°C (hexane); m/z = 753.84 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) : 4.11 (H-3, ddd, J_1= 11Hz, J_2 =11Hz, J_3 = 3 Hz), 8.82 (NH, s), 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), 86.5 (C-3), 51.4 (C-4), 48.9 (C-5), 39.4 (C-6), 90.2 (C-7), 149.0 (C-8), 146.3 (C-9), 36.9 (C-10), 202.9 (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), 36.3 (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'), 147.7 (C=N), 168.5, 169.6 (COCH3), 22.6, 22.8 (COCH3).

4α,14α-Dimethyl-5α-cholest-8-ene-3,7,11-trione-7-thiadiazoline (12). Yield: 65 %; m.p. 236-237°C (hexane); m/z = 595 ($M^{+\cdot}$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.95 (NH, s),1.62 (H-5, m), 2.45 (H-6, dd, J_1 = 16.5, J_2 = 3.6 Hz), 2.11, 2.12 (COCH3, 2s), 0.86 (H-18, s), 1.15 (H-19, s), 0.87 (H-21, d, J = 6 Hz), 0.84 (H-26, d, J = 2 Hz), 0.85 (H-27, d, J = 2 Hz), 0.98 (H-28, s), 1.25 (H-29, d, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 36.6 (C-1), 37.2 (C-2), 212.4 (C-3), 52.9 (C-4), 52.3 (C-5), 39.0 (C-6), 84.0 (C-7), 157.5 (C-8), 142.4 (C-9), 38.2 (C-10), 200.4 (C-11), 37.9 (C-12), 43.7 (C-13), 48.2 (C-14), 32.3 (C-15), 30.6 (C-16), 50.1 (C-17), 15.9 (C-18), 18.3 (C-19), 36.7 (C-20), 18.7 (C-21), 29.9 (C-22), 29.5 (C-23), 29.2 (C-24), 36.2 (C-25), 21.7 (C-26), 21.8 (C-27), 27.7 (C-28), 16.2 (C-29), 145.2 (C=N), 168.4, 169.8 ($COCH_3$), 23.1, 23.3 (COCH3).

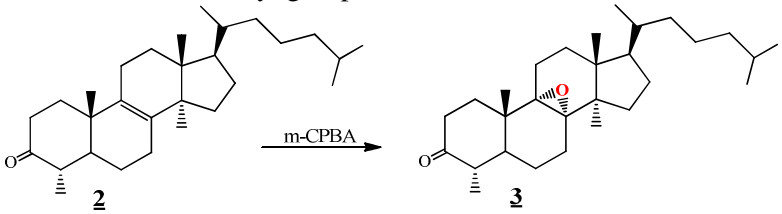
3. Results and discussion

In order to prepare new heterocyclic triterpene derivatives, we were interested to the reactivity of the ketones **1**, **2**, **3**, **4**, **5** and **6** hemisynthesized from *Euphorbia officinarum* latex [13-15]. The thiosemicarbazone [16, 17] derivatives obtained were treated with pyridine and acetic anhydride. All this allowed us, after chromatography on silica gel columns using a mixture of hexane/ethyl acetate 90/10 as eluent, to synthesize with a good yield the 1,3,4-thiadiazolines **7**-**12** (Scheme 2).



Scheme. 2: Thiadiazolic compounds **7**-**12**. [(a)] $NH_2CSNHNH_2$, EtOH, HCl (c)/5h.[(b)] Ac_2O/Pyridine/110°C/1h

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**.The reaction of acetic anhydride 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 *meta*-chloroperbenzoic acid 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 3.



Scheme 3: Epoxidation reaction of compound **2** [18]

The stereochemistry of compound **3** has been confirmed by single-crystal X-ray diffraction (figure 1).

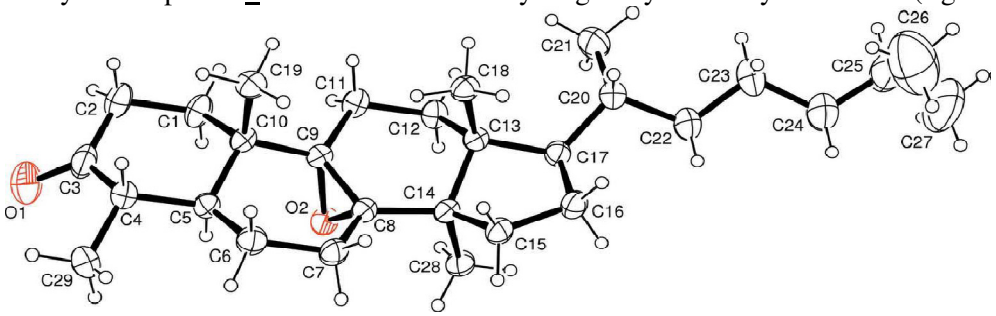


Figure 1: ORTEP drawing of compound **3**

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*., 1999), 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, precursor of compound 11. The starting material 5 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 3), we could identify all structures of this product.

A detailed study of the ^{1}H, ^{13}C NMR and HMBC spectral analyzes allowed to assign the chemical shifts of different carbons of the precursor to 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).

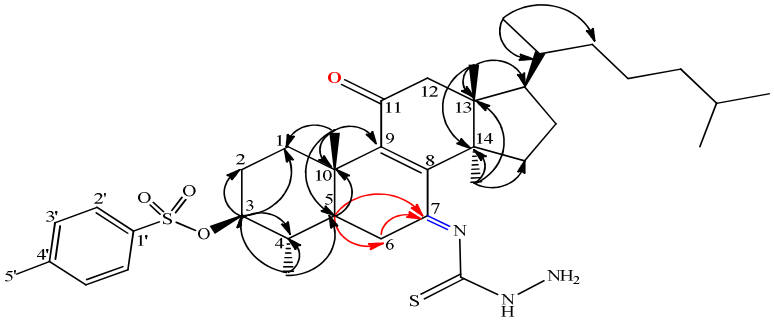


Figure 2: Principal HMBC correlations of precursor of compound **11**

The structures of newly prepared thiadiazolines **7**-**12** were fully characterized by ^{1}H NMR spectroscopic analysis. Thus, the signal for NHAc group was at 7.82, 8.75, 8.48, 8.39, 8.82 and 8.95 ppm for product **7**, **8**, **9**, **10**, **11** and **12** respectively. The ^{13}C NMR spectrums showed more precisely new signal of spiro carbon at 91.8, 100.0, 91.8, 100.5, 90.2, and 84.0 ppm for compounds **7**, **8**, **9**, **10**, **11** and **12** respectively. Table 1 summarizes the principal results obtained.

Table 1: Principal ^{13}C NMR[(a)] spectroscopic data (at 75 MHz) of compounds **7**-**12**[(b)]

C	δ ^{13}C (**7**)	δ ^{13}C (**8**)	δ ^{13}C (**9**)	δ ^{13}C (**10**)	δ ^{13}C (**11**)	δ ^{13}C (**12**)
C-3	**91.8**	**100.0**	**91.8**	81.5	86.5	212.4
C-7	28.0	28.3	117.8	34.4	**90.2**	**84.0**
C-8	132.7	129.0	142.8	40.8	149.0	157.5
C-9	134.9	131.1	143.2	50.2	146.3	142.4
C-11	21.5	22.0	119.1	20.5	202.9	200.4
C-20	36.7	36.0	36.1	**100.5**	36.3	36.7
C=N	143.7	143.2	143.5	145.5	147.7	145.2
$COCH_3$	168.6, 169.6	170.1, 171.0	168.5, 169.8	168.2, 169.4, 170.2	168.5, 169.6	168.4, 169.8
COCH3	23.4, 25.1	22.5, 24.2	22.4, 23.5	21.1, 22.4, 22.5	22.6, 22.8	23.1, 23.3

[(a)] Recorded in $CDCl_3$ (δ_C 76.9), [(b)] Chemical shifts are expressed in ppm.

Conclusion

We have described in our study a synthesis of new 1,3,4-thiadiazolic triterpene derivatives **7-12** with tetra- and pentacyclic skeletons resulting from *Euphorbia officinarum* latex.
The synthesis of 1,3,4-thiadiazolic compounds can be carried out starting from their precursors, the thiosemicarbazone derivatives [12] which were prepared by condensation of the carbonyl products **1-6** on thiosemicarbazide (TSC).
The thiadiazolines prepared were characterized by the presence of new spiro carbons and were obtained with a good yield and high regiospecificity.

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